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Abstract. Endotoxins released in the dental root by Gram-negative microorganisms can be neutralized by calcium hydroxide, when this medication is applied inside the root canal for at least seven days. However, several clinical situations demand faster root canal decontamination. Thus, for faster endotoxin neutralization, endodontists are seeking additional treatments. The *in vitro* study tested whether or not intracanal Nd:YAG laser irradiation would be able to neutralize endotoxin within the human dental root canal in a single session. Twenty-four human teeth with one root were mounted between two chambers. After conventional endodontic treatment, root canals were contaminated with *Escherichia coli* endotoxin. Then they were irradiated or not (controls) in contact mode with an Nd:YAG laser (1.5 W, 15 Hz, 100 mJ and pulse fluency of 124 J/cm^2). The endotoxin activity was measured using the limulus lysate technique and data were statistically compared ($p \leq 0.05$). The concentration of active endotoxin measured in the negative control group was significantly lower than that of the positive control group ($p = 0.04$). The concentrations of endotoxin in both irradiated groups were significantly lower than that of the positive control group ($p = 0.027$) and similar to that of negative control group ($p = 0.20$). A single session of intracanal Nd:YAG laser irradiation is able to neutralize endotoxin in the dental root tissues. [DOI: 10.1117/1.JBO.17.11.118002]

Keywords: dentin; endodontic treatment; endotoxin; Nd:YAG laser.

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1 Introduction

Gram-negative bacteria, regularly found in the oral cavity, have endotoxin, a lipopolysaccharide (LPS) complex, at the cell wall. Endotoxins released by cellular destruction or during the cell division process[1] are involved in tissue destruction and may stimulate the production of bradykinin, an important pain mediator.[2] Therefore, during endodontic treatment, with the aim of eliminating bacteria present in the root canal system, endotoxins are released, which may cause a series of undesirable post-operative local and systemic reactions.[3,4] In fact, it has been demonstrated that pulpless teeth contain high levels of endotoxin.[5,6] Moreover, endotoxic LPSs that are produced by oral Gram-negative microorganisms may be factors in pulpal and periapical diseases.[7–9]

Several drugs, particularly calcium hydroxide, have been used in order to disinfect the root canal system.[10,11] Calcium hydroxide is able to neutralize endotoxin; however, this neutralization takes time[12] and is impaired inside the root canal by the presence of dentine powder.[13] Thus, in several clinical situations when time is an issue, for example, for oral prophylaxis before surgery in patients with heart diseases, malignant tumors or other pathologies, it would be of importance to obtain faster root canal decontamination. Strategies that have been recommended to enhance disinfection after chemomechanical preparation include the use of an interappointment intracanal medication or an optimized single-visit disinfection (OSD) approach.[14,15] Thus, searching for treatments capable of faster neutralization of the endotoxin present in the root canal system is still challenging to endodontists.

Application of a laser for endodontics was first described in 1971,[16] and a variety of papers on potential applications for lasers in endodontics have been published.[17]

Lasers that use optical fibers as a delivery system, such as diode lasers (810 or 980 nm), erbium lasers (Er:YAG or Er, Cr:YSGG) or even a KTP laser can be used in intracanal treatment. However, the Nd:YAG laser already has established and accepted parameters of use based on a large number of studies showing its efficiency in microbial reduction[17–21] without causing undesirable thermal effects.[20,22–24] Thus, the laser technology has been presented as an instrument whose overall effects represent a decisive improvement in the efficiency of conservative endodontic treatment.[19]

Gutknecht et al.[19] obtained a high rate of success in repairing periapical lesions, containing Gram-negative bacteria, by using an Nd:YAG laser irradiation. Thus, it would be possible that, besides the bactericidal effect, the Nd:YAG laser irradiation could also have some effect on endotoxin neutralization. The aim of this study was to test whether or not a single session of intracanal Nd:YAG laser irradiation would be able to neutralize *Escherichia coli* endotoxin on human dental root.

