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Comparison of Antibacterial Effect of Endo activator and Diode Laser on Root Canals Infected with *Entrococcus faecalis*: an in Vitro Study

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Abstract

Objective: The antibacterial effect of Endo Activator (EA), 980-nm diode laser and sodium hypochlorite (NaOCl) as a common root canal irrigant was assessed in root canals infected with *Entrococcus(E.) faecalis*

Materials and Methods: The canals of 61 extracted single rooted maxillary incisors human teeth were prepared using rotary instruments. After docoronation, the roots were incubated. Five specimens were chosen for negative control, and the remaining teeth were incubated with *E. faecalis* suspension for four weeks. Subsequently, the teeth were divided into 4 groups of 14 teeth in each, as follows: group 1: Diode laser, group 2: EA, group 3: 5.25% NaOCl and group 4: sterile saline (positive control). Samples obtained from canals by paper points and *E.faecalis* colony-forming units (CFU) were counted in each root canal. Resulting data were analyzed using Kruskal-Wallis and Man-Whiteny U tests ($P<0.05$).

Results: There was a statistically significance difference ($P<0.001$) between groups. 5.25% NaOCl was more effective in decreasing the intracanal microbial load.

Conclusions: Although 5.25% NaOCl seems to reduce *E.faecalis* more effectively, EA also reduced the bacterial count. Therefore EA could be considered as a complementary disinfection method in root canal treatment (RCT).

Keywords: Disinfection; Laser; *Entrococcus Faecalis*; Root canal

Introduction

The main goal of RCT is the elimination of microorganisms and their by- products from the root canal system and also to avoide the re-entry of the microorganisms in to the root canal[1]. Mechanical techniques are unable to clean thoroughly this complex tubular system by itself Using mechanical methods is incapable of thoroughly cleaning this complex tubular system by itself[2,3].

E. faecalis is a common microorganism responsible for the secondary infection of the root canals[1,4]. It is resistant to the most irrigating solutions and intra canal medicaments such as calcium hydroxide. Studies showed that *E. faecalis* can form biofilm and invade dentinal tubules[5,6].

Several irrigating solutions have been used to reduce microorganisms, necrotic tissues and residual debries[7]. The most commonly used irrigating solution in RCT is NaOCl. NaOCl has become the most widely used irrigating solution in endodontics[8,9].

Various disinfection devices for irrigating solution delivery have been suggested and tested[3,10]. The EA system is a sonically driven irrigant activation system with safe, non-cutting polymer tips which has been designed to vigorously agitate irrigating solutions. It has been shown that EA increase the efficacy of irrigation better than traditional needle irrigation, and removal of smear layer and debries. Different devices for irrigation delivery have been proposed to increase the flow and distribution of irrigating solutions within the

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root canal system[3,10]. The EA system is a sonically driven irrigant activation system designed to produce vigorous intracanal fluid agitation that has been shown to increase the efficacy of irrigation better than traditional needle irrigation, smear layer removal and canal cleanliness[11-13].

Recently new methods such as lasers have been proposed to clean the root canal system thoroughly. Different types of lasers with various wavelengths have been used in endodontics[14-16]. Among various types of lasers, the properties of diode laser such as antibacterial effect have made it more popular. In recent years new methods such as lasers have been introduced in order to effectively clean the root canal system[14-16]. Among different types of lasers, the diode laser is the most desirable type due to the properties such as antibacterial effect[17]. Therefore, the present in vitro study was performed to compare the antibacterial effect of EA and 940-nm diode laser in root canals infected with *(E.) faecalis*.

Materials and Methods

Sample collection and preparation

In this experimental study fully developed maxillary central incisors extracted for periodontal and orthodontic reasons were collected and disinfected by immersion in 5.25% NaOCl for one hour. After taking the peri apical radiographs, teeth with external and internal root resorption, calcification, caries, visible cracks, fractures, more than one canal and previous root canal treatments were excluded. Crowns of all teeth were cut off using a high-speed hand piece and the root lengths were standardized to a 17 mm length. The roots with apical constriction diameter wider than a size 15 K-file (Mani, Japan) were excluded. A total of 61 teeth were selected.

All canals were prepared using the ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) and a motor controller device (Xsmart, Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacture with crown-down technique up to F_4(40 / 0.06). Then 5.25% NaOCl was as irrigant and finally all canals were rinsed with 5 mL of saline. The apical foramina of all canals were sealed using a self-cure glass-ionomer (Fuji, Japan) and the external surfaces of the teeth were covered with two layers of colorless varnish to prevent liquid penetration.

