Original Article

Comparative evaluation of photoactivated disinfection and sonic irrigation as an adjunct to conventional irrigation on *Enterococcus faecalis* in root canals: An *in vitro* study

Tushar Kohli, Namrata Mehta, Garima Garima, Alpa Gupta, Shakila Mahesh, Dax Abraham, Arundeep Singh
Department of Conservative Dentistry and Endodontics, Manav Rachna Dental College, Faridabad, Haryana, India

ABSTRACT

Objective: The aim of the *in vitro* study was to compare the antibacterial efficacy of photoactivated disinfection (PAD), sonic irrigation as an adjunct to conventional irrigation against *Enterococcus faecalis in vitro*.

Materials and Methods: A total of 75 extracted teeth were selected and prepared followed by inoculation with strains of *E. faecalis*, and a preirrigation sample was collected using sterile paper points. These teeth were then divided randomly into three groups for irrigation: Group I (Conventional irrigation), Group II (PAD), and Group III (EndoActivator) followed by postirrigation sample collection using the sterile paper points. The samples were swabbed on blood agar plates and incubated followed by the calculation of colony-forming units (CFU's).

Results: The results were statistically analyzed using the SPSS software version 18.0. On comparing the mean values among the groups, the reduction in the number of CFU's after the treatment protocol was highly significant for all groups ($P < 0.001$). With the preirrigation sample, there is a statistically significant difference in the values of Group I and Group II ($P = 0.047$). However, in case of postirrigation samples, there is nonsignificant difference between Group II and Group III.

Conclusion: PAD using 940 nm diode laser and methylene blue and endoactivator were more effective than sodium hypochlorite (NaOCl) in reducing *E. faecalis* counts.

Keywords: Antibacterial efficacy, *Enterococcus faecalis*, photoactivated disinfection, root canal irrigants, sodium hypochlorite

INTRODUCTION

The success of root canal treatment depends on the effective disinfection of the canal system and prevention of reinfection by following proper chemomechanical preparation protocol that consist of removal of infected hard tissue, disinfection by one or more irrigants, and intracanal medicaments, followed by obturation of the canal with an inert material to provide fluid impervious seal.[1,2] However, persistent microorganisms and recontamination of canals because of improper seal are the primary reason for failure.[3,4]

Enterococcus faecalis is one of the most common facultative anaerobe often found in the cases of root canal treatment failures and persistent infections. They form intraradicular and extraradicular biofilms, which are difficult to remove.[5,6]

Thorough mechanical instrumentation, either through rotary or manual technique, along with the combinations of disinfecting solutions and irrigation devices has been recommended to ensure proper disinfection of the canal

Submitted: 27-Jan-2020 **Revised:** 25-Feb-2020 **Accepted:** 14-Feb-2020 **Available Online:** 23-Mar-2021

Address for correspondence: Dr. Tushar Kohli,
Department of Conservative Dentistry and Endodontics, Manav Rachna Dental College, Faridabad, Haryana, India.
E-mail: dr.tusharkohli2010@gmail.com

Access this article online	
Website: www.endodontologyonweb.org	Quick Response Code
DOI: 10.4103/endo.endo_12_20	

How to cite this article: Kohli T, Mehta N, Garima G, Gupta A, Mahesh S, Abraham D, *et al.* Comparative evaluation of photoactivated disinfection and sonic irrigation as an adjunct to conventional irrigation on *Enterococcus faecalis* in root canals: An *in vitro* study. Endodontology 2021;33:15-9.

system.[7,8] Currently, the most frequently used irrigant in endodontics is sodium hypochlorite (NaOCl). It is a potent antibacterial agent killing off most bacteria promptly on the direct contact. It efficiently dissolves vital and necrotic remnants of pulp and collagen.[9,10] Conventionally, irrigating solutions are delivered to the canal through a syringe and needle.[11] However, there is insufficient replacement of the irrigating solution throughout the canal because the maximum streaming velocity is only present in the lumen and around the tip of the needle.[12] Furthermore, NaOCl has high-surface tension that prevents its direct contact of with the dentinal walls of the anatomical intricacies.[13]

