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Comparison between the Antibacterial Effects of 810 and 980-Nanometer Diode Lasers in Combination with Sodium Hypochlorite on Enterococcus Faecalis in the Root Canal System-*In Vitro* Study

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ABSTRACT

Background: Endodontic treatment failure can be caused by a variety of factors, including bacterial persistence, inadequate root canal cleaning or obturation, improper coronal seal, and untreated canals (missed canals). The presence of bacteria inside the root canals, such as Enterococcus faecalis, is the most common cause of endodontic failure such as (E. faecalis). These bacteria are more resistant to disinfectants, resulting in an infection that persists intra-radicularly or extra-radicularly. Because of the introduction of new antimicrobial properties of lasers in recent years, newer laser technology disinfection protocols have proposed to be effective for routine endodontic treatment.

Aim: This study compares the antibacterial effects of diode lasers with wavelengths of 810 nm and 980 nm in combination with sodium hypochlorite on Enterococcus faecalis biofilm in the root canal system in vitro.

Materials and methods: forty single canals Human permanent teeth were cleaned, shaped, and sterilized before being inoculated with E. faecalis culture and incubated for two weeks. The specimens were randomly divided into four groups after the incubation period. Forty single-canal permanent human teeth were cleaned, shaped and sterilized then inoculated with E. faecalis culture then incubated for two weeks. After the incubation period, the specimens divided randomly in 4 groups; group A (control group) specimens that have not been treated, group B (its specimens were treated with 17% EDTA and sodium hypochlorite at 5.25%), group C (specimens radiated with 810 nm diode laser after NaoCL and EDTA treatment) and group D (specimen radiated with 980 nm diode laser after NaoCL and EDTA treatment). Bacterial samples were taken by inserting paper points into the canals and counting CFU after plated on blood agar media.

Results: Laser irradiation decreased the bacterial colony count in both experimental groups. The reduction in microbial count was significantly greater in 810 nm laser group (97.9%) compared to 980 nm laser group (94.2%).

In comparison to the control group, Dunnett's T3 test revealed a significant difference between the experimental and control groups. The highest bacterial killing (97.9% CFU/ml reduction) was achieved using 810 nm diode laser in combination with NaOCL and EDTA protocol.

Conclusion: in combination with NaOCL and EDTA, 810 nm diode lasers was more effective in decreasing the intracanal microbial load than 980 nm diode laser.

Key words: NaOCL, EDTA, 980 nm diode laser, 810 nm diode laser

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INTRODUCTION

Because of its complex anatomy, bacteria's ability to penetrate dentinal tubules and the presence of a smear layer formed during mechanical instrumentation, disinfecting the root canal system is difficult [1]. Endodontic treatment's primary goal is to effectively remove bacteria and necrotic pulp tissue remnants from the root canal system [2]. However, it

has been demonstrated that after root canal preparation with a rotary Ni-Ti system, 35% of the root canal surface area remains unchanged [3]. Furthermore, a biomechanical preparation cannot completely eliminate the microorganisms in the root canal system; each technique has its own set of limitations [4]. The most commonly used root canal irrigants during routine endodontic treatment are sodium hypochlorite, chlorhexidine gluconate, and ethylenediaminetetraacetic acid (EDTA), but none of these chemicals can be considered an ideal irrigation solution [5]. These irrigants solutions cannot penetrate dentin for more than 100 micrometers due to surface tension, and it is well known that pathogenic bacteria can invade deep into dentin for up to 1000 micrometers, making these chemical irrigants ineffective against these deep-seated microorganisms [6]. The irrigation process in the apical third of the root canal has its own unique challenges, and the key issue here is achieving a balance between safety and effectiveness [6]. Diffusion of the irrigants solution through dentinal tissue is generally slow, and it is influenced by a number of factors such as irrigant concentration and temperature [7].

