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Antibacterial effect of 940 nm diode laser on *Enterococcus faecalis*-infected root canals

Amir Ardalan Abdollahi[1], Seyyed Amir Seyyedi[2], Lida Lotfollahi[3], Navid Yekadast Javan[4]

Departments of [1]Endodontics and [2]Oral and Maxillofacial Medicine, Dental School, Urmia University of Medical Sciences, [3]Department of Microbiology, Faculty of Medicine, Urmia University of Medical Sciences, [4]Student Research Committee, Dental School, Urmia University of Medical Sciences, Urmia, Iran

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Address for correspondence:
Dr. Seyyed Amir Seyyedi, Department of Oral and Maxillofacial Medicine, Dental School, Urmia University of Medical Sciences, Urmia, Iran.
E-mail: seyyediamir@yahoo.com

ABSTRACT

Background: This study compared the antibacterial effects of 940 nm diode laser and sodium hypochlorite and chlorhexidine irrigations on *Enterococcus faecalis* in human permanent single-rooted teeth.

Materials and Methods: In this *in vitro* study, 65 extracted human single-rooted teeth were prepared using the crown-down method using rotary files. The root canals were irrigated with 5.25% sodium hypochlorite, 17% ethylenediaminetetraacetic acid, and normal saline solution. After placing the roots in microtubules, they were transferred into an autoclave. The teeth were randomly divided into four groups (n = 15): laser, sodium hypochlorite, chlorhexidine, and saline. Three teeth were assigned to the positive control group and two to the negative control group. The root canals were sampled, and the colony counts were determined 24 h later. Then, antibacterial agents were applied to the canals, and immediately after, the root canals were sampled, and the colony counts were determined 24 h later. The data were analyzed using Kruskal–Wallis and Mann–Whitney *U*-tests using the SPSS software version 26. The significance level was defined at $P < 0.05$.

Results: The results showed that sodium hypochlorite, chlorhexidine, laser, and normal saline significantly reduced bacterial colony counts, confirming their antimicrobial effects ($P < 0.001$). Sodium hypochlorite and chlorhexidine showed the highest antimicrobial effects, with no significant differences between the sodium hypochlorite and chlorhexidine groups ($P = 0.512$); however, there were significant differences between the other groups ($P < 0.001$).

Conclusion: According to the results, 940 nm diode laser beams significantly reduced *E. faecalis* counts and could be used as a new, effective, and complementary treatment in disinfecting the root canal.

Key Words: Diode laser, disinfection, *Enterococcus faecalis*, root canal therapy

INTRODUCTION

The main goal of endodontic treatment is to disinfect the root canal and the three-dimensional network of dentinal tubules. Bacterial agents penetrate the deeper layers of root dentin through the infected pulp tissue, causing periapical inflammation.[1-3] One of the most important steps in root canal treatment is root canal preparation, which involves cleaning, disinfecting, and shaping the root canal system to obturate it with

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a suitable substance. In recent studies, the success rate was reported at 95% for teeth with pulpitis and 85% for necrotic teeth.[2,4] Numerous studies have shown that the prognosis of apical periodontitis after endodontic treatment is poorer in the presence of live bacteria.[5,6] Therefore, microorganisms play a significant role in endodontic treatment failures,[7,8] and chemical root canal cleaning is necessary in addition to mechanical preparation.[8,9] Ideal chemical preparation with sodium hypochlorite involves dissolving vital and necrotic tissues and removing bacteria, but the dentinal tubules cannot be sterilized.[10]

Enterococcus faecalis is particularly important in the failure of endodontic treatment.[11,12] The prevalence of this microorganism in cases of endodontic failure is 22%–77%.[13] Due to its ability to survive at pH = 11.5, this microorganism can resist calcium hydroxide, which is used as an intracanal medication between treatment sessions.[14] *E. faecalis* can resist starvation for a long time and grow alone in treated root canals without supporting coexistent bacteria.[15] Many studies have shown that the preparation of root canals with manual and rotary file systems of nickel–titanium or stainless steel cannot sufficiently prepare and clean the root canals.[16,17] However, when detergents are used in chemical preparation, with sodium hypochlorite as the most commonly used one, it is impossible to completely remove the microorganisms from the dentinal tubules.[18] Therefore, in cases of microbial resistance to conventional treatment methods, lasers can be effective as an auxiliary method to kill and reduce the microorganisms.[19] The excellent antibacterial effect of diode laser irradiation can be attributed to its greater penetration depth (up to 1000 μm into dentinal tubules) compared to the penetration power of chemical disinfectants, which is limited to 100 μm.[20]

