EX VIVO EVALUATION OF ANTIBACTERIAL EFFECTS OF ND:YAG AND DIODE LASERS IN ROOT CANALS

M. Gerek¹, S. Asci², D.I. Yaylali³
¹Yeditepe University, Faculty of Dentistry, Department of Endodontics, Istanbul, Turkey
²Istanbul University, Faculty of Dentistry, Department of Endodontics, Istanbul, Turkey
³Istanbul University, Faculty of Dentistry, Department of Microbiology, Istanbul, Turkey

Correspondence to: Muzeyyen Gerek
E-mail: muzeyyengerek@yahoo.com, kayatasm@hotmail.com

ABSTRACT
The presence of necrotic tissue and bacteria may cause the persistence of infection in root canals. The aim of this study is to compare the antibacterial efficacy of standard irrigating procedures with 2.5% NaOCl and 17% EDTA using the laser techniques of Nd:YAG and Diode lasers on 176 extracted single-rooted teeth. Strains of Enterococcus faecalis and Candida albicans were used. After the tests were performed, the number of colony-forming units per milliliter (CFU/ml) was counted and the results were statistically analyzed. According to the data evaluated, although EDTA, Nd:YAG, and Diode lasers were effective as a bactericidal agent in contaminated root canals, NaOCl had a significantly higher antibacterial effect. The Nd:YAG and Diode lasers showed more antimicrobial effect than EDTA in the E. faecalis group while the Nd:YAG and Diode lasers and EDTA showed the same level of antimicrobial action in the C. albicans group.

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Introduction
In endodontics, disinfection of the root canal system is essential for ensuring successful, long-lasting root canal therapy (6). The contamination of root canals with bacteria and its subsequent propagation in remnants of necrotic soft tissue are considered one of the main reasons for failure in endodontic treatment (5). Especially the instrumented root channel remains partially untreated; consequently, the removal of debris and necrotic soft tissue is insufficient (22). In addition to mechanical instrumentation of the root canal system, irrigating the canal with disinfectant chemicals has been proposed to enhance the removal of vital and non-vital tissue remnants, tissue breakdown products, and bacteria and bacterial by products (22). Teeth that give a negative culture for bacterial growth at the time of a root canal filling have a higher success rate than teeth that are culture positive (23).

The findings in previous studies has shown that bacteria present in treatment failures are distinct from those present in infected root canals before endodontic treatment (5, 6). Unsuccessful cases are associated with high proportions of gram-positive aerobic and facultative organisms versus the predominance of strict anaerobes upon presentation (15). Nair et al. (16) reported that fungi may be present in the apical region in therapy-resistant cases.

Moreover, Enterococcus faecalis is a facultative anaerobic Gram-positive coccus, which is part of the human oral flora and rarely present in primary apical periodontitis (18). E. faecalis has been identified in persistent root canal infections and is also related to the failure of endodontic treatment (7, 27).

Sodium hypochlorite (NaOCl) is known as a strong antibacterial agent and has been used in 0.5-5.25% concentrations in endodontic practices for many years (4, 19). The effectiveness of NaOCl is well known, although some microorganisms may hide and survive inside tubules or other inaccessible areas (2).

The presence of a smear layer after instrumentation reduces the effectiveness of irrigants and temporary dressings in disinfecting dentinal tubules (11, 28). EDTA has the effect of removing smear layers, which helps the disinfection process in root canals.

Research indicates that, besides improving the treatment of dental hard tissue and forming the root canal by using laser radiation, the ancillary effects of laser light on endodontic bacteria are postulated (8, 10). The laser radiation may be transmitted through quartz optical fibers, a property that could facilitate introducing laser light around canal curvatures and irregularities (25).

Finding a method to provide disinfection in root canals without causing a cytotoxic effect on peripheral tissue is necessary. In this study, canals contaminated with E. faecalis and C. albicans were irradiated using fiber optic directed light from a pulsed Nd:YAG laser and Diode laser or were treated in a conventional method using a NaOCl solution and EDTA. The disinfectant consequence of the test materials was investigated.
Materials and Methods

Tooth preparation

Experiments were performed on 176 extracted human teeth with single roots and single root canals. The teeth were stored in 5.25% NaOCl solution for 30 minutes to remove organic residues and left in sterile saline solution until the procedure began. The crowns were removed at the cemento-enamel junction to obtain a root canal length of 12 mm. Apical foramina were sealed with glass ionomer cement (Ionomil, Voco, Cuxhaven, Germany). The teeth were then embedded in acrylic resin blocks in order to handle the teeth during the experiment.

