

Evaluation of Antibacterial Efficacy of Elexxion Diode Laser 810nm on the Infected Root Canals (In Vitro and In Vivo Study)

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Received September 16, 2013; Revised October 08, 2013; Accepted October 09, 2013

Abstract This study investigated the antibacterial effect of Diode Laser (810nm) at different output powers in the root canals infected with *Enterococcus faecalis* in vitro and in vivo study. Root canal specimens were prepared from extracted, 50 single rooted teeth. After root canal preparation and sterilization, the root canals were infected with endodontic pathogens (*Enterococcus faecalis*). Following laser therapy, the canal contents were cultured on Enterococcus agar. The colony forming units recovered from each root canal was evaluated before and after laser treatment. This study also investigated antibacterial effect of laser in root canals associated with preiradicular lesions in vivo. In this in vivo study, 30 patients with infected root canals underwent diode laser treatment. To verify the findings, microbiological tests were performed and the results were computed. Result showed in vitro significant antibacterial difference between 1.05W, 1.5W, 1.95W and control group. In vivo there was no significant antimicrobial difference between untreated control group and 1.05W but significantly different from 1,5W and 1.95W in which there was no significant different between them.

Keywords: disinfection, 810nm diode, root canal, *Enterococcus faecalis*

Cite This Article: Njwan F. Shehab, Zaid Adel Alshamaa, and Mahmoud Y.M. Taha, "Evaluation of Antibacterial Efficacy of Elexxion Diode Laser 810nm on the Infected Root Canals (In Vitro and Vivo Study)." *International Journal of Dental Science and Research* 1, no. 2 (2013): 23-27. doi: 10.12691/ijdsr-1-2-1.

1. Introduction

It has always been a major goal of endodontic treatment to achieve a bacteria-free environment in the root canal to prevent any risk for successful root treatment. Normally, this is done with disinfecting irrigating solutions. With these solutions, infected and necrotic pulpal tissue is removed and the root canal disinfected. However, sterility of the root canal cannot be accomplished [1], because microorganisms in the lateral canals and dentinal tubules can be removed neither instrumentally nor by irrigation with disinfecting solutions because of the small diameter of the tubules [2]. Thus, the eradication of persisting bacteria in distant areas of the tubular system is a major challenge in today's treatment regimens and is crucial for the long-term preservation of the endodontically treated tooth [3].

Rinsing solutions applied during conventional root canal treatment act through direct contact with the bacteria. Due to the insufficient penetration depth of the bactericidal solutions, microorganisms in the deeper layers of dentin cannot be affected. These pathogenic microorganisms are able to penetrate the root dentin up to more than 1 millimeter, whereas rinsing solutions reach a

depth of around only 100 micrometers. Additionally, curved root canals or side branches also can be obstacles in conventional root canal treatment [4]. In addition, bacteria like *E. faecalis* is known to form intra-and extra radicular biofilms, which makes it even harder to control them [5]. It is resistant to many antimicrobial agents [6], and has been identified in infected root canals [7], acute apical abscesses, this microorganism has been associated with therapy-resistant root canal infections [8].

Because of the critical importance of disinfection of the root canal system, much research is still directed at obtaining the quickest and most consistent way of achieving this goal [9]. Therefore, several studies have done and shown the favourable effects of laser treatment in this area [10], which helps overcome these problems due to high penetration depth of the laser beam in the dentinal tissue [4].

The use of diode lasers in endodontic treatment has been increasing in recent years, especially because of the antimicrobial abilities of lasers, which has been investigated in vitro [11] and in vivo [12].

2. Materials and Methods

2.1. In Vitro Study

2.1.1. Preparation of Teeth

A total of 50 extracted, single-rooted human teeth, stored in physiological saline, were decoronated using a Diamond sectioning disc (Germany). The external root surface was cleaned with curettes to remove calculus and periodontal soft tissues. Working length was established by passing a size 10 K-file (Japan) in the canal until visible at the apex and subtracting 1mm. Instrumentation was continued until MAF 60 under irrigation with physiological saline. The apical foramen was sealed by composite resin (USA) and the root surface covered with bonding agent, to prevent bacterial leakage. Roots were then embedded in silicone impression material, covered with aluminum foil, fixed in the stainless steel boxes and sterilized by autoclaving (121°C, 15 min).

The prepared samples were then randomly assigned among four groups.

- Group 1, n=(10): Laser irradiating samples in 1.05 W.
- Group 2, n=(10): Laser irradiating samples in 1.5 W.
- Group 3, n=(10): Laser irradiating samples in 1.95 W.
- Group 4: Ten samples with bacterial suspension without irradiation were control positive, and the remainder ten sterile samples were control negative.