One of the most popular lasers in endodontics is the diode laser. This laser effectively removes the smear layer and disinfects the primary and accessory root canals.[21] Diode lasers are available in four wavelengths: 810–830, 940, and 980 nm. The antibacterial quality of diode lasers is attributed to the thermal effect and temperature increase in root canals during radiation.[22]

To date, none of the available irrigants have the ideal necessities for achieving successful endodontic treatment. Furthermore, diode lasers are more effective in cases with microbial resistance and penetration to the root canal dentin and accessory canals.[19-21] Since limited studies have investigated the antibacterial effects of 940 nm diode laser on *E. faecalis* in the root canal, this study aimed to evaluate the effect of 940 nm diode laser compared to conventional detergents against *E. faecalis*.

MATERIALS AND METHODS

This *in vitro* study was approved by the Research Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran (IR.UMSU.REC.1399.243). This *in vitro* study was performed in the Dental Faculty of Urmia University of Medical Sciences. We selected 65 extracted human single-rooted teeth. Filled root canals and root canals with caries and morphological complexities were excluded. The teeth had been extracted due to periodontal disease. The inclusion criteria consisted of fully developed single roots without caries, previous endodontic treatment, and anomalies. Furthermore, teeth with cracks and calcifications in radiographic views were excluded. After extraction, for better disinfection, the teeth were stored in 3% chloramine T solution at 4°C for 1 month. The root surfaces were cleaned with ultrasonic tips to remove residual periodontal soft tissues. Using a diamond disk (D and Z, Switzerland) and handpiece with a speed of 40,000 rpm without water cooling, the tooth crowns were separated at the cementoenamel junction. The working length was determined using a #25 K-Flexofile (Dentsply, Maillefer, Ballaigues, Switzerland), 1 mm from the apical foramen. The crown-down technique was applied for root canal instrumentation. The coronal two-thirds of the canals were prepared with #4 and #3 Gates-Glidden drills (Dentsply, Maillefer, Ballaigues, Switzerland), followed by the use of Sx, S1, S2, F1, F2, and F3 Protaper rotary instruments (Dentsply, Maillefer, Ballaigues, Switzerland). A master apical file of #40 was considered for all the root canals. Each root canal was irrigated with 1 mL of normal saline solution (Daru Pakhsh, Tehran, Iran) during the root canal preparation. The smear layer was removed using 1 mL of 17% ethylenediaminetetraacetic acid (Pulpdent Corp., Watertown, MA, USA) for 3 min, followed by a final rinse with 1 mL of 5.25% NaOCl (Taj Corp, Tehran, IRI) for 3 min. Finally, the root canals were irrigated with 5 mL of saline solution and dried with #40 paper cones (Aria Dent, Tehran, Iran). The apical foramina were sealed with

self-cured glass ionomer (Dentonics, USA), and root surfaces were coated with two layers of colorless varnish. The teeth were sterilized by autoclaving at 121°C and 15 psi pressure for 20 min. To corroborate sterilization, the teeth were incubated in brain–heart infusion broth (Merck, Darmstadt, Germany) at 37°C for 24 h. The root canals were then infected with a bacterial suspension every 48 h for 1 week.[8]

Experimental groups

The samples were randomly assigned to six groups (n = 15).

