

A comparison of the efficacy of various disinfection protocols in endodontic treatment: an *in vitro* study.

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SUMMARY

Introduction: Disinfection is the main objective of root canal preparation and whilst irrigation is most commonly used, another method is Photo-activated Disinfection (PAD).

Aims and Objectives: The aim of this *in vitro* study was to compare the efficacy of eradication of bacteria from root canals by six different disinfection protocols.

Methods: Root canals of 84 extracted human teeth were prepared to a standardised size and taper. The teeth were sterilised and then inoculated with *E. faecalis*. The teeth were randomly assigned to one of seven groups (n = 12), each of which underwent a different disinfection protocol. Dentine samples were plated onto BHI plates and incubated anaerobically. After five days, colony-forming units (cfu) were counted. The Pairwise Wilcoxon Rank Sum test and the Kruskal-Wallis test were used for statistical analysis of the data.

Results: The most effective disinfection protocol was: 3% NaOCl with 2% CHX, followed by 3% NaOCl with PAD, Chlor-XTRA and 2% CHX. The 3% NaOCl-protocol performed significantly better than PAD and distilled water. The PAD-protocol performed significantly better than distilled water.

Conclusion: The most efficient protocol in eradicating *E. faecalis* from the root canals was 3% NaOCl followed by irrigation with 2% CHX.

INTRODUCTION

The majority of persistent endodontic infections are strongly associated with the invasion of the pulp by facultative anaerobic bacteria.^{1,2,3} Chemo-mechanical root

ACRONYMS

BHI:	Brain Heart Infusion
Cfu:	Colony-forming units
CHX:	Chlorhexidine gluconate
EDTA:	Ethylene-diamine-tetra-acetic acid
PAD:	Photo-activated Disinfection
NaOCl:	Sodium Hypochlorite

canal preparation is crucial in endodontic treatment⁴ to ensure removal of the residual pulp tissue, fragments of dentine and pathogenic microorganisms.¹ The chemical dissolution of these organic and inorganic components is equally important to the physical flushing action of irrigation solutions.^{5,6,7}

Enterococcus faecalis is a gram-positive, facultative, anaerobic microorganism that is frequently implicated in persistent periapical infection.^{3,8,9}

Mechanical root canal cleaning and shaping leads to the occlusion of dentinal tubules by a deposit referred to as a "smear layer".^{10,11} If this barrier is in place, debris and organic and inorganic matter remain in the tubules and resident bacteria are not killed.^{12,13}

Removing the smear layer is accomplished most efficiently by rinsing the root canal with 0.5%-5.25% sodium hypochlorite (NaOCl) to dissolve and flush out the organic remnants, followed by rinsing with a liquid chelating agent (17% liquid ethylene-diamine-tetra-acetic acid, EDTA) to dissolve inorganic components.^{14,15,16,17}

A commercially available endodontic irrigant, Chlor-XTRA (Vista Dental Products, Racine, Wisconsin, USA), is an improved NaOCl (5.25%) solution containing also a wetting agent, surface modifying agents to reduce surface tension and alkylating agents to increase electrical capacity.¹⁸ Chlorhexidine gluconate (CHX) is a cationic bis-guanide considered to be a broad-spectrum antimicrobial agent that can be used for root canal irrigation.^{6,19} Chlorhexidine molecules bind to hydroxyapatite crystals and to soft tissues resulting in a residual bacteriostatic phenomenon known as substantivity.^{19,20,21} Chlorhexidine gluconate would in all probability be the ideal endodontic irrigant were it not for its incapacity to dissolve organic matter.²² An endodontic irrigation regime that includes

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Table 1: Disinfection protocols of the seven groups

