available at *www.jstage.jst.go.jp/browse/islsm*

ORIGINAL ARTICLES

Comparison of Antibacterial Effects of 810 and 980- nanometer Diode Lasers on Enterococcus Faecalis in the Root Canal System –An *in vitro* study

Mohamad Asnaashari [1], Leila Tahmasebi Ebad [2], Shiva Shojaeian [3]

1: Laser Application in Medical Sciences Research Center,
Shahid Beheshti University of Medical Sciences, Tehran, Iran
2: Dentist
3: Department of Endodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background and aim: Use of laser technology in endodontics has greatly increased in the recent years due to the introduction of new wavelengths and methods and optimal antimicrobial and smear layer removal properties of lasers. This in vitro study aimed to compare the antibacterial effects of diode lasers of 810 nm and 980 nm wavelength on Enterococcus faecalis (E. faecalis) biofilm in the root canal system.
Materials and methods: Fifty single-canal human anterior teeth were cleaned, shaped, sterilized and randomly divided into four groups namely two experimental, one positive and one negative control group. The experimental and positive control groups were inoculated with E. faecalis and incubated for two weeks. The experimental group one (n=20) received 810 nm diode laser irradiation (1.5W) while the experimental group two (n=20) was subjected to 980 nm diode laser irradiation (1.5W). The E. faecalis colony forming units (CFUs) were counted in each root canal before and after laser irradiation.
Results: Laser irradiation significantly decreased the bacterial colony count in both experimental groups. The reduction in microbial count was significantly greater in 810 nm laser group compared to 980 nm laser group.
Conclusion: Irradiation of both 810 and 980 nm lasers significantly decreased the E. faecalis count in the root canal system; 810 nm laser was more effective in decreasing the intracanal microbial load.

Key words: Diode Lasers · Enterococcus Faecalis · Root Canal · Antibacterial

Introduction

Efficient disinfection of the root canal system is challenging due to its complex anatomy and penetration of bacteria into dentinal tubules. The root canal system is conventionally disinfected by mechanical preparation and irrigation with chemical agents. However, it has been shown that 35% of the root canal surface area remains unchanged following root canal preparation with rotary Ni-Ti system [1)]. Root canal irrigants have limited capability to penetrate into the dentinal tubules and often fail to completely remove the smear layer and eliminate the intracanal infection [2)]. Bacterial colonies lodge in the dentinal tubules to a depth of 1150 μm while irrigating solutions can only penetrate into the dentinal tubules for approximately 100 μm [2, 3)]. In the past two decades, laser technology has gained the spotlight as an adjunct treatment in endodontics. Lasers operate in continuous wave or pulse mode or by the use of an optical fiber conductor [4)]. Specific wavelengths of laser light are capable of penetrating deep into the dentinal tubules and eliminating the microorganisms, removing the smear layer

Addressee for Correspondence:
Shiva Shojaeian
Department of Endodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Dental School, Shahid Beheshti University of Medical Sciences, Daneshjoo blv, Tehran, Iran
Tel: +98-9155191031 Fax: +98-22403194
E-mail: shiva.shojaeian@gmail.com

Received date: April 15th 2016
Accepted date: June 17th 2016

or changing the surface morphology of dentin. However, studies are ongoing to find the most appropriate, efficient, safe and accessible laser wavelength. Diode lasers have been proposed for disinfection of the root canal due to their optimal antibacterial properties and low cost in relation to most laser used in endodontic [5]. Evidence shows that E. faecalis is a common microorganism responsible for the secondary infection of the root canals. It is resistant to the most irrigating solutions and intracanal medicaments. However, it has been demonstrated that E. faecalis can be largely or completely eliminated by the application of diode laser alone or in combination with irrigating solutions [6, 7]. The available diode laser wavelengths for dental applications range from 800 to 1064 nm [8]. Some previous studies have assessed the efficacy of 810 nm and 980 nm lasers separately and their results confirm their acceptable antimicrobial efficacy. However, no previous study has compared the antibacterial effects of 810 nm and 980 nm lasers. The 980 nm diode laser was recently introduced to dentistry and soon gained popularity due to its small size, availability, having thin flexible fibers and an output power of 0.5 to 7W [9]. Also, 980 nm diode laser is well absorbed by water but is slightly absorbed by hydroxyapatite crystals; this results in light scattering in dentin [10]. Gutknech et al. reported that 980 nm laser successfully eliminated the bacteria lodged to a depth of 500 μm in dentinal tubules [11].

