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Bactericidal effect of the 908 nm diode laser on *Enterococcus faecalis* in infected root canals

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Abstract

Aim:

The aim of this study is to evaluate the bactericidal effect of 908 nm diode laser in conjunction with various irrigation regimes in disinfection of apical third of root dentin.

Materials and Methods:

Sixty prepared teeth with single canals were contaminated with *Enterococcus faecalis*. The specimens were divided into 6 groups (n = 10): Group 1 and 3 and 5 were subjected to chemo-mechanical preparation using 5.25% sodium hypochlorite (NaOCl), 17% Ethylenediaminetetraacetic acid (EDTA); 1.3% NaOCl, MTAD (mixture of doxycycline, citric acid and a detergent (Tween 80); and, 8.5% saline, respectively followed by 908 nm diode laser irradiation; Group 2 and 4, followed the same procedure as Group1 and 3, however without laser irradiation; and, Group 6, rinsed with saline solution (control). Dentin shavings from apical third were analyzed for the presence of *E. faecalis* using culture method and Polymerase Chain reaction (PCR).

Results:

One-way Analysis of variance showed statistically significant differences between the laser irradiated groups, non irradiated groups and the control group.

Conclusion:

908 nm diode used in conjunction with conventional chemomechanical techniques demonstrated a significant elimination of *E. faecalis* in the apical third of root dentin.

Keywords: Diode laser, *Enterococcus faecalis*, Polymerase *chain reaction*

INTRODUCTION

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The eradication of persisting bacteria in the distant areas of the tubular root canal system is a major challenge in treatment regimens, and is crucial for the long term preservation of endodontically treated teeth.[1] During root canal infection, the microenvironment of root canal favors the selection of few bacterial species like *Enterococcus faecalis*, *Streptococcus anginosus* and *Fusobacterium nucleatum*.[2] *E. faecalis* is a non fastidious, therapy resistant, gram positive facultative anaerobe that can proficiently invade dentinal tubules, survive chemomechanical instrumentation and intracanal medication, adapt to altered nutrient supply and continue to remain viable within the dentinal tubules.[3,4] The most potential *Enterococcus* virulence factors that promote adaptation and survival in different environments are collagen binding protein (ace), enterococcal surface proteins (esp) and adhesion-associated protein efaA (*E. faecalis* endocarditis antigen A).[5]

During chemomechanical preparation, various intracanal irrigants like sodium hypochlorite (NaOCl), Ethylene-diaminetetraacetic acid (EDTA) and MTAD (mixture of doxycycline, citric acid and a detergent (Tween 80) have been used for disinfection; however, they do not achieve a complete disinfection of root canal space and the inner layers of dentin.[6] Furthermore, it has been observed that apical third of root canal, with its high percentage of ramifications and variations escapes the debriding action of conventional chemomechanical preparation procedures leading to recurrent infection.[7] The use of lasers like high power Diode laser and neodymium-doped yttrium aluminium garnet (Nd:YAG) in endodontics is an innovative approach for disinfection, providing access to formerly unreachable parts of the tubular network, due to their ability to penetrate dental tissues better than irrigant solutions.[8–10]

Traditionally, endodontic bacteria have been studied by means of culture-based techniques. In the past decade, a major shift has occurred in oral microbiology towards microbial molecular techniques like polymerase chain reaction (PCR), which are more specific, sensitive, and more accurate and rapid than culture, and can detect uncultivable and fastidious microorganisms.[11] Hence, the aim of this study is to evaluate the efficacy of 908 nm diode laser irradiation in conjunction with different intracanal irrigants in the disinfection of the apical third of the root dentin, using culture and PCR methods.

MATERIALS AND METHODS

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Sixty extracted single rooted maxillary lateral incisors with no caries, apical fractures or resorption were collected and stored in 0.9% physiological saline. The teeth were decoronated using water cooled diamond disk and roots standardized to a length of 15 mm. A 10 K file was introduced into each canal until it appeared at the apical foramen; and, working length was established by subtracting 0.5 mm from this length. Apical third of roots were cleaned and shaped to 50 size K file using step back technique for standardization. During instrumentation, the root canals were irrigated with 3 ml of 1% NaOCl at each change of file followed by 17% EDTA solution for 1 minute for smear layer removal; and, later rinsed with saline and dried using paper points. The roots were then waterproofed externally using cyanoacrylate and the roots were set up in 1.5 mL Eppendorf tubes (Tarsons microcentrifuge tubes) embedded in heavy condensation silicone and sterilized in an autoclave at 134 °C for 15 minutes.