Enterococcus faecalis (*E. faecalis*) is the most common bacterial species in endodontic treatment failures due to resistant or recurrent infections [8]. Through various mechanisms, these *cocci* can withstand antibacterial agents such as NaOC [9]. By invading dentinal tubules and resisting nutritional deprivation, it has been successful in competition with other microorganisms [10]. It is also resistance to alkaline pH and consequently to calcium hydroxide pastes that normally inhibits other bacteria. The related mechanism could be linked to the presence of a functioning active proton pump in this bacteria's cellular membrane [11].

Because of the limitations of common irrigants in root canal treatment, new methods such as lasers have been introduced in recent years to clean the root canal system effectively. Due to properties such as high penetration depth into the dentinal tubules and proper antibacterial effect, the diode laser is the most desirable type of laser among the various types [12].

However, it has been demonstrated that by using a diode laser alone or in combination with irrigating solutions, *E. faecalis* can be largely or completely eradicated [13,14]. The available diode laser wavelengths for dental applications range from 800 nm to 1064 nm [15]. Previous studies have assessed the antimicrobial efficacy of 810 nm and 980 nm lasers separately, and the results show that they are both effective. Because of its small size, availability, thin flexible fibers, and output power of 0.5 to 7W, the 980 nm diode laser was recently introduced to dentistry and soon gained popularity [16]. Furthermore, the 980 nm diode laser is well absorbed by water but only slightly by hydroxyapatite crystals, resulting in light scattering in the dentin [17]. Gutknech et al. reported that the bacteria lodged to a depth of 500 m in dentinal tubules were successfully eliminated using a 980 nm laser [18]. Many studies have shown that the bactericidal effect of a diode laser (810 nm) is due to thermal properties, and that bacteria cannot develop resistance to laser exposure [19].

Aim of study

The antibacterial effects of diode lasers with wavelengths of 810 and 980 nm in combination with chemical irrigation solutions (NaOCL and EDTA) on *E. faecalis* biofilm in the root canal system were compared in this in vitro study.

MATERIALS AND METHODS

Preparation step

This study used forty extracted single-rooted human teeth with a single canal. All of the teeth are approximately the same size, have fully formed apices, and have never had root canal therapy. All collecting teeth are then debrided and rinsed in 5.25 NaOCl (Chlorax d 5.25%, Cerkamed, Poland) for 30 minutes before being soaked in normal saline (0.9% sodium chloride) at room temperature till the next step.

The root lengths were standardized to a 15 mm length to obtain uniform working length for all teeth specimens, and the specimens were cut off at the cement enamel junction with a disc bur using a high-speed handpiece.

#15 K-file (Dentsply Maillefer, Switzerland) was used for working length determination. The file was introduced into the canal until its tip was visible at the apical foramen.

Protaper rotary file system SX-S1-S2-F1-F2-F3

(Dentsply Maillefer, Switzerland) was used to prepare the root canals. After each file, 3 ml of 5.25% sodium hypochlorite was injected using a 30-gauge irrigation needle (Sinalident, China). The irrigation needle was placed 1 mm from the canal apices, with a delivery rate of 3 ml/min, to ensure that each canal received the same total irrigation time. Then, for 4 minutes, 2ml of 17% EDTA (17% EDTA, IL CHUNG, Korea) was injected into each canal, followed by a final irrigation with 5.25 % NaOCl. With an ultrasonic tip, both solutions (EDTA and NaOCl) were activated for 30 seconds. Finally, all specimen canals were washed with sterile water and dried with sterile paper points.

The apical foramens were sealed with composite resin fillings (Z350, 3M, USA) after the cleaning and shaping step, and all of the specimens were autoclaved for 20 minutes at 121oC under 15 psi pressure. After sterilization, each specimen was placed in an eppendorf tube with 2 mL of sterile Luria-Bertani broth (LB broth) and incubated at 37 °C for 48 hours with daily checks to ensure that the broth is free of turbidity.