To overcome this disadvantage of the traditional method, in the last few years, many mechanical methods have been established to advance the penetration and efficiency of irrigation in most areas of the root canal space. Sonic and ultrasonic devices work on the principle of hydrodynamic phenomenon created in well-prepared canals.[14,15] Active irrigation enables the disturbance of biofilms and makes the cell membrane of bacteria more porous to NaOCl.[16] The EndoActivator (Dentsply Tulsa Dental, Tulsa, OK, USA) is one such sonic device using noncutting tips. The tips made of polymer dynamically stir the solution in the canal. It is highly recommended to use NaOCl and ethylenediaminetetraacetic acid (EDTA) for final decontamination steps.[15,17]

Another method for optimal canal decontamination is the application of low-power lasers along with the dyes or photosensitizers (PSs). This method is termed as photoactivated disinfection (PAD).[18,19] This technique can be done within a range of visible red and near infrared lasers using various dyes such as toluidine blue, methylene blue (MB), and chlorine p6.[18] This technique consists of two components: A nontoxic photosensitizer and a laser. The photosensitizer first binds to the bacterial membrane and enters the cytoplasm of the target cells. It is excited by a laser light of specific wavelength producing singlet oxygen species and free radicals which are cytotoxic to the DNA and cell membrane of the target cells.[20]

Recent approaches include using high-power diode lasers along with photo-activated disinfection based on the ability to reach peripheral areas that are difficult with the traditional techniques.[21,22] High-power lasers efficiently kill bacteria based on dose-dependent heat generation. Many studies show the antimicrobial effectiveness of high-power lasers against varied microorganisms.[23]

The aim of our *in vitro* study is to compare the antibacterial action of a 940 nm high-power diode laser used with a photosensitizer dye, conventionally and sonically activated irrigation during the root canal treatment.

MATERIALS AND METHODS

Sample preparation

Seventy-five human single rooted, noncarious teeth, extracted for orthodontic and periodontal purposes were collected. Teeth with curved, dilacerated and fractured roots were excluded. Teeth were stored in 5.2% NaOCl for 30 min to remove organic residues and left in saline solution until the procedure began. Each tooth was decoronated to a standard 15 mm root segment length. Patency of apical foramina was established using hand files, and working length was determined radiographically. The canals were enlarged sequentially up to a size X3 ProTaper as per the manufacturer's recommendations, and the canals were subjected to copious irrigation with 20 ml of 3% NaOCl solution and two milliliters of 17% EDTA alternately for three minutes using 30G side vented needle. Finally, canal was washed with two milliliters of saline to remove any residual irrigant. After the preparation, the enlarged apical foramina were sealed externally and sterilized.

Inoculation of *Enterococcus faecalis* and pre irrigation sample collection

Standard strains of *E. faecalis* (ATCC29212) were subcultured in trypticase soya broth (TSB) and incubated at 37°C for 24 hours. Pure culture of *E. faecalis* (ATCC29212) grown in TSB was used to contaminate the root canals. Each root canal was inoculated with 15 µl of the turbid suspension of *E. faecalis* using sterile micropipette, while ensuring complete filling of the canal. A sterile paper point was used to obtain the sample from the canal which was deposited in a sterile Eppendorf tube containing 200 µl of TSB. Specimens were cultured on blood agar by using swab method and were incubated at 37°C for 24 hours. Colony-forming units (CFUs) of *E. faecalis* was counted for one sample in each group to ensure growth in root canals.

Irrigation of prepared teeth

The current study aims to establish the comparative analysis between experimental groups; hence, no control group is present. Groupings: The teeth were randomly divided into three groups of 25 each:

- Group I – Conventional irrigation with 3% NaOCl solution: The canals were subjected to copious irrigation with 20 ml of 3% NaOCl solution for 3 min followed by two milliliters of 17% EDTA (Dent Wash; Prime Dental, Chicago) for three minutes using 30G side vented needle kept two millimeters short of the working length
- Group II – PAD as an adjunct to conventional irrigation with 3% NaOCl: A MB dye was used. 25 µg/ml of the dye was

injected into the canals of each sample after irrigation with 3% NaOCl. The irradiation source was a diode laser (biolase) with an output power of 1.5W and a wavelength of 940 nm. A 200 μm diameter optical fiber was used. The laser hand piece was held at an angle of 10° between the fiber and root canal wall. Laser irradiation was performed three times for 5 s each with an interval of 10 s between irradiations on continuous mode delivered into the canal up to 1 mm short of the working length while moving coronally without any water spray or air cooling

- Group III – Sonic agitation as an adjunct to conventional irrigation with 3% NaOCl:

Specimens were irrigated with 20 ml of 3% NaOCl solution at the room temperature with a 30G needle syringe 2 mm short of the working length. NaOCl was left in the root canal and immediately activated sub sonically for 1 min and then EA medium tips (25/0.04) was inserted into the root canals, 2 mm short of the working length, constantly moved up and down in the canal and rinsed with normal saline. Following which the canal was activated with 2 ml of 17% EDTA for 1 min, followed by rinsing with sterile saline.

