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ORIGINAL ARTICLE

Comparative Evaluation of the Efficacy of Irrigation Delivery Systems on *Enterococcus faecalis*

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ABSTRACT

Attaining a sterile root canal system is important since microorganisms can cause persistent inflammation in the periradicular tissues. **Objectives:** This study sought to evaluate the efficacy of two irrigation delivery systems; the EndoVac and semiconductor diode laser irradiation with 5.25% sodium hypochlorite and 2% chlorhexidine. **Methods**: Fifty teeth were disinfected according to the Occupational Safety and Health Administration regulations. The teeth were then instrumented, followed by inoculation with bacterial strains of *Enterococcus faecalis* (*ATCC 29212*). The teeth were randomly assigned to five groups, Group I: Control group; Group II: Teeth irrigated with 5.25% NaOCl + Endovac; Group III: Teeth irrigated with 2% Chlorhexidine + Endovac; Group IV: Teeth irrigated with 5.25% NaOCl + laser irradiation; and Group V: Teeth irrigated with 2% Chlorhexidine laser irradiation. All samples were incubated on Muller–Hilton media plates for a period of 24 h. We determined the colony-forming units and analyzed them statistically using Fisher's exact test. **Results**: Laser irradiation completely disinfected the root canal system. The EndoVac system produced significant disinfection but was comparatively less effective than laser irradiation. **Conclusion:** Laser irradiation had a significantly greater bactericidal effect than the EndoVac system, used in conjunction with sodium hypochlorite and chlorhexidine.

Key words: chlorhexidine, disinfection, *E. faecalis*, root canal, semiconductor diode laser

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INTRODUCTION

Most endodontic treatment failures are caused by shortcomings in the disinfection process and extradition of causative microorganisms from the root canal system. Existing literature indicates that 75% of endodontic retreatment cases are successful, which is a lower rate than that associated with conventional root canal treatments.[1] This can be attributed to ineffective disinfection protocols used during treatment, which leave behind distinct microbiota.

Enterococcus faecalis (*E. faecalis*) is an anaerobic facultative bacterium that grows as a biofilm structure on the walls of the root dentin, and along the depth of dentinal tubules. *E. faecalis* is capable of proliferating both with and without oxygen.[2,3] An extra polysaccharide matrix provides mechanical stability to this microorganism and decreases the action of disinfecting adjuncts used during root canal treatments.[4-,6] *E. faecalis* can survive for up to 10 days within dentinal tubules and devoid of any source of nutrition.[5] This microorganism can also stay viable in obturated root canals until 12 months, even after root canal therapy.[7]

To completely clean the root canal system necessitates use of novel technologies such as lasers, in addition to the comprehensive chemo-mechanical approaches. Laser therapies act as adjuncts to disinfection during the process of pulp space therapy.[8–11] As evaluated in earlier studies, high-power lasers such as the diode laser and ErCr:YSGG are effective at decontaminating radicular dentin.[6,8,12] Energy is transmitted through the laser and into the root canal system through an optical fiber. This affords access to the lateral canals and ramifications, especially in the apical third of the root canal system, which is inaccessible to conventional chemo-mechanical techniques.

The purpose of this study was to evaluate the antibacterial activities of the diode laser and Endovac, used with 2.5% sodium hypochlorite and 2.0% chlorhexidine in root canals infected with *E. faecalis*.

METHODS

Fifty non-carious mandibular premolar teeth were selected for the present study. For the most part, these teeth were previously extracted for the purpose of orthodontic treatment. The anatomy of the extracted teeth was determined with the help of digital radiographs taken at various angulations. The teeth with single canals were included in the study. We excluded teeth with open or resorbed apices, teeth that were grossly decayed, and those with fractured roots. Occupational Safety and Health Administration regulations were followed when we disinfected the extracted teeth.[10] The crowns of the teeth were separated from the root transversally with the help of a high-speed diamond disc. The working length was established with direct digital radiographs. This was followed by serial preparation of the canal to a #30 K file for easier inoculation with bacteria. The biomechanical preparation was done using saline to irrigate. To seal the apex, two coats of nail varnish were smeared onto the apex. Paper points were used to dry the canal, and the teeth were sterilized by gamma irradiation.[11] The teeth were incubated for a period of 24 h after inoculation with *E. faecalis* (ATCC 29212).

Preparation of the inoculation media

A 50 µL suspension of *E. faecalis* strains were incubated in five mL of brain heart infusion broth culture medium at 37 ° C for a period of 24 h. The concentration of the inoculation media was confirmed by degree of turbidity, corresponding to the McFarland scale of 3×10^8 cells/mL bacterial concentration and 550 nm optic densities.

