Original Research Article

Effect of conventional irrigation and photoactivated disinfection on *Enterococcus faecalis* in root canals: An in vitro study

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**Abstract**

**Aims:** A study was done to evaluate the antimicrobial efficacy of sodium hypochlorite (NaOCl) and photoactivated disinfection (PAD) on *Enterococcus faecalis*.

**Settings and Design:** Random sampling, in-vitro study.

**Subjects and Methods:** Access opening and biomechanical preparation were performed on fifty freshly extracted mandibular second premolars. The specimens were sterilized; 15 µm of *E. faecalis* was inoculated into each canal and incubated at 36°C for 24 h. Later, specimens were randomly divided into two groups of fifty each and following procedures was carried out: (i) conventional irrigation with 2.25% NaOCl (ii) PAD using diode laser, and toluidine blue photosensitizer. Samples were collected from each canal using sterile paper points which were deposited in brain heart infusion broth, and microbiological evaluation was carried out.

**Statistical Analysis Used:** Student’s *t*-test was used to find the significant difference in the reduction of colony forming unit (CFU) between the groups.

**Results:** The mean CFUs of the two groups showed statistically significant difference (*P* = 0.001). Improved antibacterial efficacy was seen with PAD group compared to conventional NAOCIL irrigation.

**Conclusions:** NaOCl alone was not effective in eliminating *E. faecalis* completely from the root canals. PAD compared to conventional irrigation showed the best results in removing *E. faecalis* from root canals.

**Keywords:** *Enterococcus Faecalis*; Photoactivated Disinfection; Sodium hypochlorite

**INTRODUCTION**

The successful outcome of root canal treatment is based on the efficient disinfection of the root canal system and prevention of reinfection. *Enterococcus faecalis* has long been implicated species from root canals of teeth with posttreatment lesions.[1] The most common reason for reinfection is related to improper instrumentation and inadequacy of conventional irrigation solutions to cleanse the root canal completely. Of the irrigants used, sodium hypochlorite (NaOCl) is preferred by most clinicians as it exhibits a proteolytic effect as well as being a disinfectant.[2,3] It is a potent antimicrobial agent, killing most bacteria instantly on direct contact. It also effectively dissolves vital and necrotic pulpal remnants and collagen.[4] However, there is evidence that hypochlorite is not effective against all pathogenic bacteria, such as *E. faecalis* which is associated with recalcitrant canals.[5]

To overcome these limitations, a novel disinfecting system which includes the use of photoactivated disinfection (PAD)

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has been tested as an adjunct to conventional root canal therapy using NaOCl as a root canal irrigant.

PAD is an antimicrobial strategy in which laser energy is used to activate a nontoxic photosensitizer, the singlet oxygen released from these photosensitive dyes damages the membrane and DNA of microorganisms.\textsuperscript{[6]} The photosensitizers have a high degree of selectivity for killing microorganisms without affecting host cell viability.\textsuperscript{[7]} Hence, this study was undertaken to compare and evaluate the antibacterial efficacy of NaOCl irrigation alone, and PAD as adjuncts to conventional irrigation with NaOCl on \textit{E. faecalis} in root canals.

**SUBJECTS AND METHODS**

Fifty freshly extracted mandibular second premolars from patients for orthodontic or periodontal purposes were collected for the study. Ethical clearance was obtained by the Institution Ethical Committee.

Conventional access to the root canal system was performed. Patency of each canal was established by placing a size 10 K-file (Mani Inc., Tochigi, Japan) until it was visible in the apical foramen. Working length was established 1 mm short of the apex, and the canals were enlarged sequentially up to a size F2 protaper (Dentsply, Maillefer, Switzerland) as per the manufacturer’s recommendation. EDTA (RC Help) was used as a lubricant, and canals were irrigated with 2.25% NaOCl (VIP Vensons, India) during the preparation.

After root canal preparation, the enlarged apical foramina were sealed with epoxy resin to prevent bacterial leakage, and the specimens were then sterilized in an autoclave at 121°C for 20 min at 20 psi pressure.

Pure culture of \textit{E. faecalis} (ATCC 29212) grown in brain heart infusion (BHI), broth was used to contaminate the root canals. The root canals were inoculated with 15 µm of the turbid suspension of \textit{E. faecalis} ATCC 29212 using a micropipette (Kasablanca, Digital Variable Micropipette, Mumbai, India). The turbidity was verified using the McFarland turbidity scale, and adjusted to 0.5, corresponding to $10^8$ organisms per milliliter. The specimens were incubated at 36.5°C for 24 h. Colony-forming units (CFUs) of \textit{E. faecalis} was counted for one sample in each group to ensure growth in root canals. Autoclavable foam with punch holes was used to hold the prepared specimens. Asepsis was maintained throughout the procedures using standard precautions with two flames in a biosafety cabinet.