The root canals were enlarged using the Protaper (Dentsply Maillefer, Ballaigues, Switzerland) nickel-titanium rotary system in a crown-down sequence up to size 30# at 1 mm from the apical foramen. Physiological saline was performed using a 2-gauge needle (Monoject, Sherwood Medical, St Louis, MO, USA) to irrigate the canals after each file change. The teeth were sterilized in an autoclave at a temperature of 121°C for 15 minutes. Samples were randomly divided into a negative control group (n=16) and two experimental groups (n=80), according to the bacterial strains: Group I (E. faecalis) and Group II (C. albicans). The teeth in the negative control group were not inoculated with any bacterial suspension.

Contamination of root canals

Reference strains of Enterococcus faecalis and Candida albicans obtained from the American Type Culture Collection were used. A total of 80 root canals were inoculated with Enterococcus faecalis (ATCC 29212) for 48 hours. The bacterial strain was inoculated on the tryptic soy agar (TSA, Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 24 hours. Eighty root canals were inoculated with Candida albicans (ATCC 10231) for 48 hours. C. albicans was inoculated on sabouraud dextrose agar (SDA, Merck, Darmstadt, Germany) and incubated aerobically for 48 hours at 37°C. The grown bacterial colonies were then harvested and put into tryptic soy broth (TSB, Merck, Darmstadt, Germany) for the E. faecalis and into sabouraud dextrose broth (SDB, Merck, Darmstadt, Germany) for the C. albicans, following the same incubation conditions. The turbidity of the bacteria culture was adjusted to the McFarland Standard No. 0.5.

Ten µl of bacterial suspension were applied to the mechanically enlarged root canals with a sterile micropipette (10 µl, Reference, Ependorf, Hamburg, Germany). Root canal accesses were sealed with a temporary filling material (Coltosol®, Coltene, Whaledent).

The teeth were placed in a metal transporter. The bases of the transporters were coated with wet sterile cotton to provide a moisturized environment. The teeth were preserved in an incubator at 37°C for 24 hours, after which the temporary filling was removed and a fresh culture added in the same amounts in the root canals. The process was repeated with scaling the root canal accesses with temporary filling material and 48 hours of incubation time for total was maintained.

Treatment procedures

The experimental groups (n=80) were divided into five subgroups (n=16) according to the treatment procedure: Subgroup 1, irradiation with Nd:YAG laser; Subgroup 2, irradiation with Diode laser; Subgroup 3, irrigation with a 2.5% NaOCl rinsing of a total volume of 3ml (1ml for each 5 minutes) 15 minutes for total; Subgroup 4, irrigation with EDTA (17% rinsing of a total volume of 1 ml for 2 minutes); and Subgroup 5, positive control.

The source of radiation were the Nd:YAG and Diode lasers. The Nd:YAG laser device (Fotona, Fidelis Plus Nd:YAG laser, Slovenia) emitting pulsed infrared radiation at a wavelength of 1064 nm. The standard settings were a power output of 1.5W and a frequency of 15Hz. Light was transferred by means of a 200µm-thin flexible fiber. The Diode laser device (Fotona, XD2 Diode Laser, Slovenia) was emitting pulsed infrared radiation at a wavelength of 810 nm. The device was used at a 2W power output, with an outer diameter of the application tip at 200 µm.

For the laser irradiation, the fiber was inserted into the root canal at a distance of 1 mm from the apical foramen and moved in three consecutive cycles at 10-second applications and 10-second pauses from the apical to coronal.

For both the treatment group and positive and negative groups, the root canals were rinsed with 1 ml sterile saline solution. The bacteria specimens were taken by keeping No. 25# paper point (Roeko, Langenau, Germany) in the root canal for 15 seconds. The paper points were transferred to Ependorf vials containing 1 ml VMG II transport fluid. All collected samples were vortexed for 1 min and 10-10 seconds, and 10-1 dilutions were prepared. Aliquots of 0.05 ml suspensions were inoculated on the appropriate medium. After a 24-hour incubation at 37°C, the number of CFU/ml were counted.

Statistical analysis

The median number of bacteria for each root canal was evaluated. The total mean and standard deviation for each group were calculated. GraphPad Prism 3 software was used for statistical analysis. Kruskal Wallis test was used to compare the groups and Dunn’s multiple comparison test was used to compare the subgroups. The results were evaluated at a significance level of p < 0.05.

Results and Discussion

Control groups

The samples in the negative control group exhibited no formation of bacterial colonies.

The mean number of bacteria in the positive control group was 2.3 x 10^6 (±8.5 x 10^5) CFU/ml for E. faecalis and 8.0 x 10^6 (±6.4 x 10^6) CFU/ml for C. albicans (Table 1).

