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Apical Microleakage and SEM Analysis of Dentin Surface after 980 nm Diode Laser Irradiation

Maria Isabel Anastácio FARIA
Aline Evangelista SOUZA-GABRIEL
Edson ALFREDO
Danielle Cristine Furtado MESSIAS
Yara Teresinha Correa SILVA-SOUSA

Dental School, University of Ribeirão Preto, Ribeirão Preto, SP, Brazil

This study evaluated the effect of 980-nm diode laser on apical microleakage and intraradicular dentin morphology. Roots of 110 mandibular incisors were used in the study: 92 for microleakage test and 18 for scanning electron microscopy (SEM). Roots were randomly assigned to 3 groups according to the irrigating solution (water, NaOCl and NaOCl/EDTA) and were divided into 3 subgroups according to the laser irradiation protocol (without irradiation, irradiated at 1.5 W and irradiated at 3.0 W). Two specimens of each subgroup were prepared for SEM. The remaining roots were filled with AH Plus and gutta-percha. Apical leakage was assessed by ink penetration and data were analyzed statistically by ANOVA and Tukey-Krammer test (α=0.05). SEM analysis showed intensification of changes with increase of laser power as well as variations according to the irrigating solution. Modified smear layer was observed in specimens treated with water and irradiated with laser. Roots irrigated with NaOCl/EDTA had lower levels of infiltration (0.17 ± 0.18 mm) differing significantly ($p<0.05$) from those of roots irrigated with water (0.34 ± 0.30 mm), but similar ($p>0.05$) to those irrigated with NaOCl (0.28 ± 0.29 mm). Non-irradiated roots had lower levels of infiltration (0.10 ± 0.14 mm), differing ($p<0.05$) from those irradiated at 1.5 W (0.32 ± 0.22 mm) and 3.0 W (0.37 ± 0.32 mm). The 980 nm diode laser modified dentin morphology and increased apical microleakage.

Key Words: Apical sealing, diode laser, SEM.

INTRODUCTION

Although high-intensity diode lasers are relatively new, a diode device emitting at 980 nm has been studied for dental applications because of its low dimensions compared with other lasers and also due to its thin and flexible fiber that properly adapts to the curved shapes of root canals (1-6).

Diode laser wavelengths have good penetration potential, with high absorption peaks in melanin and hemoglobin and low interaction with water and hydroxyapatite (2,7). Its power output ranges from 0.5 to 7 W and has 3 operating modes: continuous wave (CW), pulsed power and chopped mode (6). Gutknecht et al. (1) reported that irradiation with the 980-nm diode laser can eliminate bacteria that have migrated deep into the dentin up to 500 µm, whereas chemical solutions can only reach 100 µm.

Morphological alterations in radicular dentin irradiated by 980-nm diode laser can be assessed by SEM (3-5,7). The ultrastructural features of dentin depend mainly on the laser parameters such as output power, frequency and application mode, because these parameters are directly related to the increase in temperature (8,9).

Some studies evaluated the interaction of 980-nm diode laser with the chemical substances used as irrigants during biomechanical preparation to remove smear layer and alter the dentin substrate. Wang et al. (7) verified smear layer removal, resulting in cleaner root canal walls by treating the dentin with NaOCl and 980 nm diode laser with 5 W. Dentin irrigated with NaOCl/EDTA and subsequently irradiated with 980 nm diode laser has been described as presenting absent or modified smear layer, cracks and some melted areas (4,10). These surface alterations in dentin after irradiation with 980 nm diode

Correspondence: Profa. Dra. Danielle Cristine Furtado Messias, Avenida Afonso Valera, 251, Recreio das Acácias, 14098-561 Ribeirão Preto, SP, Brasil. Tel: +55-16-3234-1025. Fax: +55-16-3603-6783. e-mail:danimessias@gmail.com

laser can be directly correlated with changes in apical leakage (7), dentin permeability (10) and adhesion of root canal sealers (11). The morphological changes in dentin walls caused by 980-nm diode laser irradiation could improve the sealing ability of endodontic sealers (11).

Although studies with diode lasers have been conducted, their influence on dentin morphology and microleakage in the apical root third is not yet fully clarified. The aim of this study was to assess the influence of 980-nm diode laser on apical leakage and intraradicular dentin morphology.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the University of Ribeirão Preto, Brazil (protocol #077/2010).