Postirrigation sample collection

Postirrigation sample was collected using sterile paper points that were deposited in a sterile Eppendorf tube containing 200 μl of TSB. Specimens were cultured on blood agar by using swab method and were incubated at 37°C for 24 h. CFUs of *E. faecalis* was counted for one sample in each group to ensure the growth in root canals.

RESULTS

The results of the present study were subjected to the statistical analysis to interpret the significant differences among various treatment groups. One-way ANOVA and *post hoc* tests were used for the statistical analysis. $P < 0.05$ was considered as statistically significant at 95% confidence level. The statistical software SPSS 18.0 (IBM Corp., Armonk, NY, USA) was used in the analysis.

On comparing the mean values among the groups, the reduction in the number of CFUs after the treatment protocol was highly significant for all groups ($P < 0.001$). Group II (PAD) and Group III (Endoactivator) showed significant mean difference than Group I (Conventional Irrigation) with over 99% disinfection ($P < 0.001$) [Table 1].

With the preirrigation sample, there is a statistically significant difference in the values of Group I and Group II ($P = 0.047$). However, in case of postirrigation samples, there is nonsignificant difference between Group II and Group III Other pairs have highly significant differences. Similarly, in percentage reduction, there is nonsignificant difference between Group II and Group III. Other pairs have highly significant differences.

DISCUSSION

The main goal of endodontic treatment is the effective control of bacterial infection within the root canal system by the elimination of pathogenic microflora, toxins, and tissue debris. The persistence of microbial infection in the root canal and the periradicular area is one of the major reasons for endodontic treatment failure.[24,25]

E. faecalis is a facultative anaerobic bacterium that is one of the most common bacteria in persistent endodontic infection.[26,27] This species is able to survive for long periods without nutrients. It invades dentinal tubules, which provide this bacterium protection against the usual irrigating agents. They are resistant to common intracanal medication when present in the form of biofilms.[28] Biofilms are microbial communities that grow in aggregates and represent the predominant growth form for bacteria in the nature.[29] Since *E. faecalis* is the predominant root canal bacteria, the current study specifically focused on the potential of eradiacation of *E. faecalis* by the experimental groups.

Inability to completely eradicate biofilm structures in proximity to host-immune cells will result in persistent infection and subsequent reestablishment of infection. Epidemiological studies have reported that 30%–50% of root canal treatments fail from residual infection.[29] This has led to the quest of novel disinfection procedures that can be an adjunct to standard endodontic antimicrobial procedure, increasing the effectiveness of orthograde endodontic treatment and retreatment procedures.[20]

NaOCl has a proteolytic effect by which necrotic tissues and debris are dissolved. Higher concentration increases the ability to dissolve necrotic and vital pulp tissue, but at the same time leads to higher risk of damage of other tissues. However, 0.5% to full strength NaOCl, if used in adequate amounts and exchanged regularly, has the capability to destroy *E. faecalis* in the root canal.[30] Concentrations ranging from 0.5%– 5.25% are widely used. Although less concentrated solutions have shown antimicrobial effectiveness, higher concentrations of NaOCl present faster and greater bactericidal effect. The "gold standard" irrigant in terms of immediate antimicrobial efficacy, with

statistically significant differences, remains the NaOCl, but without obtaining unanimity on the ideal concentration to be used, which ranges between 0.5% and 6%.[31] In the present study, 3% NaOCl and 17% EDTA alternatively were used which showed over 98% reduction in CFUs/ml. Similar findings were observed by Karale *et al*. in their study which reported that 3% NaOCl was more effective than 2% Chlorhexidine and high frequency alternating current in eradicating *E. faecalis*.[32]