E. faecalis

In the E. faecalis group, the number of bacteria was reduced significantly by Nd:YAG laser radiation in the untreated control group of 2.3 x 10^6 (±8.5 x 10^5) CFU/ml to 2.8 x 10^2
(±2.6 x 10^5) CFU/ml (p<0.001) and by Diode laser irradiation to 2.4 x 10^5 (±9.7 x 10^5) CFU/ml (p<0.001). Irrigating the root canals with NaOCl resulted in a reduction to 2.5 x 10^5 (±1.0 x 10^5) CFU/ml (p<0.001) and irrigating with EDTA to 5.7 x 10^5 (±4.0 x 10^5) CFU/ml (p<0.05) (Table 1). Table 2 delineates the comparisons of the groups according to Dunn’s multiple comparison test. Statistical analysis showed a significant difference between the NaOCl group and the Nd:YAG, Diode and EDTA groups (p<0.05) in eliminating bacteria. The number of microorganisms were lower in the nd:YAG and Diode groups than the EDTA group, but the difference was not significant (p>0.05).

In the present study we preferred to use extracted human teeth instead of acrylic blocks in order to simulate in vivo conditions. The Nd:YAG and Diode laser irradiation was performed at operational settings according to the manufacturer’s instructions to provide parameters as close as possible to in vivo conditions.

Single-rooted teeth were instrumented to create, after inoculation, a habitat for microbial growth. Preparation of the canals up to a No. 30# size at working length was selected to obtain the adequate size and easy access for the fiber tip. The final shape, therefore, allowed the precise manipulation of the tip and a correct sampling procedure.

In this study, the role of Nd:YAG and Diode lasers in root canal disinfection was defined using bacteriological testing. E. faecalis and C. albicans, which are well-known endodontic pathogens, were selected for the infection of the root canals. E. faecalis and C. albicans are of particular clinical relevance because these species have frequently been associated with therapy-resistant infections (16, 26).

Moritz et al. (13) determined that irradiation with a Diode laser in two subsequent sessions resulted in almost complete elimination of bacteria and suggested that the Diode laser could be considered equal to the Nd:YAG laser in endodontic treatment. While the number of CFU/ml represented a close estimate of viable bacteria inside the root canal system, our results indicated no statistical difference between Nd:YAG and Diode laser irradiation concerning the number of viable bacteria inside the root canal system.

Disinfectant agents such as sodium hypochloride and chlorhexidine were required in order to contact the bacteria directly and for a long duration (1). Whatever type of laser is applied, the laser’s capacity to disinfect root canals is principally a result of the heating effect. Physically, disinfection results from converting radiant energy into thermal energy inside tissues within very short timeframes. Because of its resistant to heat, E. faecalis was preferred in this study in order to investigate the effect of the laser heat on this particular microorganism. According to the results of this study, the biochemical effect of NaOCl outweighed the thermal effect of Nd:YAG and Diode lasers.

Piccolomini et al. (17) and Koba et al. (9) pointed out that the Nd:YAG laser was effective on bacteria; however, 5.25% NaOCl eliminates the bacteria completely. Hardee et al. (8) and Moshonov et al. (14), who studied the antibacterial effects of the Nd:YAG laser in vitro, concluded that this type of laser was unable to disinfect the root canal system completely. Rooney et al. also reported that NaOCl has higher antibacterial effects than the Nd:YAG laser (20). This data is in agreement with the data obtained in this study. According to the present results,
NaOCl irrigation resulted in a higher reduction of viable bacteria than laser treatment.

Studies have concluded that the Nd:YAG laser has bactericidal effects depending on its energy level (8, 12, 21). Consequently, it is efficient for root canal disinfection at energy levels of 1.5W and 30 MJ and is recommended for root canal disinfection (8, 12). Schoop et al. (21) reported that Nd:YAG and Diode lasers at 1W are efficacious for E. faecalis; while the power increased to 1.5W, only the Diode laser effected the bacteria. In this study, irrigation with the Nd:YAG laser was applied with a constant repetition rate of 15Hz and output of 1.5W, with the Diode laser was applied at 2W. Although a complete sterilization cannot be achieved, a significant reduction of viable 1.5W, with the Diode laser was applied at 2W. Although a complete sterilization cannot be achieved, a significant reduction of viable E. faecalis and C. albicans.

In a recent study, Souza LC et al. (24) reported that photodynamic therapy applied by the Diode laser did not exert a significant supplemental effect on instrumentation or irrigation procedures in terms of intra-canal disinfection. De Souza EB et al. (3) examined the antibacterial effects of combining NaOCl, EDTA and laser treatments and reported that the achieved disinfection degree was 100% while the root canals were irrigated with 0.5% NaOCl, 17% EDTA and an 830 nm Diode laser. The success rate was decreased to 98.39% without laser treatment. In addition, within the tested parameters and samples, Diode laser irradiation provided increased disinfection of the deep radicular dentin.

Conclusions
As a conclusion, the results of this study demonstrate that the Nd:YAG and Diode lasers have an antibacterial but not sterilizing capability, substantiating that laser irradiation is a possible supplement for disinfection but not an alternative. Further clinical studies should be conducted to assess possible improvements of endodontic treatment using Nd:YAG and Diode laser radiation.

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REFERENCES