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ORIGINAL ARTICLE

Photoactivated disinfection (PAD) of dental root canal system – An ex-vivo study



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Photo activated disinfection (PAD);
Photosensitiser (PS);
Tryptone Soya Broth (TSB) agar medium

Abstract *Aim:* To investigate the efficacy of photo activated disinfection (PAD) in reducing colony-forming unit (CFU) counts of *Enterococcus faecalis* (*E. faecalis*) in infected dental root canals. The study compared the efficacy of PAD with conventional endodontic treatment (CET) and also a combination of CET along with PAD.

Material and Methods: 53 maxillary incisors were taken for the study. Teeth were divided into 3 groups, CET (Group I) ($n = 11$), PAD (Group II) ($n = 21$), and a combination of CET and PAD (Group III) which consisted of ($n = 21$) samples, Group II and Group III were further divided into 2 subgroups, Group IIa, IIb and Group IIIa, IIIb. Strains of *E. faecalis* were inoculated in all the root canals. CET group samples were treated by chemo-mechanical preparation (CMP) alone, PAD samples were treated with laser alone at 2 different exposure time (4 min and 2 min). In the combination treatment, samples were treated initially by CET and then by PAD for a time period of 4 min and 2 min. Contents of the root canal were aspirated, diluted and plated in Tryptone Soya Broth (TSB) and plates were incubated for 24 h to observe the bacterial regrowth.

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Results: Showed PAD used along with CMP reduced the bacterial load of *E. faecalis* by 99.5% at 4 min and 98.89% at 2 min.

Conclusion: PAD may be an adjunctive procedure to kill residual bacteria in the dental root canal systems after standard endodontic root canal preparation.

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1. Introduction

Microbial findings of failed endodontic treatment have reported a very limited assortment of microorganisms with predominantly facultative gram positive anaerobes, especially *Enterococcus faecalis* and fungi such as *Candida albicans* (Ercan et al., 2006). It has been suggested that *E. faecalis* virulence may be related to resistance developed to intracanal medicaments and an ability to survive in the root canal as a single organism without the support of other bacteria. Irrigants used with conventional root canal treatment could eliminate those bacteria only partially. In addition bacteria such as *E. faecalis* are able to form intra and extra radicular biofilms, which makes it even harder to control them. Despite the improvement in instrumentation techniques and the use of intracanal medicaments, failure of endodontic treatment is reported in the literature (Cohen's, 2011). Considering this, disinfection of root canal, including the most distant areas of the tubular system is a major challenge in endodontic treatment and is of fundamental importance for the success of endodontic treatment (Schoop et al., 2007).

Contemporary treatment procedures include the use of ultrasonic along with NaOCl and lasers. Lasers also have been commercially available for use in dentistry since 1990 and the use of lasers in the field of endodontics is an innovative approach for meeting these requirements. In general, dental laser provides access to unreachable parts of the tubular network, owing to the fact that they penetrate the dental tissue better than rinsing solutions, consequently they have ancillary antimicrobial effects to aid in the reduction of bacteria in the root canal (Schoop et al., 2007). Low power lasers within the visible region along with dyes or Photosensitisers (PSs) have been used recently for root canal disinfection, which is termed as PAD. PAD is a newer antimicrobial strategy that involves the combination of a non-toxic PS or dyes and a non-harmful visible light source to disinfect the root canal. Low power laser in itself is not particularly lethal to bacteria, but is useful for photochemical activation of oxygen-releasing dyes. Singlet oxygen released from dyes causes membrane and DNA damage to micro-organisms. PAD technique can be undertaken with a range of visible red and near infrared lasers and dyes such as toluidine blue, methylene blue, chlorine p6, etc (Lee et al., 2004).

The aim of the present study was to explore the efficacy of PAD in reducing the CFU of *E. faecalis* and to compare the efficacy of PAD with CET and also a combination of CET along with PAD.

2. Materials and methods

A total of 53 freshly extracted human maxillary incisors with straight canals, extracted for periodontal reasons, were

gathered following an informed consent protocol approved by the commission for medical ethics of the university. The teeth were cleaned using an ultrasonic scaler (P5 Booster; Satelec, Merignac, France) and were stored in (0.5% chloramines in water) at 4 °C until employed in the experiment. The teeth were decoronated using a diamond disk (Mani Inc, Japan) and roots were standardized to a length of approximately 14 mm. Patency of apical foramen was established by inserting a size 15 endodontic k-file (Mani Inc, Japan). File measurement was taken at the point where the size 15 endodontic k-file (Mani Inc, Japan) became visible at apical foramen and 0.5 mm was subtracted to set working length. The instrumentation sequence consisted of endodontic Gates Glidden burs (Dentsply Maillefer, Switzerland) 4, 3 and 2 for a coronal 4 mm preparation, followed by an apical preparation by Master Apical endodontic File (MAF) till size 40 endodontic k-files (Mani Inc, Japan) using the hybrid technique and the root canals were irrigated and cleaned with 5 ml of 2.5% sodium hypochlorite and 5 ml of 17% EDTA solution between each endodontic file and the final flush was done with normal saline (Nirlife Health Care, Nirma Products, India).