Preparation of inoculation and contamination of the specimens

A suspension of 50 μL of *E. faecalis* ATCC 29212 strand was incubated in 5 mL of trypticase soy broth (TSB) culture medium in 37 °C incubator for 24 hours. The concentration of inoculation was then adjusted for a degree of turbidity 1 according to McFarland scale which corresponds to bacterial concentration of 3×10^8 cells/ml, and corresponding to optic density of 550 nm. The root canals were filled with inoculation and were incubated for 21 days at 37 °C in a laminar flow chamber. All samples had a portion of inoculation transferred in 5% sheep blood

Trypticase Soy Agar (TSA) plates (Hi Media M063) to check *E. faecalis* bacterial growth at several time periods, with a result of 100% positive.[9] After incubation, the contaminated roots were randomly divided into 6 groups (n = 10) according to the disinfection regimen used.

Group 1 10 specimens were prepared chemomechanically using the K3 system rotary files (Sybron Endo, Orange, CA) and crown apex technique. The sequence of instruments used was #40 (.04and.06 taper), #45 (0.02and 0.04 taper), and #50 (0.02taper). During chemomechanical preparation, each file was followed by irrigation with 1 ml of 5.25% NaOCl. Final irrigation was completed using 5 ml of 17% EDTA followed by 5 ml of 5.25% NaOCl, and the total irrigation time for the final irrigation sequence was 2 minutes. Intracanal irradiation was performed using a high power 908 nm diode laser (Fotona, EU) with a 200 μm fibreoptic tip and set at a power of 2.5 W. Using an oscillatory technique, the diode fiber (200μm fibreoptic tip) was introduced 1 mm short of the apex and recessed in helicoidal movements at a speed of approximately 2 mm/sec for 5 seconds, and repeated 6 times at intervals of 10 seconds between each one.

Group 2 10 specimens were subjected to the same procedure as Group 1, but without diode laser irradiation.

Group 3 10 specimens were prepared chemomechanically using the K3 system rotary files. The root canals were irrigated with 5 ml of 1.3% NaOCl for 5 minutes followed by 4 ml of BioPure MTAD (Dentsply, Tulsa OK) for 5 minutes, and then dried and subjected to diode laser irradiation as Group 1.

Group 4 10 specimens were subjected to the same procedure as Group 3, but without laser irradiation.

Group 5 The root canals of 10 specimens were chemomechanically prepared using K3 rotary system, irrigated with 10 ml of 8.5% saline solution and dried with absorbent points and then subjected to diode laser irradiation as Group 1 and 3.

Group 6 (Control group) 10 specimens without instrumentation, non irradiated and were rinsed with 10 ml of 8.5% saline solution.

Preparation of apical third

The apical third of the root was obtained by slicing the samples 5 mm from the apex with diamond disk and the internal diameter was calibrated to be.60-.70 mm. Dentin shavings from inner third of dentin were obtained by using Gates Glidden 1 to the apical depth followed by GG 2, 3, 4 and 5.[12,13] The preweighed shavings were then immersed in 1 ml of TSA broth in 1.5 ml Eppendorf tubes. The tubes were vibrated in Fisher Vortex equipment for 2 minutes to homogenize the samples. All samples were divided into two equal aliquots of 60 each for analysis using culture and PCR methods.

Culture methods for colony forming units

10-fold dilutions were prepared and 1 ml of aliquots of suspensions were seeded on a Petri dish with 5% sheep blood TSA plates and then incubated at 37 °C for 48 hours. The colony forming units (CFU) grown were counted using stereomicroscope and log transformation was performed.[9]

Polymerase chain reaction using *Enterococcus virulence* factor efaA gene primer (Sigma, Batch No. 5523-083)

For PCR analysis, dentinal shavings immersed in 1 ml of thioglycolate were incubated anaerobically at 37 °C for 24 hours. After overnight incubation, samples were centrifuged at 10,000 revolutions per minute (rpm) for 5 minutes. From this 1.0 μl of sample was taken for PCR. The reaction mix was prepared to a final volume of 20 μL and PCR was carried out in the Thermal cycler (Eppendorf Mastercycler Gradient 5331). The reaction mix contained efaA primers, Taq Deoxyribonucleic acid (DNA) polymerase, reaction Buffer, and dNTPs mix

(mixture of equal amounts (10mM) of dGTP, dTTP, dATP, and dCTP in a buffer (pH 7.5).