This in vitro study aimed to compare the antibacterial effects of 810 and 980 nm diode lasers on E. faecalis biofilm in the root canal system.

Methods

Fifty single-canal human anterior teeth which was scheduled for extraction due to periodontal disease, prosthodontic or orthodontic purpose, ect. with patient's consent in accordance with the ethical guidelines were collected at Oral Maxillofacial Surgery of Dental School of Shahid Beheshti Medical University, Iran. All teeth were stored in 0.9% sterile saline solution (0.9% sodium chloride) at room temperature. The teeth were decornated by a disc to yield 12 mm-length standard root canals. A #15 K-file (Dentsply Maillefer, Tulsa, OK, USA) was used for working length determination. The file was introduced into the canal until its tip was visible at the apical foramen; 0.5 mm was subtracted from this length to obtain the working length. The root canal was cleaned and shaped using the conventional crown-down technique to a #40 master apical file (Hero 642, Micro Mega, Besençon, France) with 6% taper used at working length. During cleaning and shaping, the root canals were irrigated with 2 milimolar sodium hypochlorite solution. Since the smear layer prevents the contamination of dentinal tubules with E. faecalis, the teeth were vortexed in 17% EDTA for 4 minutes. After rinsing the teeth with saline, they were vortexed in 2.25% sodium hypochlorite for 4 minutes. Tooth apices were sealed with light cure cement and the root surfaces were covered with two layers of nail varnish. Each tooth was transferred into a test tube containing sterile brain heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) and autoclave-sterilized at 121°C with 15 Psi pressure for 30 minutes.

Figure1: (A) The specimens was incubated in sterile brain heart infusion (BHI).
(B) E. faecalis suspension was injected into each canal using a sterile insulin syringe

The tubes were sealed and incubated at 37°C for 48 hours **(fig 1A)**. Five teeth were randomly selected as the negative control group and incubated in BHI broth for 24 hours. No bacterial growth in the negative control tubes indicated absence of contamination.

Frozen E. faecalis (ATCC 9854) was transferred to a Todd Hewitt broth agar plate and incubated at 37°C for 24 hours. Next, a 0.5 McFarland standard concentration of the broth containing 1.5×108 CFUs/mL was prepared; 100 µm of the suspension was injected into each canal using a sterile insulin syringe **(Fig1B)**. The root canals were then incubated for two weeks under anaerobic conditions at 37°C in order for the E. faecalis biofilm to form in the RCS. The culture medium of the tubes was refreshed daily. After the incubation time, five teeth were considered as the positive controls. The remainder of the specimens was randomly divided into two experimental groups (n=20 each). Experimental group one was subjected to 810 nm diode laser (Doctor Smile, Italy) irradiation (1.5W) while experimental group two was subjected to 980 nm diode laser (Sirona Dental Systems GmbH, Bensheim, Germany) irradiation (1.5W).

The BHI broth in the canals was dried using sterile paper points. Laser energy was directed through a 200µ diameter disposable flexible fiber to the root canal at a length 1 mm shorter than the working length in continuous wave mode. The teeth were subjected to four cycles of diode laser irradiation, with 5 seconds of irradiation and 20 seconds of resting period. The laser handpiece was held in such a way that the fiber tip and the root canal axis formed a 10° angle. Laser was irradiated in a circular fashion in an apical-coronal direction (step-back technique) without the use of water or any other coolant. After laser irradiation, saline was injected into the canals as a transformer medium.

Samples were obtained from inside the canals using a #2 peeso reamer (Dentsply Maillefer, Tulsa, OK, USA) for 20 seconds. Moreover, two sterile paper points were used for the transfer of medium and dentin chips. Paper points and peeso reamers were vortexed in a test tube containing 10mL of saline for 20 seconds. The vortexed saline was serially diluted in test tubes in 1:10 ratio to prepare 10^1 to 10^8 serial dilutions; 100 µm of these dilutes was transferred to blood agar plates and cultured, followed by incubation at 37°C for 48 hours. Eventually, number of E. faecalis CFUs in each plate was counted and reported as CFUs/mL. All phases were performed under biological hood.

The number of CFUs in each experimental group before and after laser irradiation was statistically analyzed using the wilcoxon test. Multiple linear regression was applied to assess the role of confounding factors such as the mean difference in the number of CFUs before the intervention between the two groups. Mann Whitney test was applied to compare bacterial reduction between two experimental groups.