Preparation of samples

The inoculation media was introduced into the root canals of the teeth and incubated at 37 ° C for 21 days. To check the growth of *E. faecalis* at several time periods, a fraction of the inoculation media from all the samples were transferred to 5% sheep blood Trypticase Soy Agar plates, which revealed a 100% positive result.[8]

After incubation, the 50 samples contaminated with *E. faecalis* were randomly divided into five groups according to the disinfection regimen. All the steps were conducted under sterile conditions. Group I (n = 10) served as the Control group. Here, the teeth were inoculated with strains of *E. faecalis* following biomechanical preparation, but no irrigation regimen was followed. Group II: (n = 10) Here, 5.25% sodium hypochlorite was used to irrigate the teeth with the EndoVac irrigation system. Firstly, the teeth were subjected to irrigation from a macrocannula, which was continually moved up and down from the point of apical restriction to just below the canal orifice over a time period of 30 s. The sodium hypochlorite was then left untouched in the canal for 60 s. Three cycles of microirrigation were carried out, at 30 s each. This was followed by the macroirrigation procedure, wherein the microcannula was moved up and down the entire working length. Group III (n = 10) teeth were irrigated with the EndoVac irrigation system in conjunction with 2% chlorhexidine. The procedure was otherwise identical to Group II. Group IV (n = 10) teeth were irrigated with the laser system in conjunction with 5.25% NaOCl. After the irrigant was deposited using a 30-gauge syringe, intracanal irradiation was performed using a high power 908 nm diode laser (Kavo Gentle Ray) with a 200 µm fiberoptic tip, set at a power of 2.5 W. Using an oscillatory technique, the diode fiber (200 µm fiberoptic tip) was introduced 1 mm short of the apex and recessed using helicoidal movements at a speed of approximately 1 mm/s. This process was repeated 4 times with 10 s between each repetition. Finally, in Group V (n = 10), the teeth were irrigated with the laser system in conjunction with 2% chlorhexidine (CHX). The procedure was otherwise identical to Group IV.

After irrigation, paper points were used to collect the samples from the teeth and were placed in brain heart infusion broth in microtubes and incubated for 24 h. The samples in the microtubes were transferred to petri dishes containing Muller–Hilton diffusion media using a nichrome wire loop and incubated for 24 h.

Table 1. Comparison of microbial growth among the different groups.

Growth	Sodium Hypochlorite			Fishers exact test
	Endovac	Laser	Control	p-value
No Growth	5(50.0%)	8(80.0%)	0	
$<10^3$	5(50.0%)	2(20.0%)	0	
10^3–10^8	0	0	0	<0.001*
$>10^8$	0	0	10(100.0%)	
Total	10	10	10	

*p < 0.05 statistically significant p > 0.05 non-significant, NS

RESULTS

The results obtained were statistically analyzed using Fisher's exact test and are displayed in Table 1 and Table 2.

Amongst the 10 samples subjected to treatment with the EndoVac plus sodium hypochlorite, 5 samples showed no growth whereas 5 samples showed growth of $<10^3$. Ten samples were treated with laser irradiation plus sodium hypochlorite; here, 8 samples showed no growth, whereas 2 samples showed growth of $<10^3$. All control group teeth showed growth of $<10^8$. (Table 1)

Table 2. Evaluation of EndoVac and laser irrigation delivery systems in conjunction with CHX, compared to the control group.

Growth	Group – chlorhexidine			Fisher's exact test
	Endovac	Laser	Control	p-value
No Growth	1(10.0%)	7(70.0%)	0	<0.001*
$<10^3$	7(70.0%)	3(30.0%)	0	
10^3–10^8	2(20.0%)	0	0	
$>10^8$	0	0	10(100.0%)	
Total	10	10	10	

*p < 0.05 statistically significant p > 0.05 non-significant, NS

Of the 10 samples treated with the EndoVac plus chlorhexidine, 1 sample showed no growth whereas 5 samples showed growth of $<10^3$ and 2 samples showed growth in the range of 10^3–10^8. Of the 10 samples treated with laser irradiation plus chlorhexidine, 7 samples showed no growth whereas 3 samples showed growth of $<10^3$. The control group showed growth of $<10^8$ in all the samples (Table 2).