The efaA primer sequence for PCR was 5'-CCAATTGGGACAGACCCTC-3'(forward primer) and 5'CGCCTTCTGTTCCTTCTTTGGC-3'(reverse primer). PCR cycling parameters included an initial denaturation at 95 °C followed by 30 cycles of denaturation step at 95 °C for 10 minutes; A primer-annealing step at 53 °C for 1 minute; extension at 70 °C for 1 minute and a final extension step at 70 °C for 10 minutes was carried out. The products of PCR were analyzed using Gel Electrophoresis (Brovigo) technique using ethidium bromide as DNA intercalating agent. Gel was viewed using an ultraviolet (UV) transilluminator (Remi Equipments) and documented using digital closed circuit (CC) camera. Statistical analysis for CFU was done using One-way ANOVA followed by Tukey Honestly Significant Difference (HSD) test to evaluate the degree of disinfection between the groups.

RESULTS

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Culture analysis

The mean CFU for all groups were: Group 1 = 0, Group 2 = 23.25 × 10^2, Group 3 = 0, Group 4 = 26.14 × 10^2, Group 5 = 0 and Group 6 (control) 158.83 × 10^2 CFU/mL [Table 1]. Statistical analysis demonstrated statistically significant differences between the laser irradiated groups (Group 1, 3, 5), non irradiated groups (Group 2, 4) and control group (P <.05)

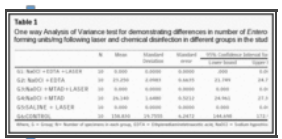

Table 1

One way Analysis of Variance test for demonstrating differences in number of *Enterococcus faecalis* colony forming units/mg following laser and chemical disinfection in different groups in the study

The degree of disinfection for experimental groups (in relation to group 6, which presented total contamination) is shown in Figure 1.

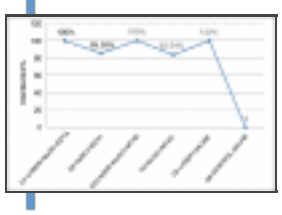

Figure 1

The degree of disinfection for experimental groups (in relation to Group 6-control, which presented total contamination) Group 1, Group 3 and Group 5 demonstrate 100% disinfection. Group 2and Group 4 demonstrate 85.36% and 83.54% disinfection, respectively **...**

Polymerase chain reaction analysis

Analysis done under PCR [Figure 2] demonstrated the absence of *E. faecalis* in all samples of diode laser irradiated Groups 1 and 3. However, Group 5 (irrigated with saline followed by irradiation) demonstrated the presence of *Enterococcus faecalis* in 4 out of 10 (40%) tested samples. The non irradiated irrigant groups 2, 4 and control group demonstrated the presence of *E. faecalis* in all samples (100%).

Figure 2

Detection of *Enterococcus faecalis* by Polymerase Chain Reaction in the experimentally treated groups and control. P - Positive Marker; N-Negative Marker. (a) Lane 1(G1)-5.25% sodium hypochlorite (NaOCl) +17%Ethylenediaminetetraacetic acid (EDTA) +5.25% **...**

The primary objective of endodontic treatment is the disinfection of the root canal, and its three dimensional network of dentinal tubules.[1,10] Diode laser has gained acceptance in laser-assisted dentinal disinfection.[8–10] The results of our study demonstrated superior bactericidal efficacy of 908 nm diode laser treated groups compared to nonirradiated canals disinfected with use of irrigants alone in the apical third of the root canal dentin. When comparing nonirradiated irrigant treated groups, Group 2- 5.25% NaOCl/17% EDTA/5.25% NaOCl exhibited greater bactericidal effect than Group 4- 1.3% NaOCl/MTAD, although, statistical analysis showed no significant difference between the two groups. The bactericidal effect of NaOCl is due to the effect of hypochlorous acid and active chlorine which exerts antimicrobial effect.[14,15] EDTA used in irrigation dissolves the inorganic smear layer and increases the permeability of dentinal tubules.[15] This combination with a final rinse of 5.25% NaOCl increases the bactericidal effect in dentinal tubules. However, despite this superior disinfection protocol, previous studies show depth of penetration of irrigants to be limited to 100 μm, whereas *E. faecalis* is known to penetrate to a depth of 600-1000 μm.[2,16] Although MTAD possesses antimicrobial and chelating properties, the lower disinfecting capability of MTAD may be attributed to the doxycycline component which is only bacteriostatic and not bactericidal.[15] This study and the previous ones[17] have demonstrated that disinfection of root dentin is not achieved by chemomechanical preparation alone. Bacteria deep in dentinal tubules are apparently protected from instrumentation and irrigation, making their removal or eradication difficult.[17]