- Group 1: The root canals were irrigated with 5 mL of 5.25% NaOCl for 1 min with a 2-mL syringe and 30G needles. Next, 5 mL of saline solution was injected into the root canals with a 2 mL syringe and 30G needles and left in the root canals for 30 s to neutralize NaOCl
- Group 2: Root canals were irrigated with 5 mL of 2% chlorhexidine for 1 min with a 2 mL syringe and 30G needles. Next, 5 mL of saline solution was injected into the root canals with a 2 mL syringe and 30G needles and left in the root canals for 30 s
- Group 3: Irradiation was carried out using a 940 nm diode laser (Epic X; Biolase Inc, Irvine, California) with a power of 1 Watt, energy of 1 J, and energy density of 2.23 J/cm^2 in continuous mode. The tip of the diode laser fiber, with a diameter of 200 μm and a length of 14 mm, was placed up to 1 mm from the apex in the root canal after placing the device in the ready state. Then, the radiation was delivered without hitting the root canal wall at a speed of 2 mm/s and moved circumferentially toward the coronal area. This cycle was repeated four times with an interval of 10 s.[23-25] Laser radiation protocol, using a 940 nm diode laser with 1 W output power and radiation in four shifts, was performed at 10 s intervals. As in previous studies, laser fiberoptics were placed directly within the root canal. The fiber tip did not directly contact the root canal walls[26]
- Group 4: The samples were irrigated with 5 mL of normal saline solution for 1 min using a 2 mL syringe and 30G needles
- Group 5: Positive group: Three teeth were selected as the positive control group after initial preparation, autoclaving, and inoculation of the bacterial suspension
- Group 6: Negative control group: Two teeth were selected as the negative control group after initial preparation and autoclaving. Inoculation of the bacterial suspension was not performed on them.

Microbial procedures

E. faecalis strains used for the study were standard strains of *E. faecalis* ATCC29212 which were cultured on agar plate in the selected medium and incubated for 24 h at 37°C.

Before inoculating the bacteria, 10 teeth were randomly sampled with #60 paper points and cultured in a blood agar medium to ensure that the root canal was sterilized. Using a pair of pliers, the paper point was inserted into the root canal and removed with a paper mover after a 90º rotation. The plates were incubated at 37°C and 5% CO_2 for 24 h. Due to the bacteria remaining in 50% of the random samples, autoclaving was performed for the second time with open microtubes. The sampling of 10 teeth was repeated randomly, and 10% of the samples remained infected. Autoclaving was performed for the third time, and sampling and culture were performed under the same conditions for the third time. Finally, all the cultured samples indicated that the root canals were free of microorganisms. Then, the bacterial suspension was inoculated.

To inoculate the bacteria, the first colonies obtained from standard strains in the blood agar culture medium, using sterile fildoplatin, were isolated from the flame of several bacterial colonies and placed in sterile physiologic serum, which was poured into a sterile test tube. We shook it and placed it on the vortex shaker to achieve a uniform suspension. Then, we used a spectrophotometer to ensure its concentration. The solution was diluted with 1 mL of normal saline solution and then placed in 1 mL of the prepared bacterial suspension in the device. When a value between 0.08 and 0.12 was obtained, the turbidity of 0.5 McFarland was confirmed.[2]

The tooth roots were then placed in 1.5 mL sterile microtubes separately, and the root canals were filled with a 10-μL sample of the bacterial suspension. After initial inoculation, the root canals were reloaded every 48 h with the same amount of bacterial suspension after aspirating the previous bacterial suspension with a 2 mL syringe and a 30G needle. The bacterial suspension was inoculated three times. The identification and purity of *E. faecalis* culture were evaluated using Gram staining and colony morphology observation in agar media before each inoculation procedure. The samples were incubated

under 5% CO_2 between each inoculation at 37°C, and the entire inoculation and incubation period lasted 1 week. After the last inoculation and 48 h, sampling was performed from all the root canals to determine colony forming units counts. The inoculation process was carried out before each intervention and after the procedure. A #60 paper point was inserted into each root canal for sampling. Immediately, on a plate containing blood agar, four concentric circles were drawn that decreased in diameter toward the center with a paper cutter. The plates were incubated at 37°C and 5% CO_2 for 24 h. Then, the colonies were counted.[8,26]