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2 Materials and Methods

2.1 *Sample Preparation*

Twenty-four human teeth with one root, provided by the Human Tooth Bank of the School of Dentistry of the University de São Paulo, were used. This project was approved by the University of São Paulo School of Dentistry Ethical Committee. The selected teeth were stored in sterile milli-Q water followed by immersion into 1% sodium hypochlorite solution (Officinalis, São Paulo, SP, BR) for 6 h for disinfecting purpose.

The crown and part of the cervical portion of each tooth were removed using diamond disks with a low speed handpiece in order to obtain 15 mm-long roots. The working length was obtained by subtracting 1 mm from the root apical end. Next, the canals were prepared by the crown-down technique, up to file number 45 with Nitiflex files (Dentsply, Maillefer, Ballaigues, Switzerland) and 2.5% sodium hypochlorite solution (Officinalis). Complementing removal of dentinal debris with 17% EDTA solution (Officinalis), final irrigation with milli-Q water was done. In 21 teeth, the cervical and 5 mm of cervical-occlusal portion of the external root surface was sealed with sticky wax. Three teeth (negative control group) had the entire external root surfaces totally sealed with sticky wax. In all other teeth, an apical barrier consisting of dentin disk (1 mm in depth) was bonded to the dental root with epoxy resin that is resistant to high doses of gamma irradiation. Then, this apical region was sealed with sticky wax. This apical barrier was placed for preventing the sticky wax displacement due to the irradiation that would compromise the seal. The teeth were placed into open-end polystyrene tubes, and each tooth-tube interface was sealed with sticky wax. Then, these tooth-polystyrene tube sets were packed in polyethylene plastic bags and sterilized by gamma irradiation (^{60}Co) to 3 Mrad (30 kGy).

From here on, all the experimental procedures were done under a laminar flow. For the experiments, the dental roots were mounted between two chambers. The upper chamber was represented by each tooth-polystyrene tube set that was mounted in a borosilicate glass container, considered the lower chamber. This lower chamber was previously sterilized in an oven at 250°C for 1 h.

To be sure that after sterilization the specimens (upper plus lower chambers set) were endotoxin-free, pyrogen-free water was added to the lower chamber (9 mL) and to the upper chamber (500 μL) of all the specimens (controls and experimental specimens). These specimens were sealed with parafilm and incubated at room temperature for 72 h. Then, 200 μL of water were removed from each lower chamber and tested to detect endotoxins. The limulus amebocytes lysate (LAL) technique (kinetic turbidimetric test; Pyrotell-T; Associates of Cape Code Incorporated, East Falmouth, MA, USA) was used for the determination of endotoxin concentration.[25] This reliable biological method uses the lyzed amebocytes of the horseshoe crab (*Limulus polyphemus*)[26] and is feasible to detect low values of endotoxin due to its great sensitivity (0.001 UE/mL). The sets that represented endotoxins were replaced, submitted to gamma irradiation and reassessed. The absence of endotoxins 72 h after the sterilization process confirmed the efficiency of the methodology used and provided reliability for the experimental sequence.

2.2 *Endotoxin Inoculation*

The upper chamber water was aspirated and the root canals were dried with sterile paper cones. Then, 500 μL of a solution containing endotoxin from *E. coli* (16 EU/mL obtained after dilution of pure *E. coli* endotoxin; Associates of Cape Code Incorporated, catalog number E0005) were placed in the upper chamber. The passage of the endotoxin through the dental root dentin was observed for 72 h. For this, the specimens were maintained at room temperature and the lower chamber endotoxin content was assessed every 24 h. When the endotoxin in the lower chamber reached a stable concentration, which occurred between 48 and 72 h, the upper and lower chambers were emptied and the root canals were dried with absorbent paper cones. Then, the contaminated specimens were assigned to the different experimental groups, as described below.