DISCUSSION

The presence of microorganisms in infected root canal systems has been acknowledged to be the fundamental factor in the ontogenesis of pulp and periapical infections.[11] Due to biofilm formation on the walls of the root dentin, the extradition of these microorganisms from the root canal system is difficult to achieve using mechanical instrumentation alone.[12,13] Consequently, a chief goal of endodontic treatment is to generate novel strategies for eliminating biofilm, thereby efficiently treating chronic lesions caused by resistant microorganisms.[14]

The fundamental goals of root canal irrigation are to deactivate biofilms and endotoxins, dissolve the smear layer and tissue remnants, and allow the irrigant to flow through the root canal system, helping loosen and flush out the debris. Although the effects of chemical disinfection are determined by the concentration of the irrigant and the duration of action, the irrigation delivery system must generate optimum streaming forces within the entire root canal system.[15]

In the present study, 5.25% sodium hypochlorite solution was used as an irrigant because it exerts potent antimicrobial actions and has powerful oxidative potential. *E. faecalis* (ATCC 29212) was used as a test organism since it is commonly associated with cases of root canal failure and persistent apical periodontitis. *E. faecalis* also has the ability to reside inside root canals without the support of other microorganisms. Under specific conditions, *E. faecalis* has the ability to infect the whole length of tubules within days.[5]

A study done by Manikandan *et al*. concluded that *E. faecalis* forms biofilm at various pH levels (7.3–12.3), and sodium hypochlorite exerts greater antimicrobial effects than chlorhexidine on *E. faecalis* biofilm.[12] The effectiveness and safety of irrigation depends on the means of delivery; hence, an increasing number of novel needle-tip designs and equipment are emerging in an effort to better address irrigation-related challenges. Recently, lasers have shown great promise in the field of endodontics and studies have demonstrated the bactericidal effects of the diode laser for root canal disinfection. Moritz *et al*. reported that an 890 nm diode laser was able to disinfect the root canal walls.[13] However, in the present study, wherein a 980 nm diode laser was used in conjunction with 5.25% sodium hypochlorite, colony-forming units were significantly reduced, compared with the control group.

The results of the present study are in accordance with studies done by Thomas *et al*., Castelo-Baz *et al*., and Mithra *et al*. These authors concluded that the diode laser, in combination with conventional irrigants, significantly eliminated *E. faecalis* in the root canals.[10,14,15] The remarkable bactericidal action of irradiation with diode lasers is attributed to its profound depth of penetration of up to 1,000 μm into dentinal tubules. This penetration is markedly deeper than that achieved by chemical disinfectants, which are limited to 100 μm.[13] Because of the progressively smaller diameters of the deep dentinal tubules, irrigant penetration of the root canal system is restricted. Diode laser irradiation allows the penetration of light into the deep dentinal tubules due to light scattering, local intensity enhancement, attenuation, and thermal photo-disruption. These factors afford the diode laser superior antimicrobial efficacy.[4,6,7,11,13]

Kreisler *et al*. evaluated the bactericidal effects of diode laser irradiation in conjunction with sodium hypochlorite, hydrogen peroxide, and saline. They concluded that diode lasers, used in conjunction with sodium hypochlorite, produced enhanced bactericidal reduction compared with lasers alone.[16] The diode laser spectrum allows for higher water absorption into the root dentin. This enhances the penetration of the diode laser through dentin, allowing the laser light to effectively act upon microorganisms present in the dentinal tubules.

In the present study, the EndoVac irrigation system was also effective in eliminating bacteria from the root canals, but not as effective as laser irradiation. The EndoVac irrigation system pulls the irrigant into the canal, then removes it using negative pressure at the working length; hence, the EndoVac could avoid entrapment of air and making a safe delivery of irrigant along the entire working length.[17] The present study is in accordance with past studies done by Hockett *et al*., Siu and Baumgartner, and Mitchell *et al*. They

concluded that EndoVac could remove bacteria more effectively from root canals than traditional irrigation systems and was a safer and more effective method for cleaning the apical third of the root canal system.[6,18,19] However, the EndoVac irrigation system does not have any inherent antibacterial properties like diode lasers and is an adjunctive mechanical aid for enhancing irrigation.

CONCLUSION

Successful endodontic treatment necessitates efficacious delivery of irrigants into the apical third of the root canal system. In the present study, laser irradiation significantly reduced microbial load in comparison with the EndoVac irrigation system, used in conjunction with sodium hypochlorite and chlorhexidine. Diode laser irradiation improves root canal system disinfection protocols due to its penetrating properties that allow its bactericidal effects to extend beyond 1 mm of dentine. However, further studies in this field will help determine an ideal irrigating protocol for use by endodontists.

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CONFLICT OF INTEREST

The authors declared no conflict of interest related to the study

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