2.1. Grouping

Teeth were divided into 3 groups, Group I for CET of root canal ($n = 11$), Group II for PAD of root canal ($n = 21$) and Group III for a combination of CET with PAD ($n = 21$). Group II and Group III were further divided into 2 subgroups, Group IIa, IIb and Group IIIa, IIIb. All samples were irrigated with 17% EDTA for 2 min followed by irrigation with saline (Nirlife Health Care, Nirma Products, India) to remove the smear layer. The apical foramina was sealed with a restorative material (3 M ESPA, FILTEC SUPREME, Germany). All the prepared samples were mounted in small vials and autoclaved (121 °C, for 15 min) (Uniclave C-79, Confident Dental Equipment Ltd, India) to obtain a sterilized system of root canals before inoculation with the microorganism, *E. faecalis*.

2.2. Bacterial growth

E. faecalis (ATCC-29212) was grown in Tryptone Soya Broth (TSB) agar medium by overnight culturing in an incubator at 37 °C to form a stationary growth phase.

2.3. Inoculation of bacteria

Bacterial growth was confirmed using a microscope. All samples were inoculated with 10 μ l of the broth containing a known number of *E. faecalis* (2.5×10^4 /ml) using a (15 μ l) micropipette (Eppendorf, Germany). All the samples were then incubated for 24 h at 37 °C.

2.4. Treatments

2.4.1. CET of root canal (Group I)

CET was performed by MAF till size 60 endodontic k-files (Mani Inc, Japan). The canals were irrigated with 10 ml of 2.5% NaOCl and 10 ml 17% EDTA solution alternatively between each file using a 28-gauge needle and syringe (Hindustan Syringes & Medical Devices Ltd, India). The final flush was done with 0.9% w/v normal saline (Nirlife Health Care, Nirma Products, India). To prevent external contamination of the root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

2.4.2. PAD of root canal (Group II)

Samples were further divided into two subgroups, Group IIa and Group IIb. In both the groups the root canals were dried using sterile paper points (Dentsply Maillefer, Switzerland) to remove any contents left inside the root canals. The canals were filled with 10 µm of PS solution of chlorine p6. The root canals were again dried with sterile paper points (Dentsply Maillefer, Switzerland) after 10 min.

2.4.2.1. Group IIa. In Group IIa disinfection of the root canal was performed with a 600-µm-thin flexible tip, at wavelength of 670 nm fiber coupled diode laser (Denfotex Light Systems Ltd) for 4 min which delivered a total power of 65 mW at the tip. The fiber was initially placed 2 mm short of the apex and moved gradually toward the middle and cervical third of the canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per minute.

2.4.2.2. Group IIb. In Group IIb disinfection of the root canal was performed with a 600-µm-thin flexible tip, at a wavelength of 670 nm fiber coupled diode laser (Denfotex Light Systems Ltd) as above but for a time period of 2 min only.

2.4.3. Combination of CET with PAD (Group III)

CET was performed by MAF till size 60 endodontic k-files (Mani Inc, Japan). The canals were irrigated with 10 ml of 2.5% NaOCl and 10 ml 17% EDTA solution alternatively between each file using a 28-gauge needle and syringe (Hindustan Syringes & Medical Devices Ltd, India). The final flush was done with 0.9% w/v normal saline (Nirlife Health Care, Nirma Products, India). To prevent external contamination of the root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

Samples were again divided into Group IIIa and Group IIIb. Canals were dried using sterile paper points (Dentsply Maillefer, Switzerland) to remove any irrigant left inside the root canals. The canals were filled with 10 µm solution of PS chlorine P6 and the root canals were again dried with sterile paper points (Dentsply Maillefer, Switzerland) after 10 min.

2.4.3.1. Group IIIa. In Group IIIa disinfection of the root canal was performed with a 600-µm-thin flexible tip, at a wavelength of 670 nm fiber coupled diode laser (Denfotex Light Systems Ltd) for 4 min which delivered at total power of 65 mW at the tip. The fiber was initially placed 2 mm short of the apex and moved gradually toward the middle and cervical third of the canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per minute.