The teeth were then randomly assigned to the mentioned groups. Antimicrobial agents were applied to the root canals. Immediately after the intervention, the teeth were sampled inside the root canal according to the mentioned protocol, and the samples were cultured. Blood agar was transferred. The plates were incubated at 37°C and 5% CO_2 for 24 h. Then, the colonies were counted.[26]

Statistical analysis

The data were analyzed using the SPSS software (SPSS, Chicago, IL, USA) version 26. The normal distribution of variables was investigated using the Kolmogorov–Smirnov test. The Kruskal–Wallis test was used to compare the percentage reduction in colony counts (%) in the study group, and the Mann–Whitney *U*-test was used to determine the group that caused the difference. For intragroup comparison, the Wilcoxon signed-rank test was used. The significance level was defined at $P < 0.05$.

RESULTS

The number of colonies in each group was examined separately [Table 1]. The results of the paired *t*-test to compare the means before and after the intervention showed a statistically significant difference in the mean colony counts between all the groups (except for positive and negative control groups) ($P < 0.001$). Furthermore, the highest reduction percentage was related to sodium hypochlorite with 99.52%, followed by chlorhexidine with 99.36% and laser with 62.06%. The lowest reduction percentage was related to normal saline with a 26.78% reduction [Figure 1].

Intergroup comparison of mean colony counts suggested that sodium hypochlorite and chlorhexidine disinfection capability were significantly higher than the laser group ($P < 0.001$). Furthermore, Mann–Whitney U test results showed that the disinfection ability of the laser group was significantly higher than the normal saline group ($P < 0.001$).

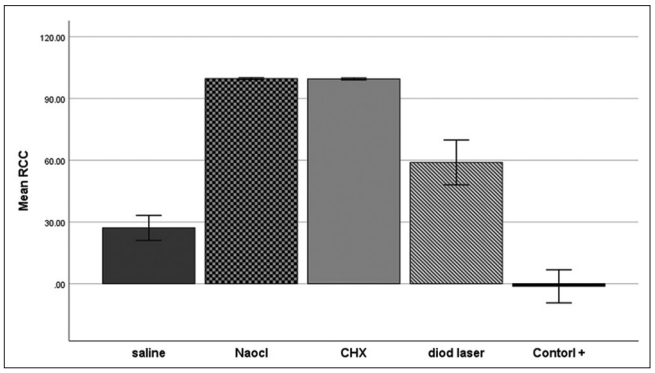

Figure 1: Comparison of mean reduction in colony counts percentages in study groups.

DISCUSSION

We designed this study to compare the effectiveness of 940 nm diode laser beams with chlorhexidine, sodium hypochlorite, and saline solution in disinfecting root canals contaminated with *E. faecalis*. The presence of bacteria in the complex morphology of the root canal and dentinal tubules is the most important reason for the failure of root canal treatment.[12,27] Therefore, eliminating bacteria and their toxins is the key to successful root canal treatment.

We used a diode laser in this study, which was more desirable due to its antibacterial properties and affordable price.[21] Regarding the antibacterial mechanism of diode laser, Moritz and Schoop[28] observed a reaction between the ions emitted by the laser and molecules on the cell wall. This reaction destroyed the protein molecules in the cell wall, which ultimately disrupted the bacterial cell membrane. Moreover, the main antibacterial effect of the laser is principally thermal effect and temperature increase in root canals during radiation, resulting in the disruption of the bacterial cell membrane.[22,29] In a study by Mehta *et al.*,[30] 940 nm laser beams had a stronger antiseptic effect than other low-power lasers and Er,Cr:YSGG laser. We used a wavelength of 940 nm to directly compare the results of this study with previous studies; the method of this study was designed to be as similar as possible to previous studies.[26,31]

The findings showed that using 940 nm diode laser beams after 24 h significantly reduced bacterial colony counts, consistent with a study by Castelo-Baz

Table 1: Intergroup comparisons of bacterial colony counts before and after irrigation