Experimental contamination and incubation step

Enterococcus faecalis bacteria were isolated from infected root canals using a #15 K-file (Dentsply Maillefer, Switzerland) with a circumferential filing motion for 20 seconds and inoculating each sample with 20 l of LB broth. After inoculating the suspension in Pfizer selective enterococcus media (PSE agar), the *E.faecalis* bacteria were identified using vitek (Vitek 2 comnpact, Bio). Spectrophotometric dilution of the bacterial suspension to match 0.5 McFarland standard turbidity (1.5 × 108 CFU/ml). After injecting this bacterial suspension into the canals of prepared teeth, the orifices were dried and sealed with light-cured temporary fillings (IL CHUNG, Korea).

The specimens placed into Eppendorf tube contain 2ml of LB broth and incubated at 37°C under anaerobic conditions for 14 days. The broth and the tube was changed every three days for nutrition purpose.

Selection of Laser parameters and pilot study

Table 1 and 2 show the maximum temperature elevation of external root surface above the room temperature during laser irradiation

Experimental specimens disinfection

After two weeks of incubation under anaerobic condition the teeth specimens brought out from the incubator and soaked in CHX solution for 2 minutes then washed with sterile water and divided into 4 groups each one contains 10 teeth specimens group A (control group) specimen with no treatment, group B (its specimens treated with 5.25% sodium hypochlorite and 17% EDTA), group C (specimen treated with 17% EDTA and 5.25% NaOCL then radiated with 810 nm diode laser) and group D (specimen treated with 17% EDTA and 5.25% NaOCL then radiated with 980 nm diode laser).

Table 1: 810 nm diode laser.

Powers	Maximum temperature elevation
0.5 watt	3.5 °C
1 watt	6.8 °C
1.5 watt	23 °C

Table 2: 980 nm diode laser.

Powers	Maximum temperature elevation
0.5 watt	2.5 °C
1 watt	5 °C
1.5 watt	19.8 °C

Group A, control group (n=10)

Assuming that the infection rate of teeth specimens with *E. faecalis* are 100% to compare it with bacterial reduction rate of other 3 experimental groups.

Group B (n=10): NaOCL and EDTA

In this group, 2 ml of 17% EDTA for 3 minutes and 3 ml of 5.25% sodium hypochlorite for other 3 minutes respectively were used for canals disinfection, using 30-gauge irrigation needle with lateral opening at the closed end of it then irrigates with 3 ml of 0.9% normal saline. all the canals were dried with sterile paper points than 0.1 ml of sterile water (09% normal saline) was introduced into each canal and sealed coronary with temporary filling and incubated for 24 hours under anaerobic conditions.

Group C (n=10): 810 nm diode laser, NaOCL and EDTA

After elimination of temporary fillings, The tooth specimens in laser group were disinfected by 810 nm diode laser with an endodontic fiber tip (200 μm) with output power of 1 watt at continuous emission mode (CW) after application of 2 ml of 17% EDTA for 3 minutes and 3 ml of 5.25% sodium hypochlorite for other 3 minutes respectively, using 30-gauge irrigation needle with lateral opening at the closed end of it then

irrigates with 3 ml of 0.9% normal saline then the canals were dried using #F3 sterile paper points. Each canal was irradiated with 5 seconds laser exposure four times, followed by 20 seconds interval for each exposure. The laser tip was inserted directly into the root canal 1 millimeter beneath the working length and moved in a helicoid pattern downward and upward. After lasing procedure, each specimen received 0.1 ml of sterile water then sealed coronary with temporary filling restoration and incubated for 24 hours under anaerobic conditions.

Group D (n=10): 980 nm diode laser, NaOCL and EDTA

The specimens irradiated with 980 nm diode laser with an endodontic fiber tip (200 μm) with output power of 1 watt at continuous emission mode (CW) with 5 seconds exposure time four times followed by 20 seconds resting interval for each exposure after treated with 5.25% NaOCL and 17% EDTA. The procedure was same in group B.