Group	Irrigant/ Treatment during minute 1	Irrigant/ Treatment during minute 2	Irrigant/ Treatment during minute 3	Irrigant/ Treatment during minute 4	Irrigant/ Treatment during minute 6	Irrigant/ Treatment during minute 7	Irrigant/ Treatment during minute 8
1	3ml 3% NaOCl	3ml 3% NaOCl	3ml 3% NaOCl	3ml 3% NaOCl	3ml distilled water		
2	3ml 2% CHX	3ml 2% CHX	3ml 2% CHX	3ml 2% CHX	3ml distilled water		
3	3ml Chlor-XTRA	3ml Chlor-XTRA	3ml Chlor-XTRA	3ml distilled water	3ml distilled water		
4	3ml 3% NaOCl	3ml 3% NaOCl	3ml distilled water	3ml 2% CHX	3ml distilled water		
5	3ml 3% NaOCl	3ml 3% NaOCl	3ml 3% NaOCl	3ml distilled water	Toluidine chloride with PAD	Toluidine chloride with PAD	3ml distilled water
6	Toluidine chloride with PAD	Toluidine chloride with PAD	3ml distilled water				
7 (control)	3ml distilled water	3ml distilled water	3ml distilled water	3ml distilled water	3ml distilled water		

both CHX and NaOCl is beneficial in that the two solutions complement each other, one making up for the shortcomings of the other.^{23,24}

A relatively new method of disinfection is Photo-activated disinfection (PAD) in which, for endodontic therapy, a non-toxic photo-sensitive agent (dye) is placed into the prepared root canal. Molecules within the dye attach to contacting bacterial cells and act as markers. A light source is applied inside the canal to initiate a chemical reaction. The molecules within the dye become excited. Highly reactive "singlet" oxygen* released from the dye has a toxic effect upon the "marked" bacterial cells, damaging their protoplasm, cell membrane and DNA. Ultimately this results in bacterial cell lysis and death.^{25,26}

The aim of this *in vitro* study was to compare the efficacy of six different root canal disinfection regimens in the eradication of *E. faecalis* from the root canals of human maxillary incisors. The disinfection efficacy was compared by microbiological culture.

*an electronically excited molecular oxygen known as dioxygen or dioxidene.

MATERIALS AND METHODS

This *in vitro* study is based on a method modified from that first used by Haapasalo and Ørstavik in 1987,²⁷ and applied successfully in many other experiments.^{5,28,29} Eighty four extracted single rooted teeth were collected. The crowns of the teeth were removed using a diamond wafering blade in an Isomet low speed saw (Buehler Ltd., Lake Bluff, Illinois, USA) leaving a standardised root canal length of 15mm.

The root canals were prepared using ProTaper Universal (Dentsply/Maillefer, Baillaigues, Switzerland) Nickel Titanium rotary endodontic files. The two shaper files S1 and S2 were used for crown-down preparation. Then the finisher files were used from the F1 to the F3 file, according to the manufacturers' instructions. A standardised taper was produced with a size 45, 6% taper ProFile (Dentsply/Maillefer) rotary file. During preparation, copious amounts

of 3% NaOCl (Rekitt Benckiser, South Africa (Pty) Ltd., Elandsfontein, Gauteng, South Africa) were used for root canal irrigation. After preparation the following sequential irrigations were completed for each canal:

- 3% NaOCl for five minutes
- distilled water for two minutes.
- 17% EDTA (Vista Dental Products, Toronto, Canada) for one minute.
- distilled water for two minutes.

The teeth were then sterilised by autoclave (Hung-Lin Medical Instruments Co. Ltd.) at 121°C for 15 minutes. Before the inoculation procedure sterility of the root canals was assessed. Sterile paper points were inserted into the root canals of five randomly selected teeth. The paper points were placed onto Brain Heart Infusion (BHI) plates (Onderstepoort Biological Products Ltd.) which were incubated under facultative anaerobic conditions using Anaerocult A® (Merck SA (Pty) Ltd.) at 37°C for three days. All cultures were negative, verifying that all the canals were sterile. The prepared teeth were randomly divided into seven groups (n=12) and the groups were placed into sterile glass containers. A McFarland standard 1 suspension (8 x 10⁸ colony-forming units) in BHI broth (Merck SA (Pty) Ltd.) was prepared from 48-hour cultures of *E. faecalis* (ATCC 49474).³⁰ A 1% inoculum of this was added to the teeth which were then incubated in a Vortex platform incubator (Ika-Works Inc. Germany) for 48 hours. Random dentine samples were taken from the prepared root canal of one tooth from each group, using a sterile round tungsten carbide bur size ISO 014 (Dentsply/Maillefer). The samples were placed onto BHI plates and incubated under anaerobic conditions (positive control). After 72 hours, numerous cfu's of the test organism were observed on the BHI plates. This served as confirmation that the root canals had been successfully inoculated with *E. faecalis*.

