

## Original Research Article

# Additive effect of photoactivated disinfection on the antibacterial activity of QMix 2in1 against 6-week *Enterococcus faecalis* biofilms: An *in vitro* study

Deepa Vaid, Nimisha Shah, Dwij Kothari<sup>1</sup>, Priyanka Bilgi

Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College, Sumandeep Vidyapeeth, Vadodara, Gujarat,

<sup>1</sup>Department of Prosthodontics, Darshan Dental College, Udaipur, Rajasthan, India

## Abstract

**Background:** Evaluation of the additive effect of photodynamic therapy (PDT) on the antibacterial activity of 2.5% sodium hypochlorite (NaOCl) and QMix against 6-week *Enterococcus faecalis* biofilms contaminated root canals.

**Aims:** To establish the most suitable irrigant for eradication of 6-week *E. faecalis* biofilms.

**Settings and Design:** *In vitro* study.

**Materials and Methods:** A 6-week *E. faecalis* (ATCC 29212) biofilm was formed in 190 extracted teeth that were subsequently subjected to irrigation protocols as follows. Group A<sub>1</sub>: normal saline, Group A<sub>2</sub>: 2.5% NaOCl, Group A<sub>3</sub>: QMix, Group B<sub>1</sub>: normal saline and photoactivated disinfection (PAD), Group B<sub>2</sub>: 2.5% NaOCl and PAD, Group B<sub>3</sub>: QMix and PAD, Group C: no irrigation. For PAD, irradiation was done three times for 5 s each with 10 s interval on continuous mode with a 980 nm diode laser. Samples from the root canals were collected and plated onto brain heart infusion agar plates to determine the colony-forming unit/ml.

**Statistical Analysis Used:** One-way ANOVA, *post hoc* Tukey's honest significant difference test.

**Results:** Maximum percentage of disinfection (99%) was seen in Group B<sub>2</sub> (NaOCl with PDT), which was similar to Groups A<sub>2</sub> (97.6%) and B<sub>3</sub> (98.8%) ( $P < 0.0001$ ).

**Conclusions:** NaOCl with PDT gave maximum disinfection.

**Keywords:** Biofilms; *Enterococcus faecalis*; photoactivated disinfection; QMix 2in1; sodium hypochlorite

## INTRODUCTION

The microbiota associated with the secondary root canal infections markedly differs from that of untreated teeth. *Enterococcus faecalis* is the most common microorganism isolated from root-filled teeth. It plays a major role in the etiology of persistent periradicular lesions after root canal treatment due to its several virulence factors and ability to

form calcified biofilms at 6-weeks. Thus, its eradication is of utmost importance for successful root canal treatment of reinfected cases.<sup>[1]</sup>

Several powerful irrigating solutions such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX), along with chelating agents and surfactants have been employed against *E. faecalis*.<sup>[2]</sup>

QMix 2in1 (Dentsply, Tulsa, USA) is a new irrigating solution that contains 2% CHX, ethylenediaminetetraacetic acid (EDTA), and a surfactant. It is recommended as a one-step final rinse for 60–90 s after irrigation with NaOCl.<sup>[3]</sup>

### Address for correspondence:

Dr. Deepa Vaid, Department of Conservative Dentistry and Endodontics, K. M. Shah Dental College, Sumandeep Vidyapeeth, Piparia, Vadodara, Gujarat, India.  
E-mail: dips167@yahoo.com

Date of submission : 22.06.2015

Review completed : 04.08.2016

Date of acceptance : 21.08.2016

### Access this article online

#### Quick Response Code:



Website:  
[www.jcd.org.in](http://www.jcd.org.in)

DOI:  
10.4103/0972-0707.209072

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

**For reprints contact:** [reprints@medknow.com](mailto:reprints@medknow.com)

**How to cite this article:** Vaid D, Shah N, Kothari D, Bilgi P. Additive effect of photoactivated disinfection on the antibacterial activity of QMix 2in1 against 6-week *Enterococcus faecalis* biofilms: An *in vitro* study. J Conserv Dent 2017;20:41-5.

In spite of the potent antimicrobial activity of individual irrigants and their combinations, no irrigation protocol can render the root canals 100% bacteria-free. Hence, newer methods need to be employed as an adjunct with conventional irrigation protocol for the additional disinfection of root canals.

