**INTRODUCTION**

The key to successful root canal treatment depends on removal of microorganisms present in the root canal system.\(^1\) \(^2\) At present techniques of root canal debridement and disinfection involve mechanical preparation in combination with the use of root canal irrigants. Despite the availability of various conventional mechanical root canal cleaning methods and chemical irrigants, root canals are not completely free of microorganisms.\(^3\)

*Enterococcus faecalis* is a facultative anaerobic gram-positive bacterium rarely found in cases of primary endodontic infections, but represent 38% to 70% of the microbiota in cases of failed root canal treated teeth. A high prevalence of *E. faecalis* is frequently observed in filled root canals associated with persistent apical periodontitis.\(^4\) \(^5\) *E. faecalis* organism has been widely used in culture and molecular methods based studies. It serves as a valuable microbiological pathogen in studies as it can form biofilm and colonize the root canal.\(^6\) *E. faecalis* penetrates up to 400 μm into the dentinal tubules and this enables it to escape the action of endodontic treatments and irritants used during chemomechanical preparation. \(^7\) Its ability to form biofilms in root canals is important for its resistance and persistence after intracanal antimicrobial procedures.\(^8\)

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution during chemomechanical preparation. It has unique properties of organic tissue dissolution, saponification and antibacterial action.\(^9\) Studies have reported that NaOCl can only penetrate into the dentinal tubules up to a depth of 130 μm.\(^10\) Root canal irrigant acts mainly through its direct contact with the root dentin. The microorganisms embedded in deeper layers of dentinal tubules cannot be effectively eliminated with irrigants alone.

Diode laser and Photo Activated Disinfection (PAD) can be used as adjuncts to conventional root canal disinfection in reducing the bacterial load.\(^11\) \(^12\) Laser assisted disinfection of root canals have been successfully used in several studies to for root canal disinfection along with conventional chemomechanical preparation.\(^13\) \(^14\) However, the use of lasers there has caused concerns about heat damage to the periapical tissues and hence low energy output settings are used for disinfection.\(^15\) The high cost of laser devices makes them unaffordable for most clinicians and hampers their widespread use.

PAD is a new antimicrobial strategy that involves the use of a nontoxic photosensitizer and a light source.\(^16\) \(^17\) The photosensitizer, methylene blue has shown to kill wide range of oral bacterial species.\(^18\) The photosensitizer dye is introduced into the canal which then attaches to the bacterial membrane of microorganisms. When a light of an appropriate wavelength is irradiated, it activates the photosensitizer and reacts with molecular oxygen of the bacterial membrane to produce highly reactive oxygen species, leading to injury and death to microorganisms.\(^19\) The successful use of photo activated disinfection in in combination with photosensitizers has been reported in the literature.\(^20\) \(^21\) \(^22\) Since endodontic therapy cannot do away with irrigants, in the present study we aimed to compare antimicrobial efficacy of Diode laser and Photo Activated Disinfection (PAD) with Sodium hypochlorite and Saline as irrigant in eradicating *Enterococcus faecalis* contaminated root canals.

**MATERIALS AND METHODS**

Thirty extracted human single mandibular premolars extracted for orthodontic purpose was used for the present study.

**Inclusion criteria:**

Premolars with single root canal

**Exclusion criteria:**

Premolars with more than one root canal or root canal curvature of more than 25°

**Teeth with root canals:**

Teeth with calcified canals.

After access opening, a #10 K-file was introduced into each canal until it appeared at the apical foramen. The working length was established by subtracting 1 mm from this length. The canals were shaped using Pro taper system up to F2 size 20; 6% using EDTA as lubricant between each instruments.

**Sterilization of teeth samples:**

Teeth samples were soaked in 5% hypochlorite solution for 30mins at 65°C and rinsed in distilled water. The Teeth sample was next soaked in 70% ethanol for 10mins and rinsed with distilled water. At this stage, Five teeth was randomly selected and was kept as negative control.