Laser is a device that is capable of mobilizing immense heat and power when focused at close range. Stern and Sognnaes (1964) and Goldman *et al*. (1964*)* were the first to investigate the potential use of ruby lasers in dentistry. One of the most commonly used lasers in dentistry is a diode laser. The available wavelengths for the use of diode laser in dentistry range from about 800–980 nm.[33]

In the present study, the 940 nm diode laser along with MB was used. MB is a well-established photosensitizer and has been used in PAD for targeting endodontic bacteria. The hydrophilicity of MB, along with its low-molecular weight and positive charge, allows passage across the porin-protein channels in the outer membrane of Gram-negative bacteria. MB predominantly interacts with the anionic macromolecule lipopolysaccharide, resulting in the generation of MB dimers, which participate in the photosensitization process.[20]

This technique appeared promising and files had the potential to prepare as well as debride root canals mechanically. According to Sluis *et al*., it proved to be difficult to control the cutting of dentine during ultrasonic preparation, which resulted in irregularly shaped root canals and also apical perforations. Passive ultrasonic irrigation utilizes an ultrasonically activated file or smooth wire within the root canal space following the completion of canal preparation.[34]

According to Caron, the EndoActivator system has been reported to provide deeper penetration of an irrigant to all areas of the endodontic space, and to effectively clean debris from lateral canals, remove the smear layer and dislodge clumps of simulated biofilm.[34,35] This was also confirmed in the present study where significantly greater efficiency of the EndoActivator against intracanal *E. faecalis* biofilm compared to the NaOCl irrigation alone was found. Similar bacterial load reduction was reported by Pasqualini *et al*.[36]

The reduction in the number of CFUs after the treatment protocol was highly significant for all groups ($P < 0.001$).Group II (PAD) and Group III (Endoactivator) showed statistically significant mean difference than Group I (Conventional Irrigation) with over 99% disinfection ($P < 0.001$). Similar findings were supported by Balakrishna *et al*. in their respective study which reported that PAD was more effective than NaOCl in reducing *E. faecalis* count.[37] Bago *et al*. who concluded that the EndoActivator and PAD succeeded in reducing root canal infection and had the capacity to eradicate *E. faecalis.*[24] Activation of irrigants through sonic, ultrasonic, internal heating, or laser devices has shown great improvement in the cleaning and disinfection of the root canal system and should be considered an important fundamental step in nonsurgical endodontic therapy.[38]

In this study, the PAD and the EndoActivator were superior to single NaOCl irrigation in eliminating intracanal *E. faecalis*. However, to determine the most effective endodontic disinfection protocol, the efficacy of the techniques should be further determined on multispecies biofilm. Finally, it is necessary to evaluate their real contribution to conventional chemomechanical preparation in *in vivo* studies. One important consideration for choosing CFU method is that only live cells, capable of forming a colony, will be counted. Although it a time-consuming method, but it is cost effective.

CONCLUSION

Within the limitations of the present study, it was found that PAD using 940 nm diode laser and MB and Endoactivator were 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.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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Table 1: Comparison of mean values between three groups

		n	Mean	Std. Deviation	Minimum	Maximum	*F*	*p*
PRE-OP	CONVENTIONAL IRRIGATION	25	2.20E+08	1.17E+07	2.07E+08	2.45E+08	3.12	0.05
	PHOTOACTIVATED DISINFECTION	25	2.15E+08	7.34E+06	2.07E+08	2.35E+08		
	ENDOACTIVATOR	25	2.14E+08	5.07E+06	2.07E+08	2.25E+08		
	Total	75	2.16E+08	8.72E+06	2.07E+08	2.45E+08		
POST-OP	CONVENTIONAL IRRIGATION	25	6.64E+06	1.61E+06	2.50E+06	9.00E+06	59.93	<0.001
	PHOTOACTIVATED DISINFECTION	25	3.32E+06	9.00E+05	2.00E+06	4.50E+06		
	ENDOACTIVATOR	25	3.50E+06	9.79E+05	2.00E+06	5.00E+06		
	Total	75	4.49E+06	1.94E+06	2.00E+06	9.00E+06		
Percentage reduction	CONVENTIONAL IRRIGATION	25	96.9785	0.71349	96.05	98.82	58.83	<0.001
	PHOTOACTIVATED DISINFECTION	25	98.4592	0.41067	97.85	99.07		
	ENDOACTIVATOR	25	98.3659	0.44603	97.62	99.07		
	Total	75	97.9345	0.8654	96.05	99.07		

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