The teeth were randomly divided into two groups of 25 each:
- **Group I** - Conventional irrigation with 2.25% NaOCl solution
- **Group II** - PAD as an adjunct to conventional irrigation with 2.25% NaOCl.

**RESULTS**

The microbiological evaluation of \textit{E. faecalis} CFU revealed a significant reduction in CFUs in the two study groups. The results were analyzed by counting the number of CFU of \textit{Enterococcus faecalis} after disinfecting the canals and calculating the mean values for the two groups [Graph 1 and Table 1].
Balakrishna, et al.: Effect of various disinfection methods on E. faecalis

DISCUSSION

Enterococci is usually isolated in root canals undergoing standard endodontic treatment because of low sensitivity to antimicrobial agents or their ability to inactivate antimicrobial agents. Authors have reported that E. faecalis has the capacity to survive under various environmental stresses. It has also been speculated that E. faecalis can enter the canal, survive the antibacterial treatment, and then persist after obturation. Hence, in the present study, the root canals were contaminated with E. faecalis (ATCC 29212) that was obtained by growing the cells in BHI broth.

The antibacterial efficacy of various irrigating solutions has been tested against E. faecalis. NaOCl is considered a gold standard for irrigants and is the most popular irrigating solution. NaOCl is commonly used in concentrations between 0.5% and 6%. However, there is considerable variation in the literature regarding the antibacterial effect of NaOCl. It is reported to kill the target microorganisms in seconds, even at low concentrations, although some reports have shown that considerable longer time for the elimination of the same microorganisms. Furthermore, there is evidence that hypochlorite is not effective against all pathogenic bacteria specifically E. faecalis which is associated with recalcitrant canals.

PAD is a new treatment modality that has been developing rapidly within various medical specialties since the 1960s and has been defined as “the light-induced inactivation of cells, microorganisms, or molecules.” On laser irradiation of an appropriate wavelength, the photosensitizer undergoes a transition from low-energy level “ground state” to a higher-energy “triplet state.” This triplet-state sensitizer can react with biomolecules to produce free radicals and radical ions or with molecular oxygen to produce singlet oxygen. These cytotoxic species can cause oxidation of cellular constituents such as plasma membranes and DNA, resulting in cell death. Another type of damage caused by PAD is the damage caused to the cytoplasmic membrane of the bacteria by cytotoxic species generated by antimicrobial PDT, resulting in inactivation of the membrane transport system, inhibition of plasma membrane enzyme activities, and lipid peroxidation.

In Group I, the mean value of E. faecalis CFUs was 163.72. Conventional irrigation with NaOCl showed the least efficacy in removing E. faecalis colonies compared to the other groups.

In Group II, the mean value of E. faecalis CFU was 14.68, which was highly significant (P = 0.001) compared to Group I, implying that PAD, used as an adjunct to conventional irrigation with NaOCl, was significantly more effective in removing E. faecalis from the root canals.

Table 1: T-test to analyze the difference between the two groups

| Groups        | n  | Mean   | SD    | T     | Degree of freedom | P     |
|---------------|----|--------|-------|-------|-------------------|-------|
| NaOCL         | 25 | 163.72 | 31.921| 22.999| 48                | 0.001*|
| NaOCl + PAD   | 25 | 14.68  | 5.558 |       |                   |       |

PAD: Photoactivated disinfection, SD: Standard deviation
The result of this study was in accordance with a study wherein PAD with Diode laser was found to be more effective at reducing or eliminating bacterial load from the canals compared to NaOCl irrigation alone. Another study demonstrated greater reduction in number of CFUs of *E. faecalis* in PAD group than conventional 2.5% NaOCl syringe irrigation.

**CONCLUSIONS**

Within the limitation of this study, it was found that PAD was more effective than NaOCl in reducing *E. faecalis* counts. NaOCl alone was not effective in eliminating *E. faecalis* completely from the root canals. However, further *in-vivo* studies are required to corroborate the present *in-vitro* study to intra-oral conditions.

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**Conflicts of interest**

There are no conflicts of interest.

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