2.1.2. Bacterial Isolation and Inoculation

Bacteria of *Enterococcus faecalis* is isolated and identified on the Enterococcus Agar (USA). The canals were inoculated with 10 µl of the bacterial suspension and incubated at 37°C for 24 hrs.

2.1.3. Root Canal Disinfection

Diode laser at a wave length 810nm (Elexxion AG-Germany) (Figure 1) was used and the output power was adjusted at 1.05W, 1.5W and 1.95W according to manufacture instruction, for 15sec. The fiber optic inserted inside the root canal (1mm from the apex) from apical to coronal in continuous circling movements to treat all dentinal tubules in one cycle for each power.



Figure 1. Elexxion Diode Laser

2.1.4. Bacterial Sampling Procedure

A file No. 60 was introduced as far towards the apex as possible, then rotated clockwise for complete apical seating and withdrawn with attached dentin. Then 15mm of its apical tip was aseptically cut off into a vial

containing 1ml normal saline, after that 200µl was cultured on Enterococcus agar plate and incubated aerobically at 37°C for 24hrs. [13].

2.2. In Vivo Study

In the present study, a total of 30 patients with infected root canals were treated. Only single-rooted teeth (upper and lower incisors and single rooted premolars) were selected in this study. All the teeth showed radio-graphical signs of an apical inflammation. After the application of a rubber dam, crowns were cleaned, then the infected root canals were prepared mechanically until size 60 and irrigating only with physiological saline solution. Microbiological samples were collected prior to laser treatment. For this purpose, MAF size 60 used to full working length to collect dentine shaving from the root canal wall. Then 15mm of its (apical tip) was aseptically cut off into a vial containing 1m BHI-broth immediately after being removed from the root canal to prepare the sample for the subsequent microbiological examination [2].

This procedure was followed by laser treatment. The root canals were irradiated with a 810 nm diode laser, at the output power 1.05W, 1.5W and 1.95W for 15sec. The optical fiber was inserted as far as the apex, the correct insertion depth was ensured by measuring. The laser was then activated and the root canal slowly irradiated from apical to coronal in continuous circling movements to treat all dentinal tubules in one cycle for each power. Following irradiation, microbiological samples were collected using MAF size 60 to full working length to collect dentine shaving from the root canal wall. Then 15 mm of its (apical tip) was aseptically cut off into the transport medium as mentioned above, after that 0.1ml of this sample transferred in the agar plate (Enterococcus agar), and incubated aerobically for 48 hours at 37°C. The colonies were then counted and the total number of bacteria (Colony Forming Units = CFU) per ml assessed before and after laser application.

3. Results

3.1. In Vitro

Statistical analysis showed that the number of bacteria was reduced significantly by Elexxion diode laser (810nm) at the selected powers compared with the untreated control group ($p < 0.001$) as in Figure 2. When comparison was made among different output powers, the results showed that at 15sec there was significant difference in the antimicrobial effect between the selected powers. The highest antimicrobial effect against *E.faecalis* was achieved by 1.95W followed by 1.5W that showed better antimicrobial effect than 1.05W using ANOVA at level ($p < 0.001$), as shown in Table 1 and Figure 3.

3.2. In Vivo

The radiographic examination confirmed the diagnosis of necrotic pulp and periapical lesions for all the patients selected, and the analysis of the first microbiological sample corroborated the presence of infection in all teeth. The initial infectious vary between individual teeth. This

variation was probably caused by differences in the internal anatomy and geometry of the individual root canal systems and the duration of the infections and the presence or absence of infiltration or caries on the teeth in the beginning of the treatment.

Treated root canal with laser at 1.05W showed reduction in the number of bacterial colonies per ml but

significantly not different from untreated control group, while treated the infected root canals with 1.95W showed statistically reduction in the mean value of bacterial colonies but significantly not different from 1.5W in which both of them significantly different from 1.05W using ANOVA at level ($p < 0.001$) as shown in Table 2 and Figure 4.