One hundred and ten mandibular incisors with fully formed roots, and no calcifications or resorptions (radiographically confirmed) were used in this study. The teeth were sectioned at the cementoenamel junction using a double-faced diamond disc (KG Sorensen, Barueri, SP, Brazil) and root length was standardized at 12 mm (incisors) and 18 mm (canines).

Conventional access was made and a #10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was introduced into the canal until its tip was seen at the apical foramen. The working length was established by subtracting 1.0 mm from this measurement.

Roots were randomly divided into 3 groups according to the irrigating solution used during chemomechanical preparation: GI- distilled and deionized water (control), GII- 1% NaOCl and GIII- 1% NaOCl combined with 17% EDTA.

Chemomechanical preparation of root canals was performed using ProTaper rotary system (Dentsply-Maillefer) in the following sequence: SX, S1, S2, F1, F2 and F3. The canals were irrigated with 2 mL of the tested irrigant (water or NaOCl) between files. In GIII, root canals were flooded by 2 mL of EDTA for 5 min. Final irrigation of all groups were done with 10 mL of distilled water.

The specimens were then randomly assigned to 3 subgroups, one control group (no laser irradiation) and 2 experimental groups, which were subjected to laser irradiation with different power parameters (1.5 and 3.0 W) and 100 Hz frequency. The laser source was a gallium-aluminum arsenide (GaAlAs) semiconductor 980 nm diode laser (SIROlaser 2.2; SIRONA Dental, Bensheim, Germany). The laser beam was delivered to the root canal walls of each specimen using a 200-µm-diameter flexible optical fiber with helicoidal movements along the canal. Only a single forward-backward movement was performed maintaining the fiber tip in contact with the root canal dentin wall for 12 s in incisors and 20 s in canines. The canals remained filled with distilled water during laser irradiation.

Two specimens of each subgroup were subjected to morphological analysis of dentin surface under SEM. The remaining teeth were filled with lateral condensation of AH Plus sealer (Dentsply DeTrey, Knstanz, Germany) and gutta-percha cones and then teeth were stored at 37°C for 24 h.

SEM Analysis of Dentin Surface

A diamond disk (KG Sorensen) mounted in a low-speed handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil) was used to make longitudinal sulcus along buccal and palatal surfaces of the tooth, with care not to reach the root canal. The teeth were fractured longitudinally with a double-tapered chisel and a surgical hammer exposing the entire root canal lumen.

The specimens were dehydrated at 37°C for 48 h, fixed on stubs with double-faced carbon tape (3M, São Paulo, SP, Brazil), sputter-coated with a 30-µm gold-platinum layer (Bal-Tec SCD 005, Zurich, Switzerland) in a vacuum apparatus (SDC050, Balzers, Liechtenstein)and examined with a scanning electron microscope (JEOL JSM model 5410; JEOL, Tokyo, Japan) operating at 15 kV. Initially, the specimens were analyzed in a panoramic vision and later SEM micrographs were obtained at ×1000 from a standardized area of intraradicular surface (at 8 mm from the apex).

Apical Microleakage

The external surface of each root was coated with cyanoacrylate adhesive (Super Bonder; Loctite Brasil Ltda, Itapevi, SP, Brazil), exceeding the cervical opening. One root was used as a positive control and one as a negative control. The roots were placed in India ink for 96 h at 37°C. After this period, the roots were subsequently washed for 1 h in running water and the cyanoacrylate layer was removed. Roots were placed in 5% hydrochloric acid (Merck KGaA, Darmstadt, Germany), dehydrated in 70, 80, 96 and 100% ethanol (1 h each), and then cleared in methylsalicylate (Vetec, Rio de Janeiro, RJ, Brazil). After clearing, apical leakage was measured in mm using a digital caliper (Digimess; Shiko Precision Gaging Ltda., China) and a stereomicroscope (Nikon, Tokyo, Japan).

Statistical Analysis

Following verification of the assumptions of ANOVA (normality and homogeneity), data obtained in the apical leakage test were evaluated using ANOVA and Tukey-Krammer multiple test. All statistical procedures were performed with Software Graph Instat (GraphPad Software Inc., San Diego, CA, USA) at a significance level of 5%.

RESULTS

SEM Analysis of Dentin Surface

Root dentin irrigated with water presented dense smear layer covering the entire surface (Fig. 1A). The same occurred in the specimens irradiated with the diode laser. However, lased-groups presented modified smear layer caused by surface heating in a less intense manner when using 1.5 W power (Fig. 1B) and in more intense manner with 3.0 W power (Fig. 1C).