Photoactivated disinfection (PAD) or photodynamic therapy (PDT) is an antimicrobial strategy consisting 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.<sup>[4]</sup>

With new irrigant combinations and strategies being introduced for root canal disinfection, it was necessary to check the efficacy of PAD as an adjunct to these new irrigants in eradicating *E. faecalis* biofilms. In addition, limited literature is available on the antimicrobial properties of various disinfecting solutions on 6-week *E. faecalis* biofilms. Thus, the aim of this study was to compare the additive effect of PDT on the antibacterial activity of 2.5% NaOCl, and QMix used as a final rinse against 6 weeks *E. faecalis* biofilms contaminating root canals.

## MATERIALS AND METHODS

In the present study, the sample size was determined to be 180 with 99% confidence interval, 90% power, 4.0 standard deviation (SD), and 1.67 differences between two groups. To this, ten samples were added to include the negative control group.

After obtaining ethical clearance, 190 extracted human single-rooted anteriors were collected and kept in NaOCl solution (Prime Dental Products Pvt. Ltd., Kalher, Thane, India) for 2 h to remove organic debris followed by storage in 10% formalin (Narsipur Chemicals Pvt. Ltd., Navi Mumbai, India) for 7 days. After disinfection, the outer surfaces were cleaned with an ultrasonic scaler.

Access preparation was made, and apical patency confirmed with #15 K-file (K-files, Mani Inc., Utsunomiya, Japan). Working length was established 1 mm short of the apical foramen. Coronal flaring was done using sequential Gates Glidden drills (K-Files, Mani Inc., Utsunomiya, Japan). Initial apical file (IAF) was selected as the first snugly fitting file at the apex, and apical preparation was done to three sizes larger than the IAF. Root canals were prepared using rotary instruments (ProTaper, Maillefer-Dentsply, Baillagues, Switzerland) by crown-down technique to an apical size corresponding to the last file used at the apex and were irrigated with 2 ml of 2.5% NaOCl between each instrumentation.

After preparation, the teeth were immersed in an ultrasonic bath of EDTA (Safe Plus, Neelkanth Health Care Pvt. Ltd., Jodhpur, India) for 10 min to remove smear layer, followed by NaOCl bath for 5 min, and normal saline ultrasonic bath for 10 min. Then, the apical foramina were sealed using light cure restorative glass ionomer cement (Fusion i-Seal, Prevest DenPro Ltd. Jammu, India), and the roots were coated with two layers of nail varnish.

Each sample was transferred to a sterile glass test tube containing sterile brain heart infusion (BHI) broth (EOS Laboratories, Thane, Mumbai, India), and all samples were autoclaved under 15 psi at 121°C for 40 min. Samples were incubated in their sealed tubes for 48 h at 37°C. Daily inspection was carried out to reveal any signs of turbidity, and the samples showing turbidity were excluded from the study. Now, all infection-free samples were taken for further evaluation.

An overnight pure culture of *E. faecalis* (ATCC 29212, KWIK-STIK Microbiologics, France) in BHI broth was prepared for inoculation. The bacterial suspension was adjusted to match the turbidity of a McFarland 0.5 scale. A 0.01 ml aliquot of the suspension was used to inoculate each canal with a sterile micropipette. The samples were incubated for 6 weeks at 37°C in aerobic conditions for biofilm formation. The inoculums inside the canal were replaced with 0.01 ml of fresh bacterial suspension every 3 days in an ultraviolet chamber within 6 inches of a gas burner. Random sampling with Gram stain kit (Biolab Diagnostics India Pvt. Ltd., Maharashtra, India) was carried out every 7 days to confirm *E. faecalis* cultures.

After 6 week biofilm formation, the samples were randomly divided into the following groups, two main groups ( $n = 90$ ) with three subgroups each ( $n = 30$ ) and one negative control group ( $n = 10$ ) and were subjected to irrigation protocol as follows:

- Group A (irrigation alone):
  - o Group A<sub>1</sub>: Irrigation with 15 ml of 10% saline for 3 min
  - o Group A<sub>2</sub> (positive control): Irrigation with 15 ml of 2.5% NaOCl for 3 min
  - o Group A<sub>3</sub>: Irrigation with 5 ml of 2.5% NaOCl solution, followed by 5 ml normal saline, and finally, 5 ml QMix 2in1 (Dentsply Tulsa Dental Specialities, USA) for a total of 3 min as per the manufacturer's instructions.
- Group B (irrigation with PDT):
  - o Group B<sub>1</sub>: 15 ml normal saline for 3 min and then irradiated with PAD
  - o Group B<sub>2</sub>: Irrigation with 15 ml of 2.5% NaOCl solution for 3 min and irradiated with PAD
  - o Group B<sub>3</sub>: Irrigation with 5 ml of 2.5% NaOCl solution, followed by 5 ml of normal saline, and then, 5 ml QMix 2in1 for 3 min as per the

manufacturer's instructions and irradiation with PAD.

