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Efficiency of Diode Laser as Root Canal Disinfectant Against *Enterococcus faecalis*: An in Vitro Study

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Abstract

The objective of the *in vitro* study is to determine the disinfection ability of diode laser 1064 nm at different powers and duration of times against *E. faecalis* and detect the optimal power and time of diode laser 1064nm. Eighty human extracted single-rooted teeth were decoronated to a length 14mm from the apical foramen to the cervical border of the root, then adjusted working length to 13 mm, autoclaved and inoculated with a suspension of *E. faecalis* at a concentration 4×10^5 cfu and incubated at 37˚C for 24 hours. Sixty samples were irradiated at different powers and irradiation time 30 and 60 sec, the remainder 20 samples were control positive and negative groups. All powers and times for laser 1064 nm had significant antimicrobial effects against *E. faecalis*. Diode laser device seems to be highly suitable for killing *E. faecalis* if appropriate energy and irradiated time used.

***KeyWords*:** Diode laser; Enterococcus faecalis.

1. Introduction

Root canal therapy is performed with the aim of removing pathogens and their products, to fill canal space with a three-dimensional filling and make a proper apical seal [1]. Microorganisms play an essential role in the development of pulpal and periapical diseases [2]. Therefore, success of endodontic therapy depends mainly on the complete elimination of bacteria and irritants from the infected root canal before obturation [3].

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Elimination of microorganisms from infected root canals is a complex procedure and a variety of instrumentation modalities, irrigation regimens, and medicaments have been used in an attempt to reduce the microorganisms in the root canal system but bactericidal potential with this treatment regimens is developed through direct cell contact [4]. *Enterococcus faecalis* has been considered one of the most resistant species in the oral cavity and one possible cause of post-treatment disease after root canal treatment [5]. One of the problems not yet solved by researchers during root canal therapy deals with the difficulties in ″sterilizing″ the endodontic space [6] due to complex morphology of root canal system including deltas and lateral canals which act as a place for bacteria, debris and necrotic tissues to harbor [7]. Thus, no chemo-mechanical technique has shown the ability to clean and/or disinfect the root canal system completely [8].laser light is capable of penetrating deep into the dentinal tubules and eliminating the microorganisms, smear layer removal [9]. Diode lasers have been available and its irradiation has recently been proposed for use in endodontic therapy [10], due to the development of fiber optic delivery system [11] which introduced directly in to the root canal that adapted to reach dimensions and curves of the radicular canals [12].

2. Materials and Methods

2.1. Sample Selection and Preparation

A total of Eighty freshly extracted human non-carious, single-rooted teeth were placed in 1.3% NaOCl for primary surface disinfection. Each tooth was decoronated at the level of cemento-enamel junction. Patency was confirmed with No.15 K-type file and pulp tissue was removed with a barbed broach. The working length for each root canal was determined to13 mm and prepared up to the size 45. Teeth were irrigated until 1.3% NaOCl and then left in sterile distilled water for 24 hrs. The apical foramen for each root was sealed with acrylic resin and the external root surface was coated with two layers of nail polish. The roots embedded in silicone impression material then covered with aluminum foil and adapted in the stainless steel boxes for autoclave sterilization.

2.2. Sample Groups

Teeth were divided into eight groups 10 teeth for each. Six groups for laser treatment, and two control groups (positive and negative).

2.3. Microbial Isolation and Identification

Enterococcus faecalis were isolated and identified on the Enterococcus Agar then prepared to final concentration of 4×10^7cfu /ml. Each root canal was inoculated with 10 µl of bacterial suspension and

incubated at 37˚C for 24 hrs.

2.4. Disinfection of the Infected Root Canals

Diode laser at a wave length 1064nm (Figure 1) was used and the output power was adjusted at (2W, 2.5W and

3W) for each selected time (5sec and 10 sec). Each root canal irradiated in non contact mode, the fiber optic inserted inside the root canal to 13 mm (1mm from the apex) with spiral continuous movement clockwise from the cervical part to the apex and anti-clockwise from apical part to cervical part. Irradiation for each root canal was repeated 6 times, for each power, two periods: 5 sec and 10 sec, with 10 sec rest intervals between each lasing cycle. Thus, the total irradiation time 30 sec and 60 sec respectively per root canal.

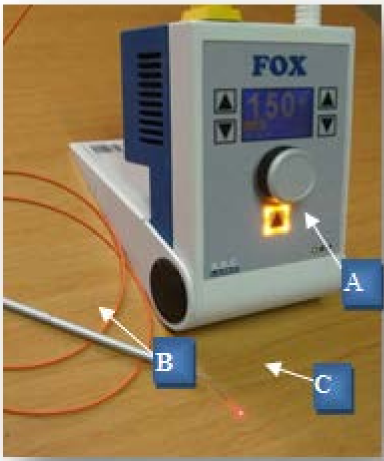

Figure 1: Photograph showing **(A)** Diode laser 1064nm, **(B)** Hand piece, (**C)** Fiber optic part at a diameter 200µm for endodontic application.