Determination of bacterial count

After 24 hours of incubation period of disinfected specimens, all specimens groups brought out and their temporary fillings were removed then received 0.1 ml of sterile normal saline to serve as transport media inside the canals. A #25 K-File was inserted inside each canal with circumferential filing motion for 30 seconds in order to disrupt the bacterial biofilm and collecting the dentin chips. A #F3 sterile paper point was used for each canal to collect the dentin chips with its transporting media. Then each paper point and k-file of each canal was inserted inside Eppendorf tube containing 2 ml of sterile LB broth. The Eppendorf tube was shaken in vortex mixer for 1 minute and incubated for 24 hours under anaerobic conditions.

After incubation, 0.5 ml was taken from each Eppendorf tube (using micropipette) and undergoes tenfold serial dilution then inoculated in a plate containing blood agar followed by incubation for 24 hours. After the incubation period, the number of colony-forming units (CFU) was determined by using the formula:

{No. of CFU X Dilution factor = No. of CFU/ml},

We compared the means of groups (B, C, D) with the mean of group A (Control group) to determine the bacterial colonies reduction in each disinfected experimental group.

Statistical analysis

Data of the study were analyzed using one way analysis of variance (ANOVA) model to compare the mean CFUs/ml among the groups. Then multiple comparisons of mean CFU/ml between groups were made using Dunnett's T3 post hoc test. P value <0.05 was considered statistically significant.

The statistical analysis was performed using SPSS for windows (SPSS INC, Chicago, Il, USA) version 21.0.0.

RESULTS

Evaluation of Bacteriological growth after disinfection

After 24 hours of incubation, group C (17% EDTA, 5.25% NaOCL and 810 nm diode laser) exhibited less bacterial growth on the blood agar media in comparison with other disinfected groups. The number of CFUs showed below in Table 3 which was counted after collecting the bacterial colonies on blood agar plate after tenfold serial dilution.

Descriptive statistics for the CFUs/ml including mean, standard deviation (SD), standard error

A	B	C	D
control group	EDTA + NAOCL	EDTA + NAOCL + 810 nm	EDTA + NA0CL + 980 nm
23200	4000	800	600
20900	3400	500	2000
18000	5700	100	1400
26500	5200	900	800
16300	4800	700	1100
13200	3200	200	1700
28700	2800	400	500
14800	2400	100	900
15100	3600	300	1200
22000	2500	200	1300

Table 4: Descriptive statistics of bacterial count among groups.

Groups	N	Mean	±SD	±SE	Minimum	Maximum
Control	10	19870	5247.232	1659.32	13200	28700
NaOCL+17% EDTA	10	3760	1145.232	362.154	2400	5700
NAOCL+17 % EDTA +810nm Diode laser	10	420	293.636	92.856	100	900
NAOCL+17 % EDTA + 980 nm Diode Laser	10	1150	474.342	150	500	2000

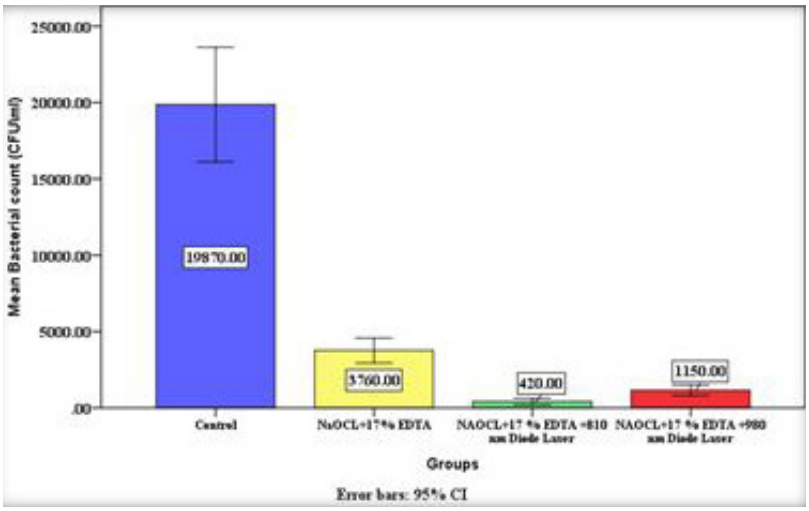

Figure 1: Mean bacterial count.