2.4.3.2. Group IIIb. In Group IIIb disinfection was performed with a 600-µm-thin flexible tip, at a wavelength of 670 nm fiber coupled diode laser (Denfotex Light Systems Ltd) as above but for a time period of 2 min only.

2.4.4. Control group

One tooth from each group was taken as the positive control where no treatment was done after inoculation of bacteria.

2.5. Bacterial evaluation

Root canals were filled with Phosphate Buffered Saline (PBS) and gently filed in a circumferential way using size 25 endodontic k-files (Mani Inc, Japan) to working length. The contents of root canals were aspirated using a sterile syringe (Hindustan Syringes & Medical Devices Ltd, India) into vials and serially diluted with PBS. 100 microliters of each dilution was plated in culture plates containing TSB agar medium. The plates were incubated at 37 °C for 24 h under anaerobic conditions. CFU were counted after 24 h in each group. The cell death or the percentage of bacterial killing was calculated from the CFU counted in the culture plates after 24 h.

Surviving fraction (%)

$$= \frac{\text{No. of CFU counts in the untreated control/ml} \times 100}{\text{No. of CFU counts in the treated group/ml}}$$

Cell death (%) = 100 – Surviving fraction.

2.6. Statistical analysis

Data were entered in statistical package for social sciences (SPSS) version 18, and analyzed using a one way ANOVA and the Turkey's HSD test with significance set as $p < 0.05$.

3. Results

The surviving fraction and the cell death of each group were calculated after 24 h, the untreated control group (3 specimen) was used as a reference to calculate the surviving fraction and cell death. Cell death denotes exactly the efficiency of treatment or the reduction of bacteria, in terms of percentage which gives a better representation of bacterial killing.

The mean and standard deviation of the bacterial reduction (%) and cell death (%) calculated from the data are (11.25 ± 1.48, 88.80 ± 1.18) for Group I (CET), (17.25 ± 3.61, 82.81 ± 3.62) for Group IIa (PAD for 4 min), (20.80 ± 2.76, 79.20 ± 2.76) for Group IIb (PAD for 2 min), (0.50 ± 0.42, 99.50 ± 0.16) for Group IIIa (CET and PAD for 4 min), (1.11 ± 0.59, 98.89 ± 0.59) for Group IIIb (CET and PAD for 2 min) respectively, after 24 h, while bacteria were found in all cases, but from the results it can be inferred that Group IIIa showed the maximum bacterial reduction (99.5%) to a mean (±SD) number of CFU, followed by Group IIIb (98.89%), Group I (88.8%), Group IIa (82.81%) and Group IIb (79.2%) which was the least [Table 1].

Data were analyzed using ANOVA followed by Tukey's HSD test. On comparison of the surviving fraction and cell death between the groups using a one way ANOVA, shows that ($p = 0.000$) for the surviving fraction and cell death which

Table 1 Surviving fraction (%) and cell death (%) – comparison between groups.

Groups	Surviving fraction (%)		Cell death (%)	
	Mean	± SD	Mean	± SD
Group I	11.25	± 1.48	88.80	± 1.18
Group IIa	17.25	± 3.61	82.81	± 3.62
Group IIb	20.80	± 2.76	79.20	± 2.76
Group IIIa	0.50	± 0.42	99.50	± 0.16
Group IIIb	1.11	± 0.59	98.89	± 0.59

implies a statistically significant difference between the Groups ($p < 0.001$). It also showed that treatment in Group III (CET + PAD) was better than Group I (CET alone) which was better than Group II (PAD alone).

Proceeding with Tukey's HSD test, the difference was statistically significant between the Sub groups for the Surviving fraction and cell death in IIa and IIb ($p = 0.006$) ($p = 0.005$), but the Surviving fraction and cell death for Sub Groups in IIIa and IIIb were not statistically significant ($p = 0.970$) ($p = 0.984$) [Table 2].

Thus PAD used along with CET reduced the bacterial load of *E. faecalis* and PAD can be recommended as an adjunct following cleaning and shaping procedure to ensure thorough disinfection and sterilization of dental root canal systems.

4. Discussion

The success of endodontics is directly influenced by the elimination of microorganisms in infected dental root canals. It is well known that microorganisms colonizing in oral environment can be conducive to pulpal and periapical pathosis. The purpose and ultimate goal of endodontics is to eliminate the bacterial infection in the dental root canal system and allow healing of apical periodontitis. Primary root canal therapy is a highly predictable procedure, however the inability to sufficiently disinfect the dental root canal system may lead to failure of root canal treatments or persistent apical pathosis (Ercan et al., 2006).