Results

Table 1 shows the number of bacterial colonies before and after laser irradiation in the two experimental groups. A significant reduction in the number of CFUs was seen after laser irradiation in the two experimental groups. Also, the two experimental groups of 810 nm laser ($3.5×10^6$) and 980 nm laser ($9.5×10^7$) showed significantly less number of bacterial colonies compared to the positive control group ($2.6×10^{12}$).

The results of statistical analyses revealed a significant difference between the two experimental groups and the reduction in microbial count in the 810 nm laser group was significantly greater than in 980 nm laser group. However, the two groups also had a significant difference in the number of CFUs before laser irradiation, which is considered as a confounding factor. To assess the role of this confounder, multiple linear regression was applied, which showed the significant efficacy of 810 nm laser in decreasing the colony

Table 1: Colony-forming units (CFUs)/mL means before and after laser irradiation

laser groups		N	Mean	Std. Deviation	Median	Min	Max
Group 1 (810nm)	Before	20	4.0×10^{11}	2.8×10^{11}	4.2×10^{11}	6.8×10^{10}	7×10^{11}
	After	20	3.5×10^{6}	6.7×10^{6}	5.2×10^{5}	2.5×10^{4}	2.9×10^{7}
Group 2 (980nm)	Before	20	4.9×10^{12}	3.7×10^{12}	1.3×10^{12}	1×10^{12}	9×10^{12}
	After	20	9.5×10^{7}	2.7×10^{7}	4.2×10^{6}	4.8×10^{4}	1.1×10^{9}

count. Regression coefficient was 0.06 and level of significance was 0.001 **(table 2)**. Under in vitro conditions, no growth of microorganisms was noted in the negative control group.

Discussion

In the recent years, application of laser technology in clinical dentistry has considerably increased, mainly due to the introduction of different laser wavelengths, methods and delivery systems. Laser therapy is known as an efficient modality in endodontic treatment due to multiple advantages such as smear layer removal, decreasing the bacterial count and reducing the apical microleakage [4, 12)]. Studies have shown that different wavelengths of lasers, particularly the diode and neodymium lasers, are effective for decreasing the intracanal bacterial count [5)]. Sundqvist et al. indicated that 38% of failed endodontically treated teeth were contaminated with E. faecalis [13)]. This Gram-positive anaerobe can proliferate alone in absence of synergistic support from other bacteria and can tolerate long-term starvation. Due to its ability to penetrate deep into dentinal tubules and forming a biofilm, it remains viable after mechanical and chemical root canal preparation [14)]. Moreover, E. faecalis tolerates high pH (as high as 11.5) and thus, it is resistant to calcium hydroxide [15)]. A meta-analysis on the effect of laser therapy on E. faecalis in the root canal showed that lasers could effectively eradicate E. faecalis [16)]. It has been confirmed that structural configuration of the cell wall affects bacterial susceptibility to laser irradiation. Several cycles of laser irradiation are required in order for the laser to affect Gram-positive bacteria; whereas, Gram-negative bacteria are eliminated faster and more easily [17)].

Studies have shown that differences in the wavelengths, power, irradiation time, spot size and number of cycles are responsible for the variable efficacy of lasers reported [18)]. Diode lasers are highly popular due to their small size and cost effectiveness. Also, they have a flexible and thin fiber, which enables easy access to narrow canals and enhances the efficacy of disinfection in the radicular dentinal tubules to a depth of 500μ [19, 20)]. It was demonstrated that 830 nm diode laser with 3W power in combination with 17% EDTA and 0.5% sodium hypochlorite irrigants resulted in complete (100%) disinfection of the root canal system [19)]. High power diode laser eliminates the microorganisms in the root canal based on a thermal mechanism [21)]. Nonetheless, in disinfection of the root canals with laser irradiation, care must be taken to use appropriate parameters and protocols to prevent thermal damage to the surrounding tissue. Scanning electron microscopic analysis showed melting and fusion of dentinal tubules in the apical region following the application of 1.25W and 2.5W diode lasers. However, temperature rise in the periodontal ligament did not exceed the safe limit (10°C) when 20-second rest periods were allowed after each cycle of laser therapy An in vivo study showed that the disinfection efficacy of 1.05W diode laser after a 15-second cycle was not significantly different from that of the control group. But, 1.5W and 1.95W diode lasers both had optimal disinfection efficacy [22)].

The recently introduced 980 nm laser with 1.5W power showed high efficacy for decreasing E. faecalis colony count and only slightly changed the morphology of dentin surface. The temperature rise was minimal at the external root surface which was within the safe threshold [10)].