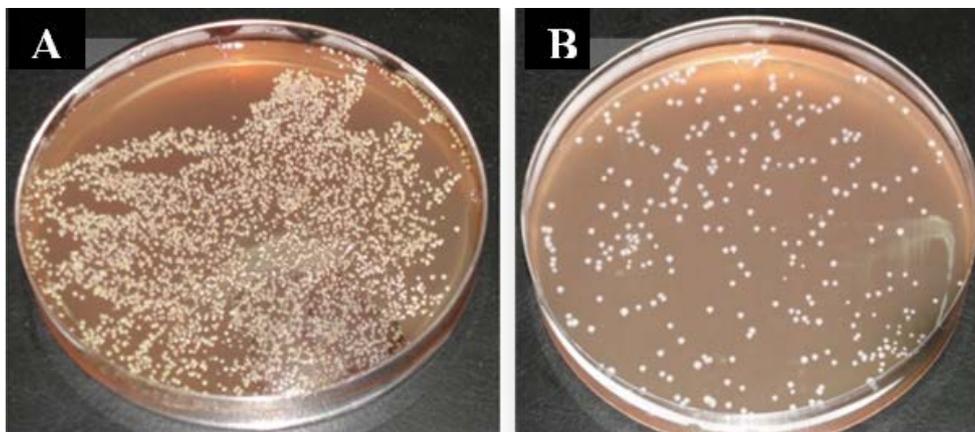


Figure 2. *E. faecalis* Count (A) Control Group. (B) After Laser Treatment

Table 1. In vitro One way analysis of variance for the differences between the antimicrobial effect of diode laser 810nm at different output powers against *E. faecalis*

Power (Watt)	No.	Mean	SD	F-value	P-value	Duncan
1.95	10	25.400	12.2945	178.34	0.000	A
1.5	10	74.900	14.3871			B
1.05	10	140.500	21.7421			C
Control	10	257.300	37.7478			D

According to ANOVA significant different exist at $p < 0.001$, means with different letters vertically have significant difference at $p < 0.001$ according to Duncan test.

SD = Standard deviation.

Table 2. In vivo One way analysis of variance for the differences between the antimicrobial effect of diode laser 810nm at different output powers against *E. faecalis*

Power (Watt)	No.	Mean	SD	F-value	P-value	Duncan
1.95	10	40.4000	11.7870	334.63	0.000	A
1.5	10	41.9000	17.6033			A
1.05	10	260.000	35.3898			B
control	10	278.000	19.3333			B

According to ANOVA significant different exist at $p < 0.001$, means with different letters vertically have significant difference at $p < 0.001$ according to Duncan test.

SD = Standard deviation.

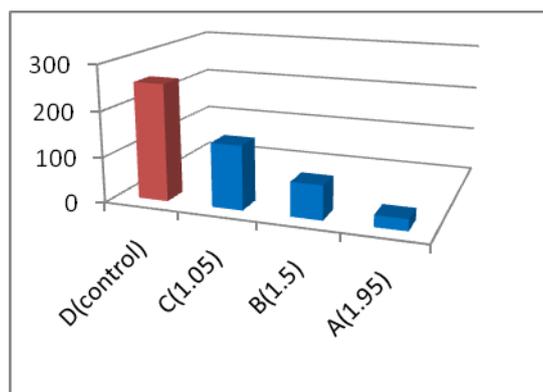


Figure 3. A histogram showing the antimicrobial activity of laser treated groups and the control group in vitro

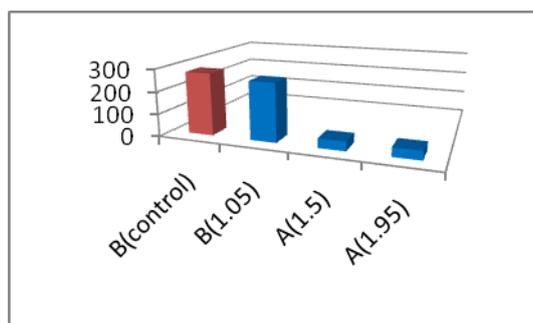


Figure 4. A histogram showing the antimicrobial activity of laser treated groups and the control group in vivo

4. Discussion

In this study, we evaluated the ability of Diode laser at a wave length 810nm to eliminate *E. faecalis* from the infected root canal and dentinal tubules. The main characteristic of *E. faecalis* is the construction of their cell wall, consisting of up to 40 layers, with a total diameter ranging from 20 to 80 nm [14] and it is associated with therapy-resistant infections [10].

The persistence of bacteria in the three-dimensional tubular network of root dentin can be regarded as the main cause for the failure of an endodontic treatment [15]. During conventional root canal treatment the infected pulp tissue, and layers of root canal dentin can only be removed to a certain extent. While root canal morphology limits the extent of mechanical preparation, chemical irrigants are only effective in dentin layers directly adjacent to the canal wall [16], bacteria are able to invade the periluminal dentin up to a depth of 1,000 mm, whereas the penetration depth of chemical disinfectants is limited to a range about 130mm [4]. Due to this lack in penetration depth of the bactericidal agents, pathogenic bacteria survive and constitute the reason for therapy resistant cases and long-term failures in endodontic treatment [17]. Therefore, root canal infection may re-occur after treatment [18].

