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Effect of different diode laser wavelengths on root dentin decontamination infected with *Enterococcus faecalis*☆

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ABSTRACT

The objective of this study was to evaluate the antibacterial effect and the ultrastructural alterations of diode laser with different wavelengths (808 nm and 970 nm) and its association with irrigating solutions (2.5% sodium hypochlorite and 2% chlorhexidine) in root dentin contaminated by a five days biofilm. Thirteen uniradicular teeth were sectioned into 100 dentin intraradicular blocks. Initially, the blocks were immersed for 5 min in 17% EDTA and washed with distilled water for 5 min, then samples were sterilized for 30 min at 120 °C. The dentin samples were inoculated with 0.1 mL of *E. faecalis* suspension in 5 mL BHI (Brain Heart Infusion) and incubated at 37 °C for 5 days. After contamination, the specimens were distributed into ten groups (n = 10) according to surface treatment: GI - 5 mL NaOCl 2.5%, GII - 5 mL NaOCl 2.5% + 808 nm diode (0.1 W for 20 s), GIII - 5 mL NaOCl 2.5% + 970 nm diode (0.5 W for 4 s), GIV - 808 nm diode (0.1 W for 20 s), GV - 970 nm diode (0.5 W for 4 s), GVI - CHX 2%, GVII - CHX 2% + 808 nm diode (0.1 W for 20 s), GVIII - CHX 2% + 970 nm diode (0.5 W for 4 s), GIX - positive control and GX - negative control. Bacterial growth was analyzed by turbidity and optical density of the growth medium by spectrophotometry (nm). Then, the specimens were processed for analysis ultrastructural changes of the dentin surface by SEM. The data was subject to the One-way ANOVA test. GI (77.5 ± 12.1), GII (72.5 ± 12.2), GIII (68.7 ± 8.7), GV (68.3 ± 8.7), GVI (62.0 ± 5.5) and GVII (67.5 ± 3.3) were statistically similar and statistically different from GIV (58.8 ± 25.0), GVIII (59.2 ± 4.0) and control groups ($p < 0.05$). SEM analysis showed a modified amorphous organic matrix layer with melted intertubular dentin when dentin samples were irradiated with 970 nm diode laser; erosion of the intertubular dentin in blocks submitted to 808 nm diode laser irradiation; and an increased erosion of the intertubular dentin when 2.5% NaOCl was associated to the different wavelengths lasers. All the therapeutic protocols were able to reduce the bacterial contingent in dentin blocks, and the association of diode laser and solutions did not significantly improve the reduction of the bacterial contingent.

1. Introduction

The disinfection of the root canal system aims to eliminate irritants such as bacteria, their products and pulp tissue remains, providing a favorable environment for the repair of the periapical tissues [1] through the action of endodontic instruments aided by irrigation solutions and intracanal medication [2,3].

However, variations in the internal anatomy of root canals such as flattening, presence of isthmuses, recesses and ramifications can interfere on the success of the disinfection, hindering the instrumentation techniques and favoring the persistence of tissue and bacteria remnants [4]. In addition, the dentin has a tubular characteristic with a conical conformation, wherein, the larger diameter of the tubules is located near the lumen of the canal, which allows the penetration of bacteria in deeper areas of the dentin [5].

The depth of bacterial penetration in the dentin tubules is on average 500 μm [6]. The bacterial species *Enterococcus faecalis*, which has a high prevalence in cases of persistent apical periodontitis [7], is known to extend to a still greater depth, penetrating 800–1000 μm in the dentinal tubules after three weeks of incubation [8].

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Therefore, diode laser at different wavelengths (970 nm and 808 nm) was developed for endodontic application, and has thin optical fibers, with a diameter of 200–320 μm, which allow adaptation to the reduced dimensions and curvatures of the root canals, enabling the decontamination along the root canal [9]. Due to the near-infrared wavelength, the diode laser is able to reach deeper layers of the dentin [10], acting where the irrigating solutions are incapable of acting. In addition, the diode laser is able to increase dentin permeability and remove the smear layer in the intraradicular dentin [11,12] without altering its chemical structure [13].

The persistence of bacteria in the root canals is a challenge for Endodontics once the control and elimination of microorganisms are necessary to reach the success of endodontic treatment [14]. Since irrigation solutions have limitations on penetration depth in the dentin tubules [15,16] and that the eradication of root canal bacterial species has not yet been achieved, further studies using auxiliary tools are necessary to ensure a successful endodontic treatment.

The purpose of the present study was to evaluate the antibacterial effect and the ultrastructural alterations of diode laser with different wavelengths (808 nm and 970 nm) and its association with irrigating solutions (2.5% sodium hypochlorite and 2% chlorhexidine) in root dentin contaminated by a five days biofilm.

2. Material and Methods

The experimental protocol was approved by the local ethical committee (19,811,113.0.0000.5083). Maxilary anterior teeth and mandibular pre-molars extracted by orthodontic or periodontal reasons were collected and stored in 0.1% thymol solution at 9 °C for 48 h. The teeth were washed in running water for 24 h and had its external surface ultrasonically cleaned (Profi II Ceramic, Dabi Atlante Ltda., Ribeirão Preto, SP, Brazil). Then, teeth were examined macroscopically with a magnifying glass (10 × magnification), and radiographed in the ortho and mesio-radial directions and later evaluated with the aid of a negatoscope. Thus, thirteen teeth were selected according to the following inclusion criteria: uniradicular teeth, with fully formed roots and single root canal, absence of calcification, curvatures and resorptions, without flattening and absence of previous endodontic treatment.