- Group C (negative control): No irrigation.

For PAD, a methylene blue dye (SDFCL Industries, Mumbai, India) was prepared by dissolution in BHI broth and was filter-sterilized immediately before use. The final concentration used was 25 µg/ml. The dye was then injected into the canals of each sample. The irradiation source was a diode laser (Sunny Optoelectronic Technology Co., Ltd., Shanghai, China, Model Number: MDL50) with an output power of 1.5W and a wavelength of 980 nm. A 200 µm diameter optical fiber was used. The laser handpiece 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. The laser irradiation was delivered into the canal up to 1 mm short of the working length while moving coronally without any water spray or air cooling.

Now, for microbiological evaluation, the canals of all the samples were dried with sterile paper points and refilled with normal saline. Using a sterile #30 H- file (H-files, Mani Inc., Utsunomiya, Japan), circumferential filing was performed for 20 s to collect dentin chips. Samples from inside the canal were collected using two sterile paper points. The sampling paper points and the sampling H-file were placed in a test tube containing 5 ml of sterile saline and vortexed for 20 s. Fifty microliters of the vortexed saline were introduced to culture plates (EOS Laboratories, Thane, Mumbai, India) and incubated at 37° C for 48 h. After 48 h, colony-forming unit [CFU]/ml was calculated for each sample by a single-blinded investigator. All data were collected, tabulated, and statistical analysis was done using one-way ANOVA and *post hoc* and Tukey's honest significant difference tests.

## RESULTS

The mean and standard deviation of CFU/ml of each group was calculated and subjected to statistical analysis [Tables 1 and 2]. The results showed Groups A<sub>2</sub>, B<sub>2</sub>, and B<sub>3</sub> to perform significantly better than other groups in reducing the total bacterial count with over 97% disinfection ( $P < 0.001$ ). Group A<sub>1</sub> performed the worst of all six experimental groups with the least percentage of disinfection of 50% while B<sub>2</sub> performed the best of all with the maximum percentage of disinfection of 99% [Figure 1].

## DISCUSSION

In the present study, *E. faecalis* was selected as it is known to form calcified monoculture biofilms at 3 weeks and can be easily cultivated *in vitro*. In our study, the samples were inoculated for 6 weeks as *E. faecalis* biofilm shows signs

of mineralization at 6 weeks making it further difficult to eradicate.<sup>[5]</sup>

For PAD, a variety of diode lasers with a wide range of wavelengths have been used. Limited literature is available on the effect of diode laser of 980 nm on root canal disinfection. Hence, in the present study, a high-power diode laser of 980 nm with 25 µg/ml methylene blue dye was utilized.<sup>[6,7]</sup>

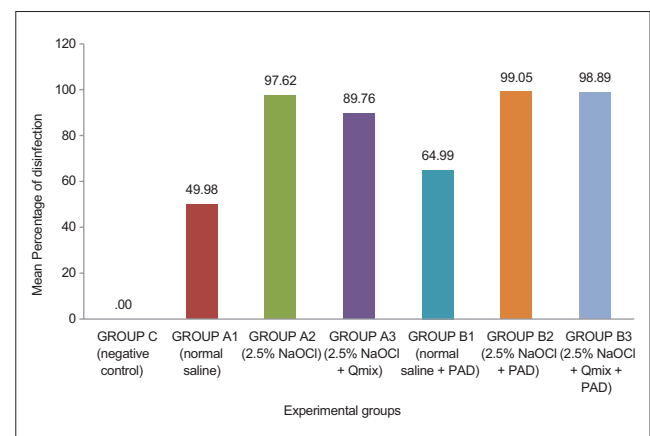
In the present study, the total volume of irrigants and the time of irrigation were kept constant for standardization. A total volume of 15 ml of irrigants was used for the disinfection of root canal of each sample in all experimental groups while irrigating for a total of 3 min.