Table 5: Multiple comparisons of CFU Between groups using Dunnett T3 post hoc test.

Multiple comparisons of Bact. Between groups using Dunnett T3 post hoc test.			
Dunnett T3			
(I) Groups	(J) Groups	Mean Difference (I-J)	p value
Control	Naocl+17% EDTA	16110	0.000 *
	810nm+NAOCL+17 % EDTA	19450	0.000 *
	980nm+NAOCL+17 % EDTA	18720	0.000 *
Naocl+17% EDTA	NAOCL+17 % EDTA+810nm Diode Laser	3340	0.000 *
	NAOCL+17 % EDTA + 980 nm Diode laser	2610	0.000 *
NAOCL+17 % EDTA+810nm Diode Laser	NAOCL+17 % EDTA + 980 nm Diode laser	-730	0.005 *
*=significant at p<0.05.			

(SE), minimum values and maximum values are in Table 4 below.

Figure 1, compares between the mean of control group with those of the treated groups can be made to assess the bacterial reduction in each treated group. Group C has the greatest bacterial reduction as 97.9% of bacteria were killed followed by group D (94.2% bacterial reduction) and then group B (81.6% bacterial reduction) respectively. It's clear that the powerful bactericidal effect against *E. faecalis* biofilm achieved by combining NaOCl and EDTA with diode laser radiation inside infected root canal. Statistical test of CFU among groups using One way Analysis Of Variance (ANOVA) revealed highly significant difference as p=0.00. Multiple comparisons of CFU/ml between groups were made using Dunnett's T3 post hoc test (Table 5 below). All comparisons with control group revealed highly significant differences.

DISCUSSION

Although several chemical agents are available with different properties, as far as cleaning of root canals is concerned, Clearly, none of the presently available irrigating solutions can be regarded as optimal, or even close to that. In clinical practice, use of a combination of solutions in a specific sequence is necessary in order to maximally contribute to the success of root canal treatment.

Due to its ability to penetrate deep into dentinal tubules and forming a biofilm, *E. faecalis* remains viable after mechanical and chemical root canal preparation. However, 20-23% of patients with endodontic failure after one year of treatment were attributed to the presence of *E. faecalis* inside their treated root canals.

The smear layer produced by files and drills during root canal instrumentation is a film of debris attached to dentin surface, composed by excised dentin particles, remnants of vital or necrotic pulp tissue, microorganisms (involving *E. feacalis*) and their byproducts, and retained chemical irrigants. The smear layer interrupts the penetration of root canal irrigants and acts as a barrier between the root filling and the canal wall, which is a potential path of leakage for bacteria contamination between the 2 surfaces. The difficulty of smear layer removal in the apical region could be caused by the inability to deliver agents such as NaOCl and EDTA, due to the smaller dimensions of the apical canal, which obstructs irrigation delivery.

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 micro leakage. 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. Diode laser is recommended for endodontic treatment because its wavelength is within the infrared range and its thin and flexible fibers help to remove the smear layer. In this vitro study, results showed that application of 810 and 980 nm diode lasers with 1 Watt power decreased the *E. faecalis* bacterial colonies in the root canal system compared to the control group. The effect of 810 nm diode laser in combination with NaOCL and EDTA on decreasing *E. faecalis* colony counts was significantly greater than that of 980 nm diode laser with NaOCL and EDTA. The reduction of bacterial count was 97.9% CFU with 810 nm diode laser while the bacterial reduction was 94.2% CFU with 980 nm diode laser under same conditions.