The samples were fixed in acrylic plates with low melt godiva (DFL, Porto Alegre, RS, Brazil). Then, teeth were attached to a fastening device in the cutting machine (Labcut 1010, Erios, São Paulo, SP, Brazil), and sectioned perpendicularly with a diamond saw to the long axis of the root in order to obtain two slices of 2 mm thickness of the cervical third of each teeth, thus obtaining 26 slices. The middle and apical root thirds and the crowns were discarded, and only the cervical slice was used in this study.

Each cervical dentin slice was sectioned twice. The first section was performed in the sagittal plane of the slice dividing it in half, and the second section in the frontal plane. The blocks were adjusted with a fine polishing disc (3 M ESPE Dental Products, St. Paul, MN, USA) and their measurements were verified using a digital caliper (Mitutoyo, Suzano, SP, Brazil) in order to obtain the standardized dimensions of 4 mm height, 4 mm width and 2 mm thickness, totalizing 104 blocks of dentin.

One hundred blocks were randomly selected for this study. The blocks were placed in a 150 mL becker with 17% ethylenediaminetetraacetic acid (EDTA) (Formula and Action, São Paulo, SP, Brazil) and kept under stirring on a tube shaker (Vortex, Model AP 56, Presidente Prudente, SP, Brazil) for 5 min. Subsequently, the same process was performed, however, using distilled water. The blocks were kept hydrated in a 150 mL becker and were incubated for 24 h.

Afterward, the becker distilled water was renewed so that the blocks were kept hydrated during sterilization in an analogue vertical autoclave (Idealclave, Stermax, Barueri, São Paulo, Brazil) for 30 min at 120 °C. After sterilization, the blocks were incubated in BHI at 37 °C for 48 h to confirm the absence of bacteria by visual analysis. The blocks were left in sterile saline until use.

2.1. Standardization of the Bacterial Indicator

The bacterial strain used in this study was *Enterococcus faecalis* (ATCC 29212) inoculated in 7 mL brain heart infusion (BHI, Difco Laboratories, Detroit, USA) and incubated at 37 °C for 24 h. Twenty-four hours prior to specimen contamination, the bacteria were again cultured on the surface of the BHI agar following the same incubation conditions. The bacterial inoculum was obtained by resuspending the cells in saline at a final concentration of approximately 3×10^8 cells mL-1, adjusted for the McFarland turbidity standard 1 standardized by UV spectrophotometer (Spectrophotometer Model Nova 1600 UV, Piracicaba, SP, Brazil) [17].

2.2. Formation of the Biofilm on the Dentin Blocks Surface

After the standardization of the bacterial inoculum, 0.1 mL of *Enterococcus faecalis* suspension was dispensed into 5 mL of BHI contained in test tubes (15 × 150mm). Hereafter, the blocks of dentin that were so far in sterile saline were transferred to this new solution containing *Enterococcus faecalis* and stored at 37 °C for five days to allow bacterial fixation.

Ten blocks were kept contaminated throughout the experiment as a positive control to verify bacterial viability, while 10 uncontaminated blocks were maintained in 5 mL of sterile BHI as a negative control to ensure sterility of the sample.

After bacterial fixation in the dentin blocks, UV spectrophotometer analyses (Spectrophotometer Model Nova 1600 UV, Piracicaba, SP, Brazil) was performed, comparing with the negative control group, which remained sterile throughout the experiment. The UV spectrophotometer allows the BHI medium without contamination to be set to zero.

After the spectrophotometer analysis of all the samples, ensuring the contamination of the samples, the specimens were randomly distributed in ten groups, according to the surface treatment to which they were submitted.

2.3. Disinfection Protocols

The groups treated with sodium hypochlorite (groups I, II and III) were immersed in 5 mL of 2.5% sodium hypochlorite (Fitofarma, Lt. 20,442, Goiânia, GO, Brazil) contained in the Petri dish (K13-0035, KASVI, Goiânia, Brazil) for 5 min. The blocks were then pinched and transferred to a gauze pad over another Petri dish to remove excess sodium hypochlorite.

Group II, after immersion in 2.5% sodium hypochlorite, the intraradicular dentin was irradiated with 808 nm laser (DMC Whitening Lase II, São Carlos, SP, Brazil), with an optical fiber tip with 200 μm diameter, perpendicularly to the surface, on all faces of the dentin blocks. In each face, irradiation with light in continuous mode (100 mW), power of 0.1 W, for 20 s, totalizing 2 W, with energy density 31,847 J/cm^2 (Table 1) was performed.

Group III, after the same immersion process as Group II, was irradiated with 970 nm diode laser (Sirona Dental, Benshein, HE, Germany)

Table 1
Parameters established for diode laser use.

Parameters	808 ± 10 nm diode laser	970 ± 15 nm diode laser
Diameter of the fiber optic tip	200 μm	200 μm
Mode of application	Continuous wave	Continuous wave
Duration	20 s	4 s
Power	2 W	2 W
Energy density	31,847 J/cm2	159,23 J/cm2