Our results demonstrated Group B<sub>1</sub>: normal saline with PAD to significantly reduce bacterial counts when compared to saline used alone. This is in accordance to the study by Mehrvarzfar *et al.*, Souza *et al.*, and Soukos *et al.* and can be attributed to the cytotoxic effect of the photosensitizer dye or the cavitation effects and heat generated by high-power diode laser in methylene blue aqueous medium. Thus, PAD alone can be considered to have some antimicrobial action, but it is significantly less than that of NaOCl or the combination of antimicrobials with PAD.<sup>[6,8,9]</sup>

**Table 1: Mean, standard deviation, minimum, and maximum values and P value of colony-forming unit/mL of all groups**

Groups	n	Mean	SD	Minimum	Maximum	ANOVA (P)
CFU/ml						
Negative control	10	2562.00	208.583	2180	2800	<0.0001
A <sub>1</sub>	30	1290.00	211.056	1060	1800	
A <sub>2</sub>	30	60.00	34.039	20	120	
A <sub>3</sub>	30	234.00	153.322	60	640	
B <sub>1</sub>	30	896.00	121.644	740	1160	
B <sub>2</sub>	30	24.00	60.207	0	200	
B <sub>3</sub>	30	28.00	34.281	0	120	
Total	190	534.63	690.436	0	2800	

SD: Standard deviation



**Figure 1:** A bar graph showing the mean percentage of disinfection of all groups after irrigation protocols

**Table 2: Comparison of colony-forming unit/ml between all groups using *post hoc* and Tukey's honestly significant difference tests**

Dependent variable	Group (I)	Group (J)	Mean difference (I-J)	SE	P	95% CI	
						Lower bound	Upper bound
CFU/ml	Negative control	A <sub>1</sub>	1272.00	46.495	<0.0001	1133.38	1410.62
	Negative control	A <sub>2</sub>	2502.00	46.495	<0.0001	2363.38	2640.62
	Negative control	A <sub>3</sub>	2328.00	46.495	<0.0001	2189.38	2466.62
	Negative control	B <sub>1</sub>	1666.00	46.495	<0.0001	1527.38	1804.62
	Negative control	B <sub>2</sub>	2538.00	46.495	<0.0001	2399.38	2676.62
	Negative control	B <sub>3</sub>	2534.00	46.495	<0.0001	2395.38	2672.62
	A <sub>1</sub>	A <sub>2</sub>	1230.00	32.877	<0.0001	1131.98	1328.02
	A <sub>1</sub>	A <sub>3</sub>	1056.00	32.877	<0.0001	957.98	1154.02
	A <sub>1</sub>	B <sub>1</sub>	394.00	32.877	<0.0001	295.98	492.02
	A <sub>1</sub>	B <sub>2</sub>	1266.00	32.877	<0.0001	1167.98	1364.02
	A <sub>1</sub>	B <sub>3</sub>	1262.00	32.877	<0.0001	1163.98	1360.02
	A <sub>2</sub>	A <sub>3</sub>	-174.00	32.877	<0.0001	-272.02	-75.98
	A <sub>2</sub>	B <sub>1</sub>	-836.00	32.877	<0.0001	-934.02	-737.98
	A <sub>2</sub>	B <sub>2</sub>	36.00	32.877	0.929	-62.02	134.02
	A <sub>2</sub>	B <sub>3</sub>	32.00	32.877	0.959	-66.02	130.02
	A <sub>3</sub>	B <sub>1</sub>	-662.00	32.877	<0.0001	-760.02	-563.98
	A <sub>3</sub>	B <sub>2</sub>	210.00	32.877	<0.0001	111.98	308.02
	A <sub>3</sub>	B <sub>3</sub>	206.00	32.877	<0.0001	107.98	304.02
	B <sub>1</sub>	B <sub>2</sub>	872.00	32.877	<0.0001	773.98	970.02
	B <sub>1</sub>	B <sub>3</sub>	868.00	32.877	<0.0001	769.98	966.02
	B <sub>2</sub>	B <sub>3</sub>	-4.00	32.877	1.000	-102.02	94.02

CI: Confidence interval, SE: Standard error

In the present study, 2.5% NaOCl (Group A<sub>2</sub>) was found to eliminate 97.6% of *E. faecalis* biofilms owing to its tissue dissolving properties.<sup>[9-11]</sup> In addition, an increase in the antimicrobial activity with the combination of 2.5% NaOCl, and PAD was seen which can be due to the increased depth of penetration of laser into dentinal tubules. Gutknecht *et al.* found 980 nm-diode laser to disinfect dentinal tubules at a greater depth of 500  $\mu$ m as compared to 100  $\mu$ m depth achieved by standard irrigants.<sup>[7,12-14]</sup>