With the introduction of lasers to the fields of conservative dentistry, the endodontic procedure was improving the chance for a successful treatment outcome [19].

The antibacterial effect of 810nm diode laser irradiation was determined in the present in vitro study. A significant bactericidal effect was detected at all selected parameters compared with untreated control group. The results of this experiment are comparable with the results obtained by Hendy [20] and Nagwan [21] who showed significant reduction of *E. faecalis* quantity in root canal following irradiated by Nd:YAG and diode (1064nm) laser that used in dentistry today. While other studies showed no effect of laser on *E. faecalis* in root canal which could be explained by experimental condition of infected teeth [22], probably due to variation in wave length, power, time and spot size [23]. Also there was a significant antibacterial differences between powers selected in our study. When the power of laser increased, the effect against this bacteria also increased. The result of this investigation is in accordance with Gutknecht *etal.* [24] who found that damage of bacteria increased with the amount of energy applied and there was significant antibacterial differences between powers.

Gutknecht *etal.* [25] showed that Nd:YAG laser at (1.5W, 40sec) was able to eliminate 99.91% of the *E. faecalis* and Rooney *etal.* [26] achieved reduction in *E. faecalis* through irradiation with Nd:YAG laser at 1.8W for 30 sec.

In vivo study showed no significant antimicrobial difference between untreated control group and group irradiated with 1.05W in one cycle of irradiation at the selected time but the antimicrobial effect was noticed when output power increased to 1.5W and 1.95W in which there was no significant difference between them at 15sec in one cycle of irradiation, this is in agreement with the results obtained by Moritz *etal.* [2] who showed that treatment of root canals with 2W diode laser (810nm), when the irradiation was repeated 5 times at each laser treatment, each time for a period of 5 sec with short breaks in-between, a maximum of two irradiations resulted in nearly complete elimination of *E. faecalis*, but in our experiment there is reduction in the number of CFU/ml of *E. faecalis* but there is no complete elimination of this bacteria, this may be due to the root canal exposed to one cycle of irradiation which may probably result in partial disruption of biofilm layer formed by this type of bacteria [7].

Our results in agreement with Silvana *etal.* [27] who showed that Nd:YAG laser at 2.5W and 3.5W did not significantly reduce the number of *E. faecalis* in the root canals, while study by Silvana *etal.* [28] showed that erbium laser did not completely sterilize any root canal. These results can be explained by the fact that the erbium laser tip was only applicable in the cervical third of the root canals, whereas the study found by Wang *etal.* [29] showed that the Er,Cr:YSGG and Nd:YAG laser irradiation at 1W and 1.5W reduce the number of bacteria but with no significant difference between the selected powers, the authors concluded that the Nd:YAG laser is more effective than the Er,Cr:YSGG laser but both lasers produced a significant bactericidal effect in the infected root canals.

The difference in the results may be attributed to differences in the methodology used in this study compared with the results of other studies. In our model, although we did not find total elimination of viable organisms (that is, sterilization), we did achieve a significant reduction in the viable bacterial load, approaching sterility. Therefore, the goal of our work was to learn whether the use of this laser could accomplish this goal of root canal sterilization, while our results did not demonstrate complete elimination of infection from root dentin, this probably due to one cycle of irradiation was not enough and require to justify more than one cycle and more than one-visit laser application for infected root canals.

Working in vivo is more complex because the variance of root canal anatomy is higher than in a controlled in vitro experiment. However, the results in vitro with the extracted teeth better than those obtained in vivo study. It is possible that in vivo the surrounding tissue could promote light backscattering, thus increasing the number of photons available to the photoreaction [30].

From all that we know of pulpal and periapical disease, the elimination of infection (that is, sterilization) and prevention of subsequent infection is at the heart of endodontic therapy. To date, no existing procedure allows the clinician to sterilize an infected root canal system quickly and easily and with absolute surety [22]. Therefore, further studies to evaluate new treatment protocols that could count for completed bacterial eradication need to be considered in the future.

5. Conclusion

1. Diode laser at a wave length 810nm has antibacterial effect against *Enterococcus faecalis*.
2. Endodontic pathogen that grow as a multilayered structure required more than one cycle for laser to be applied.

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