2.3 *Experimental Groups*

Positive control: The teeth ($n = 3$) were not irradiated.

Negative control: The completely sealed teeth ($n = 3$) were not irradiated.

Spiral laser: The teeth ($n = 9$) were submitted to an Nd:YAG laser irradiation, in accordance with the recommended kinetics[20] (slowly moving the laser from the apical to coronal surfaces in a continuous, circling fashion to irradiate all dentinal tubules at a speed of 2 mm/s).

Up-and-down laser: The teeth ($n = 9$) were submitted to an Nd:YAG laser irradiation with apical-coronal movements, each movement lasting for one second, with total irradiation time of 7 s (proportional to half the root canal length).

2.4 *Laser Irradiation*

The irradiations were done with an Nd:YAG laser equipment (Lares PowerLaseTM, Lares Research, CA, USA). This 1,064 nm laser has a pulse width of 120 μs, and operates through a silica optical fiber with core diameter of 320 μm. The irradiations were done in contact mode within the following parameters: 1.5 W, 15 Hz, 100 mJ, pulse fluency of 124 J/cm^2 for 7 s (time of use inside the root canal proportional to half the canal length that was 14 mm). Each tooth was irradiated five times, with thermal relaxation intervals of 30 s between each irradiation. Before the irradiation of a new tooth, the optical fiber was cleaned with a paper towel dampened with a 70% ethanol solution and cleaved when necessary. Before irradiating each tooth, the power was checked using a power meter (Ophir, Jerusalem, Israel).

3 Evaluation Method

After laser irradiation, all the lower chambers were replaced by other previously sterilized chambers, containing 9 mL of apyrogenic water. After 72 h of incubation at room temperature, analysis of samples collected in the lower chamber was assessed as described above using the LAL test (Associates of Cape Code Incorporated). The absorbance was obtained in the reader Tecan Sunrise (Tecan Corp, Austria, Europe) with a 405 nm filter. The data obtained in the reader were stored in the Magellan V5.0 program (Tecan Corp.).

3.1 *Statistical Analysis*

The endotoxin concentration data (EU/mL) are shown as mean ± standard error of the mean of a minimum of three samples. The data was compared by the test of Kruskal Wallis complemented with Dunn's test. The level of significance was 5% ($p \leq 0.05$).

4 Results

The concentration of endotoxin measured in the lower chamber of all groups after the final 72 h-incubation is presented in Table 1. The endotoxin concentration in the negative control group was significantly lower than that of the positive control group ($p = 0.04$). The concentrations of endotoxin in both irradiated groups were similar ($p > 0.05$). Moreover, this concentration was similar to that of the negative control group ($p = 0.20$) and significantly lower than that of the positive control group ($p = 0.02$).

5 Discussion

The goal of the endodontic treatment is to maintain the tooth in function, free of infection, and in healthy periapical tissue. For this, a significant reduction of the microbial content and their products as well as the prevention of recolonization of the root canal system with bacteria is of paramount importance. The conventional root canal chemomechanical preparation is active in diminishing the contamination inside the root canal, but not as effective in neutralizing efficiently their products, such as endotoxins, deep into the root canal dentin and cementum. This may result in a poor outcome. The release of endotoxins contaminates the root canal dentin and causes biological effects that lead to an inflammatory reaction and periapical bone resorption. Thus, root canal treatment of teeth with pulp necrosis and periapical lesions should not only eliminate bacteria[27] but also must remove the dead cells and promote the neutralization of the endotoxin. For circumventing this problem, conventionally between clinical sessions intracanal medication is usually applied as complementary clinical approach to supplement the disinfecting effects of the conventional chemomechanical procedures.