QMix (Group A<sub>3</sub>), in the present study, showed a kill rate of 89.7% against *E. faecalis* biofilms, which was significantly less than that of 2.5% NaOCl in accordance to the results of studies by Del Carpio-Perochena *et al.* and Dunavant *et al.*<sup>[11,15]</sup> In addition, Wang *et al.* found QMix to be significantly less effective than NaOCl in eradicating 3-week *E. faecalis* biofilms while they were equally effective in eliminating 1 day old *E. faecalis* biofilm. Thus, it proves that QMix is less effective than NaOCl in eradicating mature biofilms.<sup>[5,16]</sup> However, when combined with PAD, QMix showed an increase in the percentage disinfection of *E. faecalis* biofilm to 98.8%, which was similar to Group B<sub>2</sub>.<sup>[17]</sup>

In contrast to the results of this study, Mehrvarzfar *et al.* found that PAD did not show significantly better results when combined with 2% CHX. In their study, they found 2% CHX and 2% CHX in combination with PAD to be equally effective in eradicating 2 week biofilm of *E. faecalis*. This may be due to the shorter period of incubation. QMix is known to be less effective in eradicating mature *E. faecalis* biofilms. In the present study, a 6-week biofilm was used which is more resistant to disinfection procedures.<sup>[9,18]</sup>

Thus, within the limitations of the present study, it can be concluded that in lieu of the safe therapeutic window, the application of PAD might be a useful adjunct to conventional endodontic irrigation in eradicating *E. faecalis* biofilms. However, PAD must be used only as a supplement and not as an alternative to conventional irrigation protocols.

## CONCLUSION

PAD with 2.5% NaOCl proved to be the most effective in disinfecting root canals containing mature *E. faecalis* biofilms.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: A long-term light and electron microscopic follow-up study. *J Endod* 1990;16:580-8.
- Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006;32:93-8.
- Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, *et al.* The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. *J Endod* 2011;37:80-4.
- Fimpe JL, Fontana CR, Foschi F, Ruggiero K, Song X, Pagonis TC, *et al.* Photodynamic treatment of endodontic polymicrobial infection *in vitro*. *J Endod* 2008;34:728-34.
- Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. *J Endod* 2012;38:1376-9.

6. Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, *et al.* Photodynamic therapy for endodontic disinfection. *J Endod* 2006;32:979-84.
7. Gutknecht N, Franzen R, Schippers M, Lampert F. Bactericidal effect of a 980-nm diode laser in the root canal wall dentin of bovine teeth. *J Clin Laser Med Surg* 2004;22:9-13.
8. Souza LC, Brito PR, de Oliveira JC, Alves FR, Moreira EJ, Sampaio-Filho HR, *et al.* Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of *Enterococcus faecalis*. *J Endod* 2010;36:292-6.
9. Mehrvarzfar P, Saghir MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, *et al.* Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against *Enterococcus faecalis* contaminating root canals: An *in vitro* study. *J Oral Sci* 2011;53:355-60.
10. Rocas IN, Siqueira JF Jr. Minimum contact time and concentration of sodium hypochlorite required to eliminate *Enterococcus faecalis*. *J Endod* 2010;36:520-3.
11. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. *J Endod* 2006;32:527-31.
12. Poggio C, Arciola CR, Dagna A, Florindi F, Chiesa M, Saino E, *et al.* Photoactivated disinfection (PAD) in endodontics: An *in vitro* microbiological evaluation. *Int J Artif Organs* 2011;34:889-97.
13. Bergmans L, Moisiadis P, Huybrechts B, Van Meerbeek B, Quirynen M, Lambrechts P. Effect of photo-activated disinfection on endodontic pathogens *ex vivo*. *Int Endod J* 2008;41:227-39.
14. Schoop U, Kluger W, Moritz A, Nedjelic N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. *Lasers Surg Med* 2004;35:111-6.
15. Del Carpio-Perochena AE, Bramante CM, Duarte MA, Cavenago BC, Villas-Boas MH, Graeff MS, *et al.* Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. *J Endod* 2011;37:1134-8.
16. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *J Endod* 2012;38:948-53.
17. Gutknecht N, van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F. Diode laser radiation and its bactericidal effect in root canal wall dentin. *J Clin Laser Med Surg* 2000;18:57-60.
18. Schlafer S, Vaeth M, Hørsted-Bindslev P, Frandsen EV. Endodontic photoactivated disinfection using a conventional light source: An *in vitro* and *ex vivo* study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:634-41.

