High-power diode laser in the disinfection in depth of the root canal dentin

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Objective. The objective of this study was to evaluate the disinfection degree of dentine caused by the use of diode laser after biomechanical procedures.
Study design. Thirty teeth were sectioned and roots were autoclaved and incubated for 4 weeks with a suspension of *Enterococcus faecalis*. The specimens were randomly divided into 3 groups (n = 10): G1, instrumented with rotary files, irrigated with 0.5% sodium hypochlorite and 17% EDTA-T, and then irradiated by 830-nm diode laser at 3 W; G2, the same procedures as G1 but without laser irradiation; and G3, irrigation with saline solution (control). Dentin samples of each third were collected with carbide burs and aliquots were sowed to count viable cells.
Results. The disinfection degree achieved was 100% in G1 and 98.39% in G2, when compared to the control group (G3).
Conclusion. Diode laser irradiation provided increased disinfection of the deep radicular dentin in the parameters and samples tested. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e68-e72)**

The success of endodontic therapy is solidly based on efficient disinfection of the root canal system.[1-4] Persistent contamination following chemical preparation is due to the inadequate execution of the technique and to the anatomical difficulties present in the radicular canal system.[5-8]

Among the most commonly found microorganisms in root canal re-intervention with or without periradicular lesions is the coccus gram-positive *Enterococcus faecalis*, which is resistant to endodontic therapy procedures.[9-13] Many resources, such as interappointment dressings, irrigation solutions, and different types of laser radiations are used to act against these microorganisms, which are resistant to chemical surgical preparations.

Disinfection of the main canal through use of laser radiation has been demonstrated by many studies.[14-17] It is worthwhile to analyze its effectiveness of decontamination deep in the dentin (dentinal tubules) where microorganisms that remain following endodontic treatment may cause failure.[18,19]

The high-power diode laser has been tested in several areas of dentistry, with promising results in relation to dentinal disinfection.[20-26] Because of the laser's properties and its low cost in relation to most lasers used in endodontics, the diode laser has proved to be a resource worth testing. This study aims to verify the degree of disinfection deep in the dentin caused by the use of high-power diode laser irradiation, following chemomechanical procedures against *Enterococcus faecalis.*

MATERIAL AND METHODS

Thirty single-rooted teeth, supplied by the Human Tooth Bank from the College of Dentistry at the University of São Paulo, were cut to a standard length of 15 mm, cleaned, and emptied with the help of file K-type #15 and 1% sodium hypochlorite in order to remove the pulp remains. Following these procedures, the apical third of these roots was gradually filed until reaching the K-type #40 (Dentsply-Maillefer, Ballaigues, Switzerland) in order to standardize the specimens. The roots were then waterproofed externally using cyanoacrylate (Super Bonder, Loctite Henkel, Itapevi, SP, Brazil) and dried at room temperature for 24 hours. The roots were set up in 1.5 mL Eppendorf tubes coated in heavy condensation silicone (Zetaplus, Zhermack, Rovigo, Italy) and the set underwent sterilization in a 134°C autoclave for 15 minutes.

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Preparation of inoculation and contamination of the specimens

A suspension of 50 μL of the *E. faecalis* ATCC 29212 strand was incubated in 5 mL of tryptic soy broth (TSB) culture medium (Difco, Sparks, MD, USA) in a 37°C incubator for 24 hours. The concentration of the inoculation was then adjusted for a degree of turbidity 1 according to the McFarland scale (Bio Mérieux, Marcy l'Etoile, France), which corresponds to a bacterial concentration of 3×10^8 cells/mL referent to an optic density of 550 nm.

Next, the specimens had their roots filled with the inoculation and were incubated for 4 weeks in a 37°C incubator (with the culture medium being filled every day). Each handling, seeding, and inoculation was done in a laminar flow chamber.

All of the specimens had a portion of the inoculation transferred in TSB medium in order to check bacterial growth at several time periods, with a result of 100% positive.