Up to present, calcium hydroxide is the only intracanal medication able to neutralize endotoxins at a distance.[12,28–34] This is due to its alkaline characteristic that causes hydrolysis of the A lipid, converting it into fatty acid chains and atoxic sugars.[12,28] However, the neutralization of endotoxins by calcium hydroxide is obtained when applied in more than one session.[35] The action time of calcium hydroxide to neutralize the effects of endotoxin varies in the literature. According to a series of authors this substance requires at least seven days for neutralizing the endotoxins[12,31–33,36,37]; however, in a clinical study, reduction of bacteria and endotoxin was not observed in this period.[27] Moreover, calcium hydroxide's effect on the neutralization of endotoxins is impaired inside the root canal by the presence of dentine powder due to the buffering capacity of this substrate.[13]

Table 1 Mean endotoxin content (EU/mL) in the lower chamber in the end of the experiments after the final 72 h incubation.

Experimental groups	Endotoxin content (EU/mL) Mean ± SEM
Positive control	0.0450 ± 0.0092^{a}
Negative control	0.0070 ± 0.0017^{b}
Helical laser	0.0036 ± 0.0022^{b}
Up-and-down laser	0.0047 ± 0.0032^{b}

Different letters (a, b) indicate statistical differences.

Bacterial colonization of the root canal and its associated tubular network can represent a serious challenge to successful endodontic treatment. Bacteria that penetrate dentin to a shallow depth may be removed by mechanical preparation of the root canal or be destroyed by the chemical action of irrigating solutions.[38,39] However, those localized deep in dentin may remain even after cleansing and shaping procedures.[40] Overall, the removal of debris during chemomechanical preparation and the amount of root canal enlargement seem to play an important role in reducing oral bacterial endotoxin during endodontic treatment.[38–42] Therefore, the features of the bacteria and endotoxin due its distribution in the dentinal tissues also are important for the establishment of an effective instrumentation protocol.

Gram-negative microorganisms are able to penetrate approximately 275 μm within the dentin tubules,[18] whereas the endotoxin penetrates up to 800 μm.[43] Thus, the authors concluded that endotoxin penetrates in dentin four times more than the microorganisms due to their low molecular weight. In theory, if the instrumentation based on minimal three apical enlargements can still leave 50% of endotoxin-infected dentin, instrumentation higher than 500 μm is necessary, but it would be unfeasible because it does not respect the internal anatomy of the root canal.[41]

There are clinical situations where the endodontic treatment must to be done in just one session. This makes the use of calcium hydroxide impractical due to the time needed by this substance to neutralize the endotoxin in the dental root which is in a minimum of 14 days.[27] These clinical situations mostly occur in patients with heart diseases, malignant tumors or other pathologies. In these cases, when the patient is hospitalized, an oral prophylaxis has to be done as fast as possible in order to eliminate any source of infection. Nowadays, endodontic treatment is being done in one session also in other kind of patients, for example, special needs patients that receive the odontologic treatment under general anesthesia and must have the endodontic treatment finished at the same session. For these situations, new supplementary disinfection clinical approaches, effective in just one session, must be researched to be applied in optimized single-visit disinfection (OSD).[14,15] Thus, due to the characteristics of the Nd:YAG laser radiation this laser is a good candidate to be used as supplementary disinfection approach in the one session endodontic treatment.

The Nd:YAG laser irradiation of the root canal produces bacterial reduction,[18–21,44–47] without causing undesirable thermal effects.[20,21,23,48,49] With the aim of testing whether irradiation with an Nd:YAG laser would also be capable of neutralizing the endotoxin present in the dentin of human dental roots, endotoxin was inoculated into the root canal of the human dental roots in amounts high enough to allow its passage to the external part of the root where it was collected and quantified. These endotoxin contaminated roots were then submitted or not (controls) to an Nd:YAG laser irradiation using two different irradiation kinetics. Under the experimental conditions used, endotoxin quantification after irradiation showed that it was

possible to observe a complete neutralization of endotoxin after therapy with an Nd:YAG laser, irrespective of the kinetics applied.