When the 1% NaOCl was used as an irrigating solution, smear layer covering the dentin surface (Fig. 2A) was observed in non-irradiated specimens. The dentin of specimens irradiated with laser with 1.5 W presented modified smear layer, flat surface and cracks (Fig. 2B). The dentin surface of specimens irradiated with 3.0 W exhibited modified smear layer, some cracks and areas with exposed tubules (Fig. 2C).

In the non-irradiated specimens irrigated with 1% NaOCl plus EDTA 17%, dentin surface showed a typical pattern with flat surface, no smear layer and open dentinal tubules (Fig. 3A). The 980-nm diode laser irradiation with 1.5 W resulted in absence of smear layer and partially obliterated dentinal tubules (Fig. 3B). When the laser power was increased to 3.0 W, it was observed smear-free dentin surface and partially obliterated tubules, suggesting an initial melting process (Fig. 3C).

Apical Microleakage

Table 1 shows the mean values and standard deviation of apical infiltration (mm) for each group. There was statistically significant difference ($p<0.05$) for both tested factors: irrigating solution and laser irradiation.

Tukey's post-hoc test showed that roots irrigated with 1% NaOCl and 17% EDTA had less apical leakage, differing significantly from the those irrigated with distilled water ($p<0.05$). Specimens irrigated with distilled water showed the highest values of apical leakage, statistically similar ($p>0.05$) to those irrigated with 1% NaOCl.

Regarding the use of laser, the non-irradiated roots had lower levels of infiltration, statistically different ($p<0.05$) from the irradiated specimens, regardless of the power used. Specimens irradiated with 3 W were statistically similar to those irradiated with 1.5 W ($p>0.05$).

DISCUSSION

Laser irradiation has the capacity to reduce the number of microorganisms within the root canal system (1) and also removing debris and smear layer from the root canal walls following chemomechanical instrumentation (8,9,12,13). This study assessed the effect of 980-nm diode laser on apical leakage and intraradicular dentin morphology.

The choice for the two power settings (1.5 and

Table 1. Mean and standard deviation of apical infiltration (mm) for each group.

Group	No irradiation	Diode laser (1.5 W)	Diode laser (3.0 W)
Water (control)	(0.22 ± 0.19) Aa	(0.30 ± 0.24) Aa	(0.51 ± 0.39) Aa
1% NaOCl	(0.05 ± 0.10) Bab	(0.39 ± 0.24) Bab	(0.41 ± 0.35) Bab
1% NaOCl plus 17% EDTA	(0.04 ± 0.06) Bb	(0.27 ± 0.22) Bb	(0.20 ± 0.15) Bb

Different uppercase letters indicate statistically significant difference within columns (laser irradiation) ($p<0.05$). Different lowercase letters indicate statistically significant difference within rows (Irrigation solution) ($p<0.05$).

3.0 W) and the frequency (100 Hz) used in the present study was based on the results of Alfredo et al. (9), who demonstrated that these parameters yielded a temperature raise of almost 10°C, which does not exceed the threshold supported by the periapical tissues without causing thermal damage.

The results of ultrastructural analysis by SEM showed that laser irradiation resulted in alteration of dentin morphology, different from the non-irradiated specimens. In general, the laser effects were intensified when the power was increased from 1.5 W to 3.0 W. The current study also revealed that the effect of diode laser was dependent on the chemical action of each substance on dentin.

In the specimens irrigated with NaOCl, it was predominantly observed modified smear layer by laser