Contemporary techniques for root canal disinfection consist of ultrasonics and lasers used as an adjunct along with the conventional CMP. Ultrasonic devices were first introduced in endodontics. Lasers have been available commercially in dentistry since 1990. The various applications of lasers include caries detection (Diagnodent), diagnosis of pulpal blood flow, in the treatment of dental hypersensitivity, pulp capping, pulpotomy, smear layer removal, root canal

sterilization, tooth preparation, enamel etching, gingivectomy, bleaching, periodontal pocket disinfection, calculus removal, and laser photosensitization of the root canal (Kimura and Wilders, 2000). In 1986 Zakariassen and colleagues for the first time demonstrated that lasers could be used in endodontics with a good bactericidal effect. This laser yields a bactericidal effect on root canal surfaces and the deeper dentin layers. All the high power laser systems function by dose dependent heat generation, but, in addition to killing bacteria they have the potential to cause collateral damage such as charring of dentin, ankylosis of root, melting of cementum, root resorption, and periradicular necrosis.

To overcome these problems a new antibacterial strategy that involves the combination of a non toxic PS and a laser light source within the visible region between 400 to 700 nm was introduced which is termed as PAD. (Dickers et al., 2009) demonstrated that after 150 s of PAD irradiation the average temperature rise was 0.16 ± 0.08 °C, the recorded values were lower than 7 °C which was within the safety level for periodontal injury. So use of PAD in root canals could be considered harmless for periodontal tissues also. PAD is a medical treatment that utilizes light to activate a photosensitizing agent PS in the presence of oxygen. The exposure of the PS to light results in the formation of oxygen species, such as singlets or free radicals, causing localized photo damage (Walsh, 1997). Photodynamic antimicrobial chemotherapy (PACT) represents an alternative antibacterial, antifungal and antiviral treatment for drug resistant micro-organisms. It is unlikely that bacteria would develop resistance to the cytotoxic action of singlet oxygen or free radicals. Applications of PAD are growing rapidly in the treatment of oral cancer, bacterial, fungal infections and diagnosis of malignant transformation (Konappa and Goslinski, 2007). The long-term use of chemical antimicrobial agents, however, can be rendered ineffective by resistance developing in the target organisms. PAD is being investigated for treatment of root canal infections. In most of the studies effect of PAD alone has been investigated and the efficacy of treatment is poor as bacterial regrowth has been observed and also further studies are required against more clinically relevant organisms such as *E. faecalis* (Garcez et al., 2007).

The present study explored the efficacy of PAD in the reduction in CFU counts of *E. faecalis* and the objective was to compare its efficacy with CET and also a combination of CET along with PAD. *E. faecalis* (ATCC-29212) was used as the test organism as this gram positive facultative anaerobic bacterium is the most common isolate found in failed cases. (Almyroudi et al., 2002) found it easy to maintain and culture *E. faecalis* under laboratory conditions although this organism makes up a small percentage of the root canal flora. It may be favored by ecological challenges and establish infections difficult to treat and demand for retreatment.

In the CET group, only CMP was done using NaOCl 2.5% and saline till MAF 60 size, to simulate the clinical situation 2.5% NaOCl was used as the irrigant which was a potential antimicrobial agent used in conventional root canal therapy as described by Garcez et al. (2007), which showed that CMP alone reduced the bacterial load by about 90%. NaOCl is an oxidizing and hydrolyzing agent, it has bactericidal and proteolytic actions and dissolves proteins. NaOCl has been used as an irrigant as early as 1920s. Concentrations ranging from 0.5% to 5.2% have been recommended for use in endodontics (Clarkson et al., 1998). In Group I the bacterial

Table 2 Surviving fraction (%) and cell death (%) – comparison within groups.

Groups	Surviving fraction (%)		Cell death (%)	
	Mean	p-Value difference	Mean	p-Value difference
Groups IIa and IIb	-3.5500*	0.006*	3.6100*	0.005*
Groups IIIa and IIIb	-0.6100	0.970	0.5100	0.984

* Denotes significant at 5% level.

reduction of about 88.8% or antibacterial action was solely because of CMP.

In samples treated only with PAD, treatment was done with a 670 nm diode laser (Denfotex Light Systems Ltd) because chlorine based PS has got the best absorption in this wave length. The 600 μm fiber optic gave a power density of 65 mw, the total energy fluence dose was 12.6 J/s which was used for disinfecting canals for a period of 4 min and 2 min in Group IIa and Group IIb, respectively. This was enough to activate the PS as described by Fimple et al. (2008) in which 100 mW for 5 min was considered enough for the disinfection of the microorganisms in the canal. In Group II the PAD alone reduced the bacterial load by about 82.81% for a 4 min exposure and 79.20% for a 2 min exposure to the laser. Laser in itself is not particularly lethal to bacteria, but aids in photoactivation of oxygen-releasing dyes which tag bacteria.