**Inoculum Preparation**

A standard strains of *E. faecalis* (ATCC 29212) was obtained for the study. The bacterium was sub-cultured in nutrient broth at 37°C for 48 hours before inoculating in the root canals.

**Inoculation**

Five teeths was randomly selected and was kept as negative control. The samples were Group 1: PAD and Saline; Group 2: Diode laser and Saline; Group 3: NaOCl and PAD; Group 4: NaOCl and Diode laser; Group 5: Positive control. The samples were incubated in 37°C for 48 h in BH agar and colony forming units were counted.

Statistical analysis: Descriptive analysis for CFUs as Mean & SD. Kruskal Wallis Test & Mann whitney Post hoc Analysis for comparison between groups.

**Results:**

Diode laser with NaOCl and PAD with NaOCl showed similar reduction in the growth of *E. faecalis* (P<0.001).

**Conclusions:**

A combination of NaOCl irrigant activated with Diode or PAD showed similar outcome. PAD can be considered as an alternative to Diode Laser for root canal disinfection.

**ABSTRACT**

To compare the antimicrobial efficacy of Diode laser and Photo Activated Disinfection (PAD) against *E. faecalis*.  

**Methodology:** Thirty extracted premolars were instrumented upto Protaper rotary file size F2 and contaminated with a known species of *E. faecalis*. The samples were Group 1: PAD and Saline; Group 2: Diode laser and Saline; Group 3: NaOCl and PAD; Group 4: NaOCl and Diode laser; Group 5: Positive control. The samples were incubated in 37°C for 48 h in BH agar and colony forming units were counted.  

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STUDY MODEL

Inoculation of teeth samples:

*Enterococcus faecalis* was inoculated into freshly prepared Brain heart infusion broth and was incubated at 37 °C for 24 hrs.20µl of cultured *Enterococcus faecalis* broth was added to the teeth sample on 1st, 3rd, 5th and 7th day respectively using 1ml of sterile insulin syringes without overflooding and placed in upright position and was incubated at 37°C.

Diode laser

A Diode laser with wavelength of 940nm (Photon Dental Diode Laser, Zolar Technology, Canada) was used in the present study.

Photo Activated Disinfection (PAD)

A light-emitting diode (LED) lamp emitting light in the red spectrum with a power peak at 628 nm. (FotoSan;CMS Dental, Denmark) was used in the present study.

Experimental Groups

**Group 1:** Photo activated disinfection and Saline

Five samples were filled with methylene blue dye 15µg/mL. The tip of the lamp was inserted into the canal orifice and irradiated for 30 seconds by LED lamp 630 nm (Fotosan 630, CMS Dental) with an output power of 2 - 4 mw/cm2 according to manufacturer instructions. The specimen was then irrigated with 5 ml saline.

**Group 2:** Diode laser and Saline

Five samples were filled with methylene blue dye 15µg/mL. 5ml of Saline solution was agitated with a #15 K file and left undisturbed in the canal for 2 minutes as a pre-irradiation time. This was followed by irradiation with 1.5 W diode laser. The optical fiber was introduced 1 mm short of the apex and recessed in helicoidal movements at a speed of approximately 2 mm/s for 5 seconds. This was repeated 4 times with a 10 second rest period between irradiation to avoid heat build-up.

**Group 3:** Sodium hypochlorite and photo activated disinfection

Five samples were first irrigated with 5 mL 3% NaOCl for 30 seconds and then treated as the Group 1.

**Group 4:** Sodium hypochlorite and Diode laser

Five samples were first irrigated with 5mL of 3% NaOCl followed by irradiation with 1.5 W as the Group 2.

**Group 5:** Positive control

No experiment was done in this group. The positive control served to determine the total bacterial number and was used to determine the percentage killing by the various experimental treatments.

Sample culturing

The teeth were placed in vials, which contained 2 mL of the nutrient broth. Dilution of the samples was maintained at 10^-3. 6 mL of broth, in duplicate, from all the samples together was collected and seeded on a Petri dish containing BHI agar in order to count the CFUs. It was incubated in 37°C for 48 h in BHI agar. The colony forming units CFUs grown were counted with the aid of a magnifying lens.