2.5. Microbiological Sampling from Treated Root Canals

Sample was taken from each root canal using K-type file size 45 which was inserted inside the root canal to full working length.

Then the file is rotated 360° in clock wise direction for engagement in dentine.

The sample was transferred immediately to screw-capped vial containing 1ml normal saline.

The solution was agitated for 30 sec, then 200 µl was cultured on agar plate [13], culturing was done by spreading using swab on Enterococcus agar and incubated aerobically at 37°C for 24hrs.

Then the number of bacterial colonies were counted.

3. Results

Statistical analysis showed that the antimicrobial effect of diode laser at different powers and times was highly significant compared with the control group using unpaired t- test at level ($p<0.001$), as shown in Table (1).

Table 1: A comparison between the antimicrobial effect of diode laser (1064 nm) as root canal disinfectant against *E. faecalis* compared with control group.

Power/Watt	Total irradiation time /sec	Mean	SD	p-value
2	30	1134.0	241.47	0.000*
2	60	789.50	43.17	0.000*
2.5	30	1033.5	142.24	0.000*
2.5	60	132.00	30.29	0.000*
3	30	297.00	38.17	0.000*
3	60	1.50	3.37	0.000*
Control		2823.5	71.69	

Unpaired t-test for diode laser at different output powers and times compared

with control group. *Significant difference existed at $p<0.001$. SD= Standard deviation.

When the comparison was made among different output powers at the selected time using ANOVA for each time at level ($p<0.001$), the results showed that at 5 seconds (6 cycles) total time (30sec) there was no significant difference in antimicrobial effect between 2W and 2.5W in which both of them were significantly different from 3W, while at 10 seconds (6 cycles) total exposure time (60 sec), there was significant antimicrobial difference between 2W, 2.5W and 3W as shown in Table (2).

Table 2: A comparison between the antimicrobial effect of diode laser 1064 nm at specific time for different output powers against *E. faecalis*.

Time (sec.)	Power (Watt)	Mean	SD	F-value	P-value	Duncan
30	2	1134.00	241.47	78.319	0.000	B
	2.5	1033.5	142.24			B
	3	297.00	38.17			A
60	2	789.50	43.17	1916.158	0.000	C
	2.5	132.00	30.29			B
	3	1.50	3.37			A

According to ANOVA $p<0.001$ for each time, means with different letters vertically for each level of time have significant difference at $p<0.001$ according to Duncan test..

SD= Standard deviationUsing unpaired t-test for each output power at level ($p<0.001$), the results revealed that the diode laser at 10 seconds (6 cycles) total time (60 sec) had significantly higher antimicrobial effect than 5 seconds (6 cycles) total time (30 sec) of exposure at all powers output as shown in Table (3).

Table 3: A comparison between the antimicrobial effect of diode laser 1064 nm against *E. faecalis* at specific output power between different times and among all laser disinfected groups**.**

Power (Watt)	Time (Sec.)	Mean	SD	p-value	F-Value	P-value	Duncan
2	30	1134.0	241.47	0.000	169.652	0.000	D
	60	789.5	43.17				C
2.5	30	1033.5	142.24	0.000			D
	60	132.00	30.29				AB
3	30	297.00	38.17	0.000			B
	60	1.5	3.37				A

Unpaired t-test to compare between 5 sec and 10 sec for each power.

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

SD= Standard deviation. Further comparison of antimicrobial effect among different output powers and times of diode laser using ANOVA at level (p< 0.001), the results revealed that 2.5W /10 sec (6 cycles) had antimicrobial effect similar to that of 3W /5 sec and 10 sec (6 cycles), although there was a significant difference between these times at 3W, while at 5 seconds (6 cycles) there was no significant antimicrobial difference between 2W and 2.5W, but they were statistically different from all other groups as shown in Table (3) and (Figure 2).

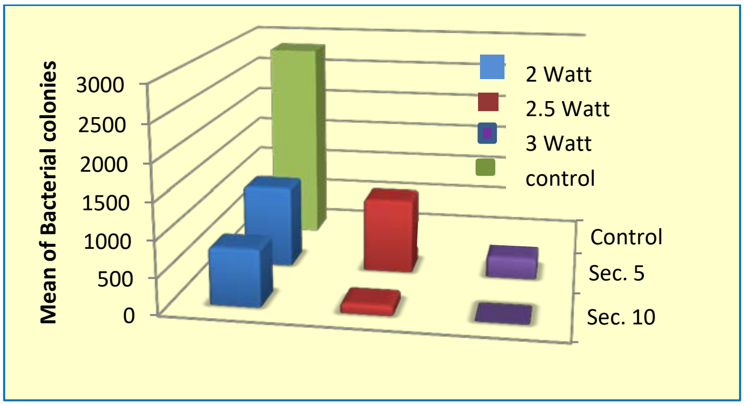

Figure 2: A histogram showing the antimicrobial activity of diode laser treated groups and control group.