Group	*n*	Time	Bacterial colony counts, mean±SD	Disinfecting efficacy (%)	*P**
Laser	15	Preoperative	83,733.33±30,548.47	62.06	<0.001
		Postoperative	31,766.66±14,368.69		
Sodium hypochlorite	15	Preoperative	88,866.66±24,683.32	99.52	<0.001
		Postoperative	423.33±844.77		
Chlorhexidine	15	Preoperative	80,466.66±27,508.09	99.33	<0.001
		Postoperative	531.33±100,972		
Normal saline	15	Preoperative	77,666.66±19,263.83	26.78	<0.001
		Postoperative	56,866.66±17,864.03		
Positive control	3	Preoperative	86,333.33±11,503.62	1.15	0.58
		Postoperative	87,333.33±11,372.48		
Negative control	2	Preoperative	0	0	NA
		Postoperative	0		

*Independent *t*-test. NA: Not assigned; SD: Standard deviation

et al.[23] In this study, the disinfecting power of the laser was 62.06%, which was less than that reported by Ashofteh *et al*.[32] with the 980 nm diode laser after 48 h with a frequency of 91.4%, which was higher than that in a study by Benezra *et al*.[26] with a frequency of 30.28%. These findings show that the disinfecting power of laser beams at intervals of >24 h can have a significant effect, and also, at the same power of 1 W, the 940 nm diode laser has a higher antibacterial effect than the 810 nm laser.

The findings of this study showed that the use of laser disinfectant power was significantly less than that of sodium hypochlorite and chlorhexidine, consistent with the findings of a peer-reviewed study[33] in which the disinfecting power of sodium hypochlorite and chlorhexidine was significantly higher than the 940 nm diode laser. Buraihi and Alkurtas[33] showed that the antiseptic power of sodium hypochlorite after 24 h was significantly higher than the 940 nm laser, consistent with the current study. Furthermore, Bitter *et al*.[34] showed that the efficiency of 2% chlorhexidine in root canal disinfection of *E. faecalis* was higher than the 940 nm laser and sodium hypochlorite, with 1% and 0.9%, consistent with the current study. Furtheremore, Mehta *et al*.[30] showed that diode laser had a lower antimicrobial effect than sodium hypochlorite in removing *E. faecalis*, consistent with the current study. In addition, Ozkocak *et al*.[35] showed that sodium hypochlorite and chlorhexidine had a significantly better antimicrobial effect than the 940 nm laser, consistent with the present study. However, in the study by Benezra *et al*.,[26] the 1 W 810 nm diode laser did not differ significantly from the saline solution in reducing bacterial colony counts, indicating that at equal power, the 940 nm diode laser performed better than the 810 nm laser. However, the 810 nm diode laser with a power of 1.5 W significantly decreased bacterial colony counts compared to normal saline solution, consistent with the current study.

Laser beams were significantly better than the normal saline solution in removing *E. faecalis*. In addition, laser performance was significantly better than that of 0.5% and 1% sodium hypochlorite solution.[34] According to a study by Dai *et al*.,[36] 100% disinfection of the root canal requires the simultaneous use of laser beams and sodium hypochlorite.

The use of laser beams in root canal treatments also raises considerations. If the laser settings are incorrect, the laser beam's heat can damage the periapical tissues.[32]

This study also had some limitations. Conducting it under laboratory conditions and not in clinical settings was one of its most important limitations. Furthermore, in this study, the studied biofilm was identified as a single species with only *E. faecalis*, while under clinical conditions, the biofilm is multifaceted with a combination of different microbial species. Furthermore, in this study, the role of the laser beam on the pure form was examined, and it is suggested that in future studies, the combined effect of laser beams and other disinfectants should be evaluated. In this study, the antimicrobial effects were examined only within the main root canal, while the microorganisms that remained within the dentinal tubules could reduce the success of treatment.[13] Unlike conventional irrigation solutions, lasers can penetrate dentinal tubules. The examination of dentinal tubules by electron microscopy was not possible to examine the effects of antibacterial agents

on dentinal tubules. Moreover, it is recommended that future studies be designed with modifications in laser irradiation protocols and in the form of clinical studies to investigate the disinfecting effect of laser beams in the root canal systems.

CONCLUSION

This study showed that 940 nm diode laser beams significantly decreased *E. faecalis* counts; therefore, they can be used as a new, effective, and complementary treatment modality for disinfecting root canals.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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