Group design

The specimens were randomly split into 3 groups, as follows:

Group 1. Ten specimens were prepared with 0.5% sodium hypochlorite and urea peroxide cream (Endo PTC cream, Oficinalis Farmácia de Manipulação, São Paulo, SP, Brazil). Chemomechanical procedures were carried out using the K^3 system rotary files (Sybron Endo, Orange, CA) crown-apex technique. Final irrigation was done using 17% EDTA-T (Oficinalis Farmácia de Manipulação, São Paulo, SP, Brazil). In order to prevent apical overfilling, and to maintain the root canal shape, the sequence of instruments used was #40 (0.04 and 0.06 taper), #45 (0.02 and 0.04 taper), and #50 (0.02 taper). The specimens were later rinsed using saline solution and dried with absorbent sterile paper points (Cell Pack Dentsply, Petrópolis, RJ, Brazil). There was 10 mL of sodium hypochlorite solution used per tooth; the same amount of 17% EDTA-T was used.

Intracanal irradiation was performed using a high-power diode laser (Opus 10, OpusDent, Sharplan, Norwood, MA) at a wavelength of 830 nm, and set at a power of 3 W. The diode laser device was loaned by the Special Laser Laboratory of Dentistry, LELO-FOUSP. Irradiation followed the oscillatory technique developed by Gutknecht et al.[15]: the fiber is introduced 1 mm short of the apex and is recessed in helicoidal movements at a speed of approximately 2 mm/sec for 5 seconds, repeated 4 times at intervals of 10 seconds between each one.

Group 2. Ten instrumented specimens, rinsed and dried in the same way as group 1, but without irradiation.

Group 3. Ten specimens without instrumentation, nonirradiated, rinsed in saline solution with a syringe, and dried with absorbent paper points. Total saline solution used per tooth was 20 mL.

Sample collection

The specimens were sliced into thirds, crosswise, with diamond bur at high speed. Each third was drilled with a sterile bur, which was not reused. The handpiece was refrigerated with sterile saline solution in a container that had been disinfected beforehand together with the interior of the hoses. To collect the samples, drilling was done with a sterile Batt-type drill (at low speed), from the lumen of the main canal to halfway through the root, using centrifugal drilling, an adaptation of the methodology used by Haapasalo and Ørstavik[6] in 1987. Each third provided two samples: circumpulpal dentin (dentinal dust) and deeper dentin from the root canal (cylinder remaining), with the exception of the apical third, which was not drilled because it was less thick.

Thus, each specimen provided 5 portions, which were immersed in a 1 mL peptonated water solution, previously distributed in Eppendorf tubes. The tubes were then vibrated in Fisher Vortex equipment (Genie 2, Fisher Scientific, Bohemia, NY) to homogenize the samples. In total there were 50 portions collected from each group, which underwent dilutions of 10^{-1} and 10^{-2}, providing 150 samples.

We collected 25 μL, in triplicate, for each sample and seeded on a Petri dish with selective medium for *E. faecalis*—mEnterococcus Agar (Difco)—in order to count colony-forming units (CFUs). Incubation lasted 48 hours in a 37°C incubator.

Statistical analysis was performed to evaluate the degree of disinfection obtained in the radicular dentin.

RESULTS

Colony counts were done with the aid of a magnifying lens. Sample data did not show normal distribution, so the statistical test used was the nonparametric Kruskal-Wallis test, with comparison using the Dunn method of means. The mean numbers for CFUs/mL of groups were: group 1 = 0, group 2 = 2.77×10^2, and group 3 = 171×10^2 CFU/mL. Statistical analysis demonstrated significant differences among the groups ($P < .05$). The samples (circumpulpal dentinal dust and deep dentin) were analyzed, and the CFUs/mL means are shown in Table I. The mean numbers of CFUs/mL in the groups' dental root thirds are shown in Table II. The degree of disinfection for the groups (both in

Table I. Colony-forming units (CFUs)/mL means of circumpulpal dentin and deep dentin

Experimental groups	*Circumpulpal dentin (dentinal dust)*	*Standard deviation*	*Deep dentin (external cylinder)*	*Standard deviation*
Group 1	0		0	
Group 2	205.1	439.0	171.5	401.8
Group 3	17817.3	24497.3	11369.2	20677.7

Table II. CFUs/mL means of the dental root thirds

Experimental groups	*Cervical*	*Standard deviation*	*Middle*	*Standard deviation*	*Apical*	*Standard deviation*
Group 1	0		0		0	
Group 2	277.5	521.5	77.1	216.8	16.0	24.8
Group 3	17642.1	24693.6	11040.3	20454.2	8803.8	16652.5

relation to group 3, which presented total contamination) is shown in Table III.