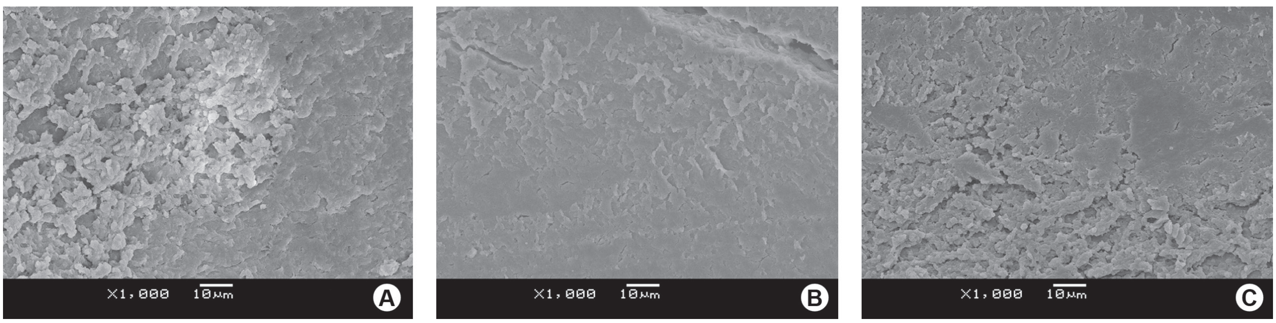

Figure 1. SEM micrographs of intraradicular dentin in specimens irrigated with water. A= Non-irradiated specimen showing the surface covered by smear layer. B= Surface irradiated with 1.5 W exhibiting the modified smear layer covering the surface. C= Surface irradiated with 3.0 W exhibiting modified smear layer and cracks (1000 ×).

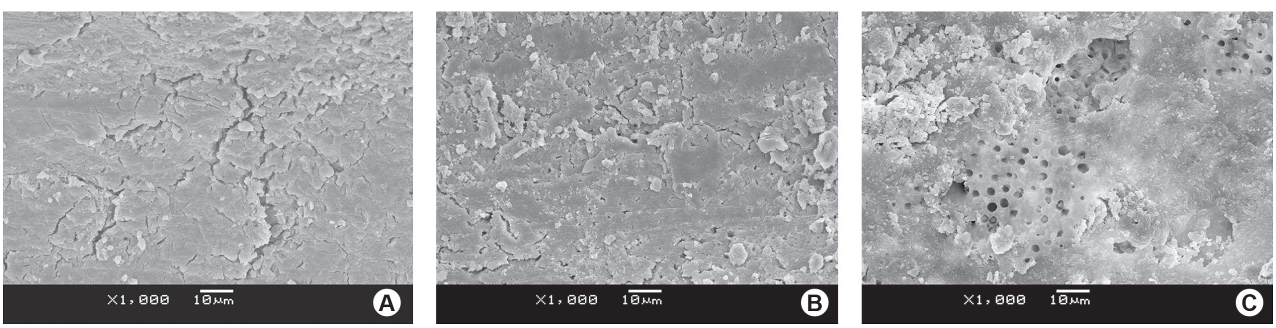

Figure 2. SEM micrographs of intraradicular dentin in specimens irrigated with 1% NaOCl. A= Non-irradiated specimen with the surface covered by smear layer. B= Dentin irradiated with 1.5 W exhibiting flat surface covered by modified smear layer and presence of cracks. C= Surface irradiated with 3.0 W exhibiting modified smear layer, with cracks and some areas of exposed tubules (1000 ×).

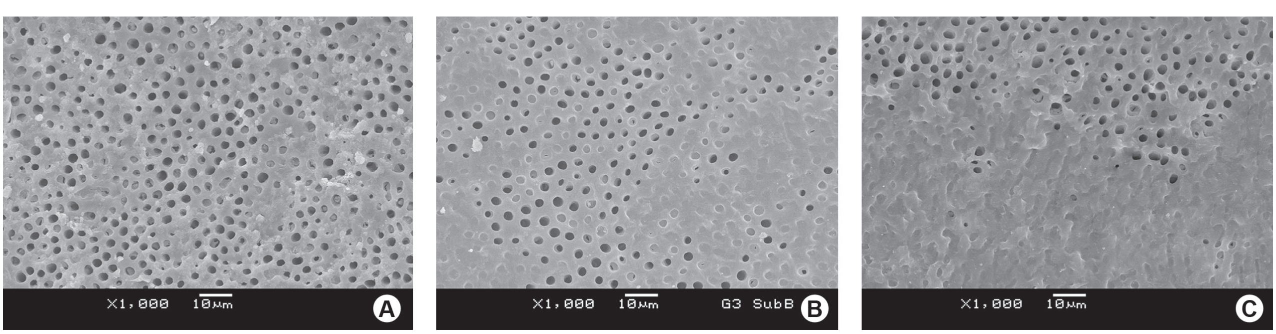

Figure 3. SEM micrographs of intraradicular dentin in specimens irrigated with 1% NaOCl + 17% EDTA. A= Non-irradiated specimen showing flat surface free of smear layer and open dentinal tubules. B= Dentin irradiated with 1.5 W showing absence of smear layer and partially obliterated tubules. C= Surface irradiated with 3.0 W showing no smear layer with partially obliterated tubules (1000 ×).

irradiation and cracks on dentin surface. Dentinal tubules were not visible. When NaOCl was combined with EDTA, absence of smear layer and open dentinal tubules were verified. With 3 W, an initial melting process was observed. These changes may be attributed to the thermal effect caused by overheating and subsequent cooling of the surface when the laser interacts with dentin (3,4,14,15). Esteves-Oliveira et al. (5) found signs of fusion and partially open tubules when dentin was irradiated with a 808 nm diode laser in continuous mode with 2.5 W. Gonçalves et al. (16) verified fused lava-like areas sealing the apical foramen when root canal was treated with CO_2 laser with 5 W.