Our study results showed that application of 810 and 980 nm diode lasers with 1.5W power significantly decreased the E. faecalis bacterial load in the root canal system compared to the control group. The effect of 810 nm diode laser on decreasing E. faecalis colony counts was significantly greater than that of 980 nm diode laser. Our results are similar to those of Beer et al, who showed that both 810 and 940 nm wavelengths of laser decreased E. faecalis and Escherichia coli (E. coli) colony counts by approximately 98% when including access cavity in irradiation. However, the efficacy of both laser wavelengths in reduction of microorganisms was almost the same and 810 nm laser was slightly more effective [6)].

Gutknecht et al, in two different studies assessed the effects of 810 and 980 nm diode lasers on intracanal E. faecalis and demonstrated that 810 nm laser decreased the bacterial count by 74% to a depth of

Table 2: Difference in CFU at base line between two laser groups

Model	Standardized Coefficients	T	P-value
	Beta		
(Constant)		-.578	.567
difference in CFU at base line between two laser methods	.076	.578	.567
laser groups	.630	4.771	.001

500μ; 980 nm laser, despite using higher distal output power, decreased the bacterial count by 57% [11, 20].

In the current study, only diode laser irradiation was used for disinfection of the root canal and the efficacy of diode laser in two different wavelengths was compared. However, several studies have demonstrated that laser irradiation as an adjunct and in conjunction with other methods is more effective for root canal disinfection [23]. Mehrvarzfar et al. showed that combined use of irrigants such as sodium hypochlorite and chlorhexidine with 810 nm diode laser effectively eradicated E. faecalis from the root canal system. In particular, MTAD in conjunction with laser therapy eliminated 100% of E. faecalis bacteria from the canal [24].

Assnashari et al. demonstrated significant reduction of intracanal E. faecalis count after photodynamic therapy with 810 nm diode laser. In photodynamic therapy, simultaneous application of laser and a photosensitizer decreases the thermal effect of laser and increases its antibacterial activity [25]. Optical properties of bacteria and their microenvironment play critical roles in the efficacy of laser for root canal disinfection. Use of photosensitizers improves the mechanism of action of laser especially against non-pigmented bacteria such as E. faecalis and E. coli [26]. Ahmeduddin et al. (2012) compared the efficacy of 980 nm diode laser and Nd:YAG laser in different conditions and reported that Nd:YAG laser decreased the number of CFUs to zero; whereas, 980 nm diode laser although had an acceptable inhibitory effect on proliferation of E. faecalis clinically, it could not decrease the number of CFUs to zero; however, increasing the power of this laser will enhance its effect [27].

Conclusion

Irradiation of 810 and 980 nm diode lasers can significantly decrease E. faecalis colony count in the RCS and both wavelengths are efficient for disinfection of the RCS. However, 810 nm diode laser caused a greater reduction in E. faecalis colony count. Considering the increasing popularity of lasers due to their variable wavelengths, further investigations are required on different lasers and optimal parameters to maximize their efficacy. The current study had an in vitro design, and in vivo studies are required to assess the efficacy of these wavelengths of laser in the clinical setting.

Conflict of Interest:

The authors have no conflict of interest to declare

References

1: Peters O, Schönenberger K, Laib A (2001):Effects of four Ni–Ti preparation techniques on root canal geometry assessed by micro computed tomography. International Endodontic Journal, 34:221-230.
2: Berutti E, Marini R, Angeretti A (1997): Penetration ability of different irrigants into dentinal tubules. Journal of endodontics, 23:725-727.
3: Kouchi Y, Ninomiya J, Yasuda H, Fukui K, Moriyama T, Okamoto H(1980): Location of Streptococcus mutans in the dentinal tubules of open infected root canals. Journal of dental research, 59:2038-2046.
4: Kimura Y, Wilder−Smith P, Matsumoto K. Lasers in endodontics: a review (2000): International Endodontic Journal, 33:173-185.
5: Asnaashari M, Safavi N(2013): Disinfection of Contaminated Canals by Different Laser Wavelengths, while Performing Root Canal Therapy. Journal of lasers in medical sciences, 4:8-16.
6: Beer F, Buchmair A, Wernisch J, Georgopoulos A, Moritz A (2012). Comparison of two diode lasers on bactericidity in root canals--an in vitro study. Lasers in medical science, 27:361-364.
7: Preethee T, Kandaswamy D, Arathi G, Hannah R (2012): Bactericidal effect of the 908 nm diode laser on Enterococcus faecalis in infected root canals. Journal of conservative dentistry, 15:46-50.
8: Camargo SCC (2012). The antibacterial effects of lasers in endodontics. infection.1:8-14.
9: Marchesan MA, Brugnera-Junior A, Ozorio JE, Pecora JD, Sousa-Neto MD (2008):. Effect of 980-nanometer diode laser on root canal permeability after dentin treatment with different chemical solutions. Journal of Endodontics, 34:721-724.
10: Schoop U, Kluger W, Dervisbegovic S, Goharkhay K, Wernisch J, Georgopoulos A, et al (2006): Innovative wavelengths in endodontic treatment. Lasers in surgery and medicine, 38:624-630.
11: Gutknecht N, Franzen R, Schippers M, Lampert F(2004): Bactericidal effect of a 980-nm diode laser in the root canal wall dentin of bovine teeth. Journal of clinical laser medicine & surgery. 22:9-13.