When 908 nm diode laser was used in conjunction with either of these two irrigation regimes (Group 1and 3), or even saline alone (Group 5), a complete elimination of *E. faecalis* was demonstrated when analyzed using culture methods. PCR analysis of the laser irradiated samples confirmed the absence of *Enterococcus faecalis* in Group1 and 3; however, Group 5-diode laser with saline demonstrated the presence of *Enterococcus faecalis* in 4 out 10 samples. This highlights the importance of intracanal antimicrobial irrigants in improving the efficacy of laser irradiation.

It may be suggested that subsequent to removal of debris and smear layer using chemomechanical preparation, diode laser compounded with a fibre optic tip could provide greater accessibility to formerly unreachable parts of the tubular network resulting in the superior bactericidal effect in the root canal dentin. These results were congruent with the study by Eliana Barbaosa[9] where NaOCl/EDTA irrigation followed by diode laser irradiation demonstrated complete disinfection compared to the disinfectant treated group alone. Contrary to the results of our study, D. Jha *et al*. (2006) demonstrated the inability of laser and rotary instrumentation to bring about the complete disinfection of root canals.[12] The less favorable results could be possibly due to lesser disinfecting capability of the Erbium, Chromium doped Yttrium Scandium Gallium Garnet (Er,Cr:YSGG) laser set at a lower power (1.5W); and, that the infected teeth were laser instrumented first followed by irrigation. Similar results supporting our study were obtained when M. Kreisler (2003) investigated the bactericidal effect of a semiconductor laser used in combination with NaOCl/hydrogen peroxide (H_2O_2) irrigation, or saline alone, and found that the former resulted in significant bactericidal reduction compared to the use of laser alone.[18]

The superior bactericidal effect of diode laser irradiation could be attributed to its greater depth of penetration (up to 1000 μm into dentinal tubules) when compared to the penetration power of chemical disinfectants, which is limited to 100 μm.[14] It has been found that with progressive decrease in diameter of the deep dentinal tubules, the penetration of irrigants is restricted. However, laser irradiation with its inherent properties of light scattering, local intensity enhancement and attenuation allows light penetration deeper in the dentin tubules contributing to a superior antimicrobial efficacy.[9,10,14] Diode laser spectrum (GaAlAs -Gallium Aluminum Arsenide) allows for greater absorption by water in dental tissues when compared with Nd:YAG laser.[9,14] This results in greater laser light penetration through dentin with little interaction with it, making it possible to act on microorganisms

present in the dentinal tubules.[8,9,10,14,16] In addition, the diode laser causes a thermal photodisruptive action in the unreachable parts of dentin, resulting in an enhanced bactericidal effect in the root canal dentin.[14,16]

The results of our study confirmed the efficiency of both culture and PCR to detect the presence of *Enterococcus faecalis*. However, the disparity between culture and PCR methods was evident in Group 5 (irrigated with saline followed by irradiation) where culture technique did not demonstrate *E. faecalis* colony forming units in any of the 10 samples, while PCR detected the presence of *E. faecalis* in 4 out of 10 samples. This difference is attributed to higher sensitivity of PCR which has a potential bacterial detection level of as low as 10 bacterial cells.[11,17,18,19,20,21] There are other several possible explanations also for the higher prevalence values of *E. faecalis* as detected in PCR compared with culture. One possible reason for differences is the ability of molecular methods to detect DNA from dead cells as well. However, it is unlikely that the DNA from dead cell can remain intact in a complex background like the infected root canal.[11,19] After cell death, the DNA molecule faces an onslaught of nucleases and other chemical processes like oxidation and hydrolysis, which can contribute to DNA damage over time, causing an irreversible loss of nucleotide sequence information.[11,19]

Recent studies indicate that in addition to the bactericidal effect, diode laser has a biostimulative effect which is of great importance in regard to healing of periapical tissues. It stimulates cell proliferation and shows an inhibiting effect on inflammation propagating enzymes like matrix metalloproteinases implicated in tissue destruction.[14] Within the parameters of this study, it was concluded that 908 nm diode used in conjunction with conventional chemomechanical techniques aids in complete elimination of the highly resistant bacterial species of *Enterococcus faecalis* in the apical third of the root canal.

Footnotes

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Source of Support: Nil

Conflict of Interest: None declared.

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