As a rule, the irradiation of diode lasers is poorly absorbed by hard dental tissues and thus allows propagation, scattering, or diffused transmission of light through dentin (1,8). However, Marchesan et al. (3) observed sparse lava-like areas of dentin fusion after irradiation with 3 W with the same laser of this study, in root canals irrigated with water, suggesting that diode laser at 980 nm has good interaction with the dentin tissue.

The morphological changes observed in laser-irradiated surfaces affected the results of apical microleakage. The roots irradiated with 980-nm diode laser had the highest values of marginal apical leakage, regardless of the power used. The best results of apical sealing were obtained with the irrigation with NaOCl and EDTA, probably due to the removal of the smear layer by EDTA (17,18), which enables higher contact of the sealers to root dentin, favoring the mechanical interlocking of the sealer into the dentin tubules. Conversely, the presence of the smear layer in specimens irrigated with water or NaOCl allowed greater apical leakage. The smear layer impairs the contact of the sealer with root canal walls, resulting in higher leakage (19). Using Er,Cr:YSGG laser, other authors (20) also verified that the laser treatment does not enhance the sealing ability of the sealers compared with EDTA.

Good results of apical leakage with the application of 980 nm diode laser were obtained by Wang et al. (7); however, those authors applied the laser in dentin pretreated with 5% NaOCl and 3% hydrogen peroxide and irradiation was done with 5.0 W for 2 s at the apical stop and for 5 s along the root canal walls. According to Alfredo et al. (9), the use of power higher than 3.0 W in continuous mode exceeds the maximum acceptable temperature for maintaining the integrity of periapical tissues.

The laser parameter settings used in this study did not interfere in apical microleakage because the energy increments were probably not sufficient to promote severe ultrastructural alterations. Overall, this study showed that the application of 980-nm diode laser on intraradicular dentin resulted in ultrastructural alterations ranging from modifying smear layer to initial melting, and these effects were intensified with the increase of power. However, these alterations were not able to improve apical sealing, regardless of the power and irrigating solution used during the biomechanical preparation.

Sealing capacity is one of the main aspects of the quality of root canal sealer (20,21). Therefore, further research with other laser systems and fiber designs should be conducted searching for better technique to obtain the most effective sealing of the root apical area.

RESUMO

Este estudo avaliou o efeito do laser de diodo 980 nm na microinfiltração apical e na morfologia intrarradicular da dentina. Raízes de 110 incisivos inferiores foram utilizadas no estudo: 92 para o teste de microinfiltração e 18 para microscopia eletrônica de varredura (MEV). As raízes foram divididas em 3 grupos de acordo com a solução irrigante (água, NaOCl e NaOCl/EDTA) e subdivididas em 3 de acordo com a irradiação laser (sem irradiação, irradiados com 1,5 W e irradiados com 3,0 W). Duas amostras de cada subgrupo foram preparadas para MEV. As raízes restantes foram preenchidas com AH Plus e guta percha. A infiltração apical foi avaliada nas raízes por meio da penetração do corante e os dados foram analisados por ANOVA e teste de Tukey-Krammer (α=0,05). Os resultados da MEV mostraram intensificação das mudanças quando se aumentou a potência do laser e variações de acordo com a solução irrigadora. Camada de *smear* modificada foi observada em espécimes tratados com água e irradiados com laser. Raízes irrigadas com NaOCl/EDTA tiveram níveis menores de infiltração (0,17 ± 0,18 mm) estatisticamente diferente (p<0,05) das raízes irrigadas com água (0,34 ± 0,30 mm), mas semelhante (p>0,05) aos irrigados com NaOCl (0,28 ± 0,29 mm). As raízes não irradiadas tiveram níveis menores de infiltração (0,10 ± 0,14 mm), diferente (p<0,05) de 1,5 W (0,32 ± 0,22 mm) e 3.0 W (0,37 ± 0,32 mm). O laser de diodo 980 nm alterou a morfologia da dentina e aumentou a infiltração marginal apical.

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