Sterilization

The samples were placed in acryl and were sterilized using an autoclave (20 min, 121°C, 20 psi). To get sure of sterilization, specimens were incubated at 37°C for 24 hours and samples from canals of 5 specimens were obtained using # 35 Hedstromfile (Mani, Japan) and cultured. No bacterial growth was observed. To induce infection, pure *E. faecalis* (ATCC 29212) suspension in Brain Heart Infusion (BHI) broth with a concentration of 1 Mc Farland (3 x 108 bacteria per ml) was injected into canals using insulin syringes. Five specimens received sterile BHI broth and served as negative control group. All specimens were placed in an incubator at 37°C for 4 weeks. During this period, canals were replenished with fresh bacterial suspension every 48 hours.

After incubation, the canals were divided randomly (simple randomization method) into four groups of 14 canals each. They were filled with sterile saline and then each canal was dried by 3 sterile # 30 paper points with intervals of 30 seconds. The paper points were transferred into test tube containing 1 mL sterile saline. To obtain a suspension of bacteria, the test tubes were placed in Vortex Mixer shaking machine for 20 seconds. After preparing 10^{-1}, 10^{-2} and 10^{-3} dilutions, 0.1 mL of the suspensions were cultured in BHI agar and the number of CFU was determined.

Experimental and positive control groups

Subsequently, the samples were submitted as follows: In group 1(n = 14), diode laser (Doctor Smile, Italy) with wavelength of 980 nm, continuous wave mode, output power of 1 Watt which was checked by power meter was used. A fiber optic 200 µm diameter was inserted into the canal at the 1 mm shorter than the root canal length and after activation of the apparatus; the fiber was guided outward with a circumferential motion with a speed of 2 mm / s. This process repeated 4 times. There was a 15 seconds interval between the cycles.

In group 2 (n = 14), 5.25% NaOCl was agitated with the EA (DentsplyMaillefer, Ballaigues, Switzerland) blue tip (35 / 0.04) at 10000 cycles / min for 60 seconds.

In group 3 (n = 14), rinse with 5 ml 5.25% NaOCl for 5 min using 28 gauge needles (DentsplyRinn, Elgin,IL) was performed.

In group 4 (positive control, n = 14), rinse with 5 ml sterile saline for 5 min using 28 gauge needles (DentsplyRinn, Elgin,IL) was performed.

Finally all of the canals were rinsed with 5 ml sterile saline. To determine the amount of reduction of intra canal bacteria, all canals were filled with sterile saline and the same procedures explained in relation to determination of CFU were repeated.After preparation of 10^{-1}, 10^{-2} and 10^{-3} dilutions, the samples were cultured in BHI agar at 37°C. All phases were performed under biological hood.

Statistical analysis

The data were entered and analyzed with SPSS 20 software (SPSS Inc., Chicago, IL, USA). For continues variable were reported (mean ± standard deviation). The statistical analysis was performed with one-way analysis of variance test when distribution of variable was normal, otherwise was applied Kruskal-Wallis and Man-Whiteny U tests with Bonferroni adjustment. In addition linear regression was used for present of size of effect of methods. The level of significance was set 5% for all tests.

Results

There is no significant difference between mean of bacterial growth in groups (Table 1) and No bacterial growth occurred in the negative control group.

Table 1: The mean (SD) of bacteria in groups

Group	Mean(SD)	F	p-value	Statistical Test
Laser irradiation	162.71 (58.92)	2.27	0.09	
	ANOVA test			
EA	156.5 (67.56)			
5.25% NaOCl	193.21 (84.23)			
positive control	215.92 (60.99)			

There was a statistically significance difference ($P < 0.001$) between groups. (Table 2 and figure 1).

Table 2: The mean (SD) of CFU per ml in groups.

Group	Mean(SD)	Disinfection (%)	p-value	Statistical Test
Laser irradiation	18.3×10^4 (12.1×10^4)	88.25	<0.001	Kruskal-Wallis test
EA	4.8×10^4 (10.2×10^4)	96.23		
5.25% NaOCl	0.1×10^4 (0.4×10^4)	99.95		
positive control	123.7×10^4 (51/4 $\times 10^4$)	42.90		

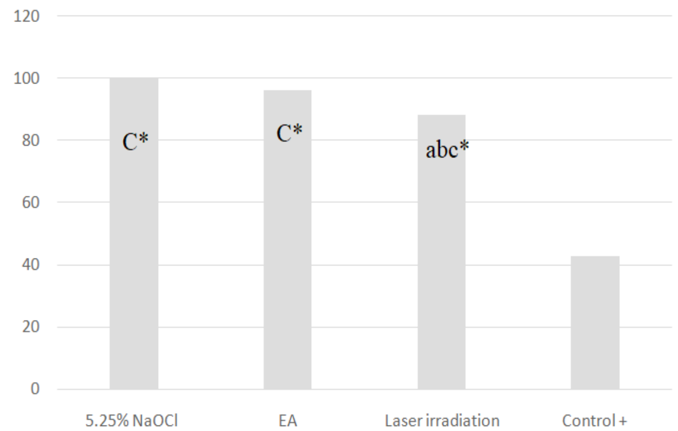

Figure 1: Disinfection in groups with Man-Whiteny U tests with Bonferroni adjustment

5.25% NaOCl was more effective in decreasing the intracanal microbial load.