12: Muhammad OH, Rocca JP, Fornaini C, Medioni E (2015): Evalution of the role of phototherapy during endodontic decontaminaton. journal of laser therapy, 24:291-302.
13: Sundqvist G, Figdor D, Persson S, Sjogren U(1998): Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics, 85:86-93.
14: Figdor D, Davies JK, Sundqvist G(2003): Starvation survival, growth and recovery of Enterococcus faecalis in human serum. Oral microbiology and immunology, 18:234-9.
15: Bystrom A, Claesson R, Sundqvist G(1985): The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endodontics & dental traumatology, 1:170-175.
16: Sadik B, Arikan S, Belduz N, Yasa Y, Karasoy D, Cehreli M(2013): Effects of laser treatment on endodontic pathogen Enterococcus faecalis: a systematic review. Photomedicine and laser surgery, 31:192-200.
17: Moritz A, Jakolitsch S, Goharkhay K, Schoop U, Kluger W, Mallinger R, et al (2000): Morphologic changes correlating to different sensitivities of Escherichia coli and enterococcus faecalis to Nd:YAG laser irradiation through dentin. Lasers in surgery and medicine, 26:250-261.
18: de Paula Eduardo C, Gouw-Soares S (2001): The Use of Lasers for Endodontic Applicationsin Dentistry. Medical Laser Application, 16:231-243.
19: de Souza EB, Cai S, Simionato MR, Lage-Marques JL. High-power diode laser in the disinfection in depth of the root canal dentin. Oral Surgery Oral Medicine Oral Pathoogyl Oral Radiology Endodontics, 106:e68-72.
20: Gutknecht N, van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F(2000): Diode laser radiation and its bactericidal effect in root canal wall dentin. Journal of clinical laser medicine & surgery, 18:57-60.
21: Udart M, Stock K, Graser R, Hibst R (2011): Inactivation of bacteria by high-power 940 nm laser irradiation. Medical Laser Application, 26:166-171.
22: Shehab NF AZ, Taha M (2013): Evaluation of Antibacterial Efficacy of Elexxion Diode Laser 810 nm on the Infected Root Canals (In Vitro and In Vivo) International Journal of Dental Sciences and Research,1:23-27.
23: Mithra NH, Krishna RS, Shishir S, Veenna SA (2013): Comparative evaluation of bactericidal effects on Enterococcus faecalis using diode laser irradiation, sodium hypochlorite and chlorhexidine gluconate irrigation"- an in vitro study. Oral health and dental management, 12:145-150.
24: Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, et al (2013): Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against Enterococcus faecalis contaminating root canals: an in vitro study. Journal of oral science, 53:355-360.
25: Asnaashari M, Mojahedi SM, Asadi Z, Azari-Marhabi S, Maleki A (2016): A Comparison of the antibacterial activity of the two methods of photodynamic therapy (using diode laser 810 nm and LED lamp 630 nm) against Enterococcus Faecalis in extracted human anterior teeth. Photodiagnosis and photodynamic therapy,13:233-237.
26: Pirnat S, Lukac M, Ihan A (2011): Study of the direct bactericidal effect of Nd: YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria. Lasers in medical science, 26:755-761.
27: Ahmeduddin M, Nagesh B, Reddy K, Raj K (2012): An assessment of bactericidal effect of two different types of lasers on enterococcus faecalis: An in vitro study. Journal of Dental Lasers, 6:2-6.