4. Discussion

The bactericidal effect of diode laser 1064 nm irradiation was detected in the present experiment which varied with the powers output and times. A significant bactericidal effect was observed at all selected parameters compared with untreated control group. The results of this study are comparable with the results obtained by others [14,15] which showed significant reduction of *E. faecalis* quantity in root canal following irradiated by different laser wavelengths (Nd:YAG, diode, and Er:YAG) that most commonly used in dentistry today for disinfection of root canals at different output powers and times. While other studies showed no effect of laser (Nd:YAG) on *E. faecalis* in root canal which could be explained by experimental condition of infected teeth [16]. One possible explanation for such differences may be due to variation in wave length, spot size, output powers and exposure times [17].

The results of the current study indicated the ability of diode laser to kill *E. faecalis* in the infected root canal and therefore it could be effective against any organism found in endodontic infections and this technology might have clinical application in disinfecting root canals during endodontic therapy [18].

The possible mechanisms regarding the antibacterial effect of diode laser are summarized in the following: thermal and photodisruptive effects were considered the principal reasons for the laser to eliminate the bacteria [19]. Lethal damage includs destruction of cell wall integrity and possibly denatured of protein. The damage of cell wall will cease the cell growth and successive cell lysis [20]. On the other hand, the cellular protein is highly sensitive to thermal changes [21]. Another possible explanation could be due to possibility of occluding dentinal tubule which results from melting of dentine and there by entrapping invading microorganisms [22].

Further explanation of antimicrobial effect of laser is believed to be due to the fact that with laser, it is possible to achieve expansion of intratubular water and the collapse of water vapor as deep as possible, which is capable of producing acoustic waves strong enough to disrupt intratubular bacteria [18].

There was a significant antimicrobial difference between output powers at 10 sec (6 cycles) exposure to laser. When the power of laser increased, the effect against this bacteria also increased. The result of this investigation is in accordance with others [23,24] who found that the damage of bacteria increased with the amount of energy applied and there was significant antimicrobial differences between powers output after laser irradiation using Nd:YAG 1064 nm and diode 980nm laser. Other study showed different result, with no significant difference despite the effectiveness of diode laser 1064nm at different output powers on the bacteria in the root canal [15]. The results of the study showed that 10 sec (6 cycles) exposure time to laser, was more effective than 5 sec (6 cycles) at each output power, and no significant difference between 2W and 2.5W at 5 sec (6 cycles). These results indicated that time plays an important role in root canal disinfection when using laser. Thus, our observations suggested that the lethal effect of laser irradiation on microorganisms is based on the time of irradiation inside the root canal more than the increased power. The exposure time at 5 sec (6 cycles) was not enough for the laser light to reach all bacteria at all surfaces of main canal and within the dentinal tubules compared with 10 sec (6 cycles) which was better, probably due to more surface area of root canal exposed to irradiation. For the same reason this could be explained that the effect of 2.5W / 10 sec (6 cycles) was comparable with 3W / 5 sec and 10 sec (6 cycles). In the present experiment the fiber optic of laser tip was constantly kept with continuous movements inside the root canal to reduce the thermal effect of laser. Lee and his colleagues *(*2006) found that continuous movement of fiber tip reduced the thermal effect and simultaneously reached high bactericidal efficiency [25]. While in the present work during irradiation with 3W / 10 sec (6 cycles) small black spot (carbonization) formed in three samples from 10 samples, this may be due to concentration of heat which resulted from uncontrolled movements of fiber optic inside the root canal. Similar finding was also recorded by Kreisler and his colleagues (2003) who found that carbonization of root canal was observed in single root canal at 3W and 4.5W from 12 specimens for each power [26]. More than one cycle of laser irradiation inside the root canals used in this study, probably due to formation of bacterial biofilm which required more than one cycle to reach the deeper layer and disrupt it. Using one cycle may probably result in partial disruption of biofilm layer and extensive bacterial reduction was achieved in all cases by repeating laser treatment with a high power diode laser [27]. Moritz and his colleagues (2000) found that Gram-negative organism showed immediate structural injury, whereas the Gram-positive organism (*E. faecalis*) required repeated application of laser irradiation [23]. The heat causes non-lethal reversible damage, which is then converted into lethal damage during repetition of the stress. Bergmans and his colleagues (2006) showed that using two cycles of Nd:YAG (1064nm) laser irradiation resulted in the destruction of superficial layers and a partial disruption of the biofilm of *E. faecalis* and demonstrated that the extent of damage was in a line with total amount of energy (the number of irradiation cycle) applied [28]. However, complete eradication of *E. faecalis* in 75% of the samples was noticed using diode and Er:YAG lasers at 1.5W with five irradiations of 5 seconds for each[14].

5. Conclusion

1. Diode laser 1064 nm has significant antibacterial effect against *E. faecalis* infected root canals. and bactericidal effect of it depends on the laser parameters and the results vary with different powers.

2. Time of laser irradiation plays a significant role during irradiation with flat fiber optic tip of laser.

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