Statistical analysis

Statistical Package for Social Sciences [SPSS] was used to perform statistical analyses. The Descriptive analysis included expression of CFUs in terms of Mean & SD. Kruskal Wallis Test followed by Mann whitney Post hoc Test was used to compare mean CFUs between the study groups. The level of significance was set at P<0.05.

RESULTS

The results showed a maximum reduction in microbial load in the Group 4: NaoCl and Diode laser followed by Group 3: NaoCl and PAD. The two experimental Group 3 and Group 4 showed comparable results in reduction of *E. faecalis* microorganisms.(p<0.01).

**Table 1:** Represents the number of colony forming units (CFUs) in each group

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Positive control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>43</td>
<td>44</td>
<td>1</td>
<td>0</td>
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<tr>
<td>2</td>
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<tr>
<td>4</td>
<td>135</td>
<td>60</td>
<td>43</td>
<td>1</td>
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</tr>
<tr>
<td>5</td>
<td>120</td>
<td>74</td>
<td>51</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>79.2</td>
<td>50.0</td>
<td>46.4</td>
<td>0.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Table 2:** Comparison of mean CFUs between groups using Kruskal Wallis Test

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5</td>
<td>30.0</td>
<td>18.4</td>
<td>25</td>
<td>74</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Group 2</td>
<td>5</td>
<td>46.4</td>
<td>3.2</td>
<td>43</td>
<td>51</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group 3</td>
<td>5</td>
<td>0.8</td>
<td>0.8</td>
<td>0</td>
<td>2</td>
<td>0.03*</td>
</tr>
<tr>
<td>Group 4</td>
<td>5</td>
<td>0.4</td>
<td>0.5</td>
<td>0</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>Group 5</td>
<td>5</td>
<td>79.2</td>
<td>46.9</td>
<td>24</td>
<td>135</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* - Statistically Significant

Note: Group 1 - Saline/PAD, Group 2 - Saline/Diode Laser, Group 3 - NaoCl/PAD, Group 4 - NaoCl/Diode Laser, Group 5 - Positive Control

**DISCUSSION**

Many investigators have explored elimination of *E. faecalis* in the root canal for its ability to resist conventional antibiotics.
In a systematic literature review Siddique et al. reported that photodynamic therapy with lasers effectively reduced *E. faecalis* counts in infected root canals compared with traditional endodontic instrumentation/irradiation treatment protocols. (36) Mehrvarzfar et al. suggested that a combination therapy of chemical irrigation and laser irradiation could totally eliminate all root canal pathogens including *E. faecalis*. (37) A study by Gutknecht et al. used diode laser with 980 nm wavelength for disinfection of root canal contaminated by *E. faecalis*, eliminated the microorganism up to 500 μ depth. (38) In a study by Rios et al. showed that Photo Activated Disinfection using a dye and a LED lamp has the potential to be used as an adjunctive antimicrobial procedure in conventional endodontic therapy. (39)

Our study combined chemomechanical irrigation and photodynamic therapy which effectively eliminated *E. faecalis* microorganism further validating previous studies. (40) (41) The results obtained in our study showed that Diode laser and NaOCl and PAD and NaOCl were equally effective for disinfecting the root canals containing *E. faecalis*.(P<0.01) While lasers generate heat that may be harmful and can potentially injure periapical tissues. LED light used in Photo Activated Disinfection serves as a safer alternative because it does not generate significant heat as compared to laser. (42) LED lamps have lower thermal productivity and minimal injuring of tissue. LED lamps are also more cost effective since, the energy consumption is lower. Comparing to laser, they are cheaper and also portable and easy to use. (43) The LED lamp used in the present study provides a considerably higher energy output, emitting 1 J/s. The temperature rise at the root surface was not measured in this study, but it is unlikely that any heat damage. (44)