Root canal decontamination by an Nd:YAG laser basically occurs by vaporization of the organic material or remaining debris, diminishing the possibility of microorganisms proliferating. Thermal action also provides decontamination because it penetrates beyond the surface region of the dentin wall, interacting inside the dentinal tubules. Irradiation with an Nd:YAG laser promotes fusion and resolidification of dentin, closing the tubules, reducing dentinal permeability[50–52] and increasing dentin microhardness. Its characteristics have made this laser capable of producing vaporization and removing the existent dentinal debris inside the root canals.[22,53,54] The irradiation produces a melted surface and decreases dentin permeability which can reduce bacterial penetration into the canal space or into the canal wall after filling.[50]

The Nd:YAG laser irradiation does not occur in a homogeneous fashion inside the root canal when the optical fibers are moved by hands. For this reason we have tested two irradiation kinetics (spiral and up-and-down) in order to find the most appropriate irradiation methodology for endotoxin decontamination. Independent of the kinetic used, the irradiations were always done with the same laser irradiation parameters. In the spiral kinetics, recommended by Gutknecht et al.,[20] the irradiation is done by slowly moving the laser from the apical to coronal surfaces in a continuous, circling fashion to irradiate all dentinal tubules, whereas in the up-and-down kinetics, the irradiation is done with apical-coronal movements. The manner in which the optical fiber moves inside the root canal did not seem to influence the laser irradiation effect once both irradiations kinetics resulted in total neutralization of the endotoxin.

We expected to have reduction in the endotoxin in the lased groups; however, surprisingly, the endotoxin concentration in these groups was similar to those from the negative control group. These data point out the success of therapy with an Nd:YAG laser in degrading endotoxins inside the root dentin. This result may have been obtained by neutralizing the endotoxin inside the dentin, by either making it impossible for the endotoxin to diffuse beyond the cement in the direction of water in the lower chamber, or by a more distant action of the irradiation not only degrading the endotoxin within the root dentin, but also that contained in the water in the lower chamber. If the latter hypothesis is correct, it may be expected that *in vivo* irradiation with an Nd:YAG laser would decontaminate not only the tooth root, but also the periapical tissue. Corroborating this hypothesis, Hamaoka et al.,[54] have shown that the inflammatory response to the implantation of dental roots into the rat subcutaneous tissue were milder in root previously irradiated by an Nd:YAG laser, which would improve the periapical tissue regeneration.[54]

The difference between the endotoxin content of the lased groups and the positive control group, where no further treatment was done after endotoxin contamination of the root canal system, was expected because the Nd:YAG laser irradiation is able to act throughout the dentin walls.[55] In fact, previous studies with intracanal Nd:YAG laser irradiation showed efficiency in obtaining bacterial reduction at distance.[18,21,44,45] Moreover, the neutralization of endotoxin shown in our study may justify the high rate of success in repairing periapical lesions, even those containing Gram-negative bacteria, when using intracanal Nd:YAG laser irradiation.[19] This better outcome with endotoxin neutralization can be also related to the decrease in the production of bradykinin, which is an important pain mediator, once the production of such protein is stimulated by endotoxins.[2] Thus, given the harmful characteristics of endotoxins and the possibility of the Nd:YAG laser in neutralizing this substance, clinical success would be expected with the use of this irradiation as a supplementary disinfection clinical approach applied in optimized single-visit disinfection.

6 Conclusion

Under the *in vitro* experimental conditions of this study, it was possible to conclude that a single session of an Nd:YAG laser irradiation is capable of neutralizing endotoxin from *E. coli* in the root dentin and cement irrespective of the irradiation kinetic technique used. Intracanal laser irradiation does not intend to substitute the conventional endodontic treatment; however, this technique has the capability to increase the benefits of the conventional endodontic treatment, especially when time is a concern in the treatment of the patient. Thus, the intracanal Nd:YAG laser irradiation emerges as a new possibility for improving endodontic treatment, increasing the endodontic success rates.

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