Singlet oxygen released from dyes causes cell membrane and DNA damage to micro-organisms, which was the reason for bacterial reduction in this group (Konappa and Goslinski, 2007). In Group IIa and Group IIb there was significant difference in bacterial reduction because the efficiency of bacterial killing was more with more duration of exposure time to the laser beam.

In a combination of CET with PAD, the CMP reduced the initial bacterial load by about 89%. This was in accordance to studies done by Garcez et al. (2008), which reduced the bacterial load by about 90% when only CET was done. Samples were further treated with PAD for 4 min and 2 min for Group IIIa and Group IIIb respectively which reduced the bacterial load by 99.5% and 98.89% respectively which was similar to studies done by Garcez et al. (2006), where he suggested that PAD used as an adjunct to the CET can lead to reduction of pathogens in a short period of time. The initial reduction in bacterial load in Group III was due to the bactericidal action of the 2.5% NaOCl used in the CMP. The remaining viable bacteria after the CMP, were killed by the PAD.

The fiber was initially placed 2 mm short of the apex and moved gradually toward the middle and cervical 3rd of the root canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per minute as described by Garcez et al. (2007).

In the present study, chlorine based PS chlorine p6 was used as it is anionic in nature and was more effective against gram positive microorganisms like *E. faecalis*. Various PS used in PAD are acridine orange, methylene blue, porphyrin derivative (HPD) (photofrin), 5-amino levulinic acid (ALA), chlorine derivatives such as chlorine p6, chlorine p6 (Konappa and Goslinski, 2007). The photosensitivity of bacteria appears to be related to the charge of the sensitizer. In general neutral or anionic PS binds effectively to and inactivates gram positive bacteria while they bind to some extent to the outer membrane of gram negative bacteria. Relatively a porous layer of peptidoglycan and lipoteichoic acid outside the cytoplasmic membrane of gram positive species allows the PS to diffuse into the sensitive sites (Hamblin and Hassan, 2004). The outer membrane of gram negative bacteria acts as a physical and functional barrier between cells and its environment. Affinity of negatively charged PS for gram negative bacteria may be enhanced by linking the PS to a cationic molecule (Konappa and Goslinski, 2007; Soukos et al., 1998) (e.g. poly-L-lysine-chlorine p6). A PS that is taken up slowly by micro-organisms may cause only cell wall damage after activation with light,

whereas nucleic acid strand breakage, will be apparent on a longer incubation time of PS (Wainwright, 1998).

Results of the present study suggested that a combination of the CET procedure followed by PAD can reduce the bacterial load of *E. faecalis* by about 99.5%. The CMP alone reduced the bacterial load by about 88.9% while PAD alone reduced the bacterial load by about 82.81%. Results were almost similar to a study by Garcez et al. (2007), in which a combination of CMP and PAD reduced the bacterial load (*Pseudomonas aeruginosa* and *Proteus mirabilis*) by about 99%.

There are two mechanisms of action that have been proposed for lethal damage caused to bacteria by PAD, (i) DNA damage (ii) damage to cytoplasmic membrane, allowing cellular contents or inactivation of membrane transport systems and enzymes. There is good evidence that treatment of bacteria with PS and light leads to DNA damage. Breaks in both single-stranded and double-stranded DNA, the disappearance of the plasmid super-coiled fraction have been detected in both gram positive and gram negative species after PAD. There is some evidence that PS that can more easily intercalate into double-stranded DNA can easily cause damage. Thus inactivation of membrane enzymes and receptors is also possible (Wainwright, 1998). Results of the present study suggest that the use of PAD as an adjuvant to the CET leads to a statistically significant further reduction of bacterial load and in particular reduces the amount of bacterial regrowth after 24 h compared to either treatment alone. The initial bacterial load was reduced initially by the CET procedure and the remaining viable bacteria were disinfected by PAD.

Further in vivo studies especially in retreatment cases are required to validate the use of PAD as an adjunct to conventional CMP of the root canal. The effect of PAD and various PS may help in the complete eradication of all bacteria and ensure successful endodontic treatment.

5. Conclusion

Within the limitations of the present study, it can be concluded that PAD used along with CMP reduced the bacterial load of *E. faecalis*. Hence PAD can be recommended as an adjunct following cleaning and shaping procedures to ensure thorough disinfection and sterilization of dental root canal systems.

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