Table III. Disinfection degree, in relation with group 3 (control)

Experimental groups	*Disinfection, %*	*Means*	*Standard deviation*
Group 1	100.00	0	
Group 2	98.39	277	369.1
Group 3	0	17100	21529.1

DISCUSSION

E. faecalis is a microorganism frequently found in cases of secondary infection and has proven resistant to commonly used interappointment dressings.[27-31] The results obtained in this experiment show how promising the clinical application of the diode laser irradiation can be in endodontics. Its performance in deep dentinal tubules is also recommended where anatomical difficulties (apical delta, accessory canals) do not permit the instrument to be used within the dentin walls.

In this experiment, the cell concentration in the inoculation was approximately 10^8 cells/mL; in the control group the average was at 10^4 CFUs/mL (maximum of 8.9×10^4 and a minimum of 5.2×10^2 CFUs/mL). These data lead to at least 2, nonexclusive hypotheses: the number of microorganisms in the canal decreased (1) due to cellular death caused by the ecosystem's adversities (fewer nutrients, age of the culture by incubation time, intratubular penetration with less culture medium for the physical conditions, the very dilution provoked by the culture medium's daily complementation, and so forth) and (2) the degree of precision of the methodology used to count the CFUs. The latter hypothesis also probably explains why there were no CFUs/mL in the group undergoing laser irradiation. This methodology's level of sensitivity is believed to be insufficient for detecting possible viable cells in lower concentrations. Even so, in addition to being widely used for some time now, the method has proven to be effective when comparing the groups, which showed that the diode laser irradiation provoked a higher level of disinfection than in the other 2 groups tested, with significant statistical differences. In this methodology, the study was done in 2 parts of the radicular dentin (circumpulpal dentin and deeper dentin) to test the capacity of the diode radiation laser to deeply disinfect this dentin. Batt-type drills were used because of the smaller root volume of the human teeth used in the study, and for maintenance of the conicity of the remains, so that the drilling could, as faithfully as possible, split the root crosswise into 2 portions of equal volume. The division into thirds was also done for the same reason: it makes it easier to control drilling. Similar to the results from previous studies, greater likelihood of infection was found in the cervical third, followed by the middle and apical thirds. This result is mainly due to the larger amount of dentinal tubules from the cervical to the apical in the root canal system.[1,21,22]

It is well known that chemomechanical preparation proves to be highly effective against contamination of the main canal, which was corroborated by this study, where the prepared group's rate of disinfection reached 98.39%. This shows the importance and the effectiveness of the precepts of the endodontic technique, which when executed appropriately is quite efficient in controlling *E. faecalis*. It also showed that the use of rotary systems during endodontic therapy promoted results that were completely satisfactory in regard to disinfection, which is shown in other studies.[3,4,13]

The high-power diode laser reduces dentine perme-

ability, although it did not provoke the dentine melting characteristic of neodymium laser irradiation on the dentin surface.[20,21,23,24] Among the other advantages of the diode laser are its size as a compact device and its use in diverse areas of dentistry.[19,21,26] The diode laser device is composed of 2 layers of semiconductor material interlaced with a nonconductive layer (bandgap layer). Its light presents a spectrum that allows for greater absorption by water than dental tissues when compared with Nd:YAG laser.[32] This characteristic means greater laser light penetration through the dentin with little interaction on the dentin, making it possible to act on the microorganisms present in the dentinal tubules.[25] Its effectiveness at disinfecting in relation to diverse microorganisms has been demonstrated by many authors, even against *E. faecalis.*[14,16-19]

The parameters used in this study were considered safe in accordance with Radaelli et al.[19]; however, other studies should be done in vivo to check whether this radiation can cause cellular damage to the periodontal ligament when acting in depth, for example.

CONCLUSION

Diode laser irradiation provided increased disinfection of the deep radicular dentin in the parameters and samples tested.

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