Different studies showed the effects of 810 nm and 980 nm diode lasers on intracanal *E. faecalis*, In 2018, Martins et al. reported the reduction of the bacterial count in deep layers of the infected root canal wall up to 74% by means of the diode laser (810 nm).while 980 nm laser, despite using higher distal output power, decreased the bacterial count by 57%.

The absorption coefficient of diode lasers in water is low (µa =0.04–0.05 cm−1) and as a consequence, they have low absorption in dentin. The greater depth of penetration of diode laser irradiation can be the reason for its superior bactericidal effect (more than 1000 µm into dentinal tubules).

These lasers can interact with pigments (e.g. melanin) of the root canal pathogens directly and exert a great bactericidal effect.They also because thermal photo disruptive action in the unreachable parts of root canal dentin, resulting in an enhanced bactericidal effect there. In 2014, Ashofteh et al performed a study on infected root canals to compare the antibacterial effect of intracanal irrigants and diode lasers. They used an 830 nm diode laser and output power of 1.5 W and a frequency of 20 Hz. They concluded that diode lasers were not as effective as irrigants in disinfecting the root canal but they showed increased disinfection in deep dentin due to deeper penetration.

Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant. Among the current available endodontic irrigating solutions, sodium hypochlorite is known for its ability to digest organic tissues during chemo mechanical debridement of the root canals. The optimal chemical concentration of NaOCl is between 1% and 6%. Studies have shown that a concentration of 5.25% NaOCl can kill *E. faecalis* and *C. albicans* within 15–30 seconds. Ethylenediaminetetraacetic acid (EDTA) is a synthetic amino acid, it is often used as a chelating agent. EDTA demineralizes the inorganic components of dentin by chelating calcium ions, which reduces the micro hardness. The EDTA solution can completely remove the inorganic components from the smear layer and open dentinal tubules within 1 minute. However, a prolonged treatment (>10 minute) may lead to erosion of the intertubular and peritubular dentin. Sodium hypochlorite is still the most effective 'gold standard irrigant'. Unlike with sodium hypochlorite, the extrusion of iodine and chlorhexidine is thought to be more forgiving to the soft tissues as they do not dissolve organic tissue. Chelators in liquid form are not a replacement for antimicrobial irrigants like NaOCl. The antimicrobial properties of chelators are low yet they can be used to remove the smear layer, increasing the penetration of other irrigants such as NaOCl and hence increasing

their antimicrobial effects The inorganic portion of smear layer can be removed by the use of 15–17% concentrations of EDTA and the organic portion can be removed by NaOCl in concentrations exceeding 1%.

In this study, 2 ml of 17% EDTA with 3 ml of 5.25% sodium hypochlorite were used respectively for root canal disinfection. The bacterial reduction was 81.6% CFU. In a similar study, de Souza et al. concluded that diode laser irradiation following chemomechanical irrigation was more effective than NaOCl irrigation alone in root canal disinfection and elimination of *E. faecalis*.

The antibacterial effect of the diode laser on the infected root canal wall. The laser used in this study was diode laser. The results showed that a combination of irrigation with NaOCl and laser irradiation is more effective than conventional endodontic therapy for a reduction in bacterial flora from the root canal system [20-47].

CONCLUSION

In comparison between the effects of diode laser radiation on the *E. faecalis* in the root canal system at 1 watt, the bactericidal effect of 810 nm was greater than 980 nm. The reduction of bacterial count was 70.8% CFU with 810 nm diode laser while the bacterial reduction was 29.1% CFU with 980 nm diode laser under same conditions. However, 5.25% sodium hypochlorite with 17% EDTA has better antibacterial effect on *E. faecalis* biofilm (The reduction of bacterial count was 81.6%) than diode lasers. Results showed greater effect of diode lasers on *E. faecalis* reduction when they applied in combination with other chemical irrigants like NaOCL and EDTA.

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