Discussion

The present study compared the efficacy of a 980-nm diode laser, EA and 5.25% NaOCl in removing *E. faecalis* from the root canal. Presence of bacteria within the root canal and dentinal tubules is considered to be the most important cause of endodontic treatment failure[1,14]. Thus, complete elimination of bacteria and their by-products is critical for successful endodontic treatment.

Several studies have used *E. faecalis* to evaluate the disinfection potential of antibacterial agents or different types of laser[9,14,17] this cocci is highly resistant to many disinfecting agents and also is particularly important in persistent endodontic infections and failed RCTs[1,18]. Thus, in the present study the antibacterial effect of EA, diode laser and NaOCl evaluated on *E. faecalis*. Baumgartner *et al*. showed that 3 weeks of incubation of *E. faecalis* in root canals, lead to a dense infection in dentinal tubules[19]. To get sure of adequate infection, we inoculated the bacteria for 4 weeks.

In our study, the apical foramina were sealed and the external surfaces of the teeth were covered because it more accurately simulates in vivo conditions[20]. Groups were shaped to a ProTaper F_4(apical size 40, taper 6%) to increase volume exchange of irrigants at the working length[21,22].

Recently, in order to the introduction of different laser wavelengths, delivery systems and tip designs, application of laser technology in dentistry has notably increased. Laser therapy is an effective method in endodontics because of different advantages such as reduction of apical microleakage bacterial count and dentine permeability and removal of smear layer. In the recent years, use of laser technology in dentistry has increased, mainly due to the introduction of different laser wavelengths, methods and delivery systems. Laser therapy is known as an efficient method in endodontic treatment due to different advantages such as smear layer removal, decreasing the bacterial count and reducing the apical microleakage[8,15,16]. Studies have shown that different wavelength of lasers, particularly the diode is effective for decreasing the intra canal bacterial count[14,17,23]. Diode lasers are very chosen because of their advantages such as flexible fiber, small size and cost effectiveness. Due to a thermal mechanism, high power diode laser reduced the microorganisms counts in the root canals[24]. Nevertheless, in disinfection of the root canals with laser irradiation, proper parameters and protocols should be used to prevent thermal damage to the surrounding tissues. Diode lasers are highly popular due to their small size, cost effectiveness and flexible fiber. High power diode laser eliminates the microorganisms in the root canals based on a thermal mechanism[24]. 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 tissues. In this study, the laser irradiation protocol was selected based on factory setting and similar previous investigations[23,24].

The present study is in agreement with Baumgartner *et al.*[19] who showed the greatest number of samples with no bacterial growth in group of NaOCl. In this study, group of NaOCl showed the greatest number of specimens with no bacterial growth. This agrees with the results of Baumgartner *et al.*[19], Ashofteh *et al.*[25], Krause *et al.*[26] and Sohrabi *et al.*[27] studies. Giardino also showed that 5.25% NaOCl was the only irrigant that could disrupt the biofilm of *E.faecalis* within 5 minutes[28]; so in group 3 we rinsed canals with 5 ml 5.25% NaOCl for 5 min.

Results of present study are in contrast to the results obtained Mancini *et al.*[12], who found EA is effective in canal cleanliness. This issue may be explained by different evaluation method; they evaluate smear layer removal using electron microscopic, but we used bacterial counts method.

The present study is in agreement with Bago *et al.*[29] who assess the antimicrobial effect of different methods such as diode laser, photo-activated disinfection, conventional and sonic activated irrigation with 2.5% NaOC in root canals infected with *E. faecalis*. The present study is in agreement with Bago *et al.*[29], who evaluate the antimicrobial effect of a diode laser irradiation, photo-activated disinfection, conventional and sonic activated irrigation with 2.5% NaOCl *E. faecalis*. Their results showed that EA was more successful in reducing the root canal infection than the diode laser.

Results of present study are in contrast to the results obtained Neelakantan *et al.*[30], who found diode laser was effective in reducing *E. fecalis* biofilms. This issue may be explained by using higher power (maximum output power of 7 W) in their study.

Conclusion

The results of the present study showed that 5.25% NaOCl had significantly stronger antibacterial effect compared to a 980-nm diode laser and EA; however, the effectiveness of EA in bacterial reduction was acceptable. EA can be considered as an alternative method for root canal disinfection.

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Conflict of interest: 'None declared'

Ethical Approval: 'None declared'

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