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

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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

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. 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. An extra polysaccharide matrix provides mechanical stability to this microorganism and decreases the action of disinfecting adjuncts used during root canal treatments. E. faecalis can survive for up to 10 days within dentinal tubules and devoid of any source of nutrition. This microorganism can also stay viable in obturated root canals until 12 months, even after root canal therapy.

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. As evaluated in earlier studies, high-power lasers such as the diode laser and ErCr:YSGG are effective at decontaminating radicular dentin. 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.
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. 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. 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 × 10^6 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.

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.

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

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

<table>
<thead>
<tr>
<th>Growth</th>
<th>Sodium Hypochlorite</th>
<th>Laser</th>
<th>Control</th>
<th>Fishers exact test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Growth</td>
<td>&lt;10^3</td>
<td>5(50.0%)</td>
<td>8(80.0%)</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>&lt;10^1</td>
<td>5(50.0%)</td>
<td>2(20.0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10^0–10^6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>&gt;10^8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10(100.0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 statistically significant p > 0.05 non-significant, NS
The present study was 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. 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. 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.

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. 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. 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. 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

**REFERENCES**


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