To each group was assigned a specific disinfection regime and the teeth treated according to that protocol (Table 1). The teeth were then split longitudinally. Three dentine samples were taken from one of the two sections (coronal, middle and apical) using a sterile round tungsten carbide bur size ISO 014. The dentine powder was

Table 2: Kruskal Wallis test comparing the variable cfu counts of all seven treatment groups (H_0 = null hypothesis)

Treatment Group	N	Sum of Scores	Expected Under H_0	Std Deviation Under H_0	Mean Score
3% NaOCl	36	6123.00	4554.0	386	170.
2% CHX	36	3113.00	4554.0	386	86
Chlor-XTRA	36	2619.00	4554.0	386	73
3% NaOCl / 2% CHX	36	2370.50	4554.0	386	66
3% NaOCl / PAD	36	2485.50	4554.0	386	69
PAD	36	7342.50	4554.0	386	204
Control	36	7824.50	4554.0	386	217

Average scores were used for ties.

Kruskal–Wallis Test

Chi-Square	205.7565
DF	6
Pr > Chi-Square	<0.0001

Table 3: Significant differences between the cfu counts of all seven disinfection treatment groups. (Level of significance : $p < 0.05$)

	3% NaOCl	2% CHX	Chlor-XTRA	3% NaOCl / 2% CHX	3% NaOCl / PAD	PAD
3% NaOCl		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	
2% CHX				$p < 0.02$		
Chlor-XTRA						
3% NaOCl / 2% CHX						
3% NaOCl / PAD						
PAD	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	
Distilled Water	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.0487$

collected over separate sterile pre-weighed Bijou bottles (Merck SA (Pty) Ltd.). The weight of the collected dentine was determined and the weight/volume concentration of each sample was calculated. This concentration was used to quantify the amount of viable *E. faecalis* that survived in each root canal.

One millilitre of sterile saline was added to the dentine in the Bijou bottles. The cfu were determined as follows: ten-fold dilutions were made in sterile quarter-strength Ringers solution. A quantity of 1ml of 10^{-3} to 10^{-7} of these dilutions was plated onto BHI agar plates.³¹ The BHI plates were incubated at 37°C for five days in facultative anaerobic conditions using Anaerocult A®. Colony forming units were counted after a period of five days.

Data were collected and submitted to a statistician. A pairwise comparison of the cfu counts of all seven treatment groups was done using the Pairwise Wilcoxon Rank Sum test and a non-parametric analogue of the one-way ANOVA test, the Kruskal Wallis test.

RESULTS

The results of this study are presented in Tables 2 and 3. The results were analysed using the Pairwise Wilcoxon Rank Sum test and the Kruskal-Wallis test. A comparison of the cfu counts of all seven treatment groups is presented in Table 2. Of the six test groups, the irrigation regimen of 3% NaOCl combined with 2% CHX was the most effective in eradicating *E. faecalis* from the root canals. In descending order of efficacy, this protocol was followed by: 3% NaOCl in combination with PAD, Chlor-XTRA and 2% CHX. The protocols that performed the most poorly in this *in vitro* study were 3% NaOCl alone and PAD alone.

The Pairwise Wilcoxon Rank Sum test showed that there were no statistically significant differences between the

disinfection effects of 2% CHX, Chlor-XTRA or 3% NaOCl/PAD protocols. That using 3% NaOCl combined with 2% CHX was significantly more efficient than 2% CHX alone. PAD, 3% NaOCl and distilled water were significantly less efficient than the other disinfection protocols. The results of the group in which PAD was used did show that this method was statistically more effective in eradicating the test organism than was distilled water.

DISCUSSION

In this *in vitro* study comparing the efficacy of six different root canal disinfection regimens, *E. faecalis* was chosen as the test organism for its resilient, resistant nature,^{32,33,34} whilst the extracted tooth model has been shown to be a reliable method in the evaluation of the bactericidal effects of root canal irrigants.³⁵ In an attempt to remove the smear layer which is formed during root canal preparation, 2.5% NaOCl was used for irrigation during preparation of the samples and 17% EDTA as the final rinse (continuous passive irrigation) for one minute.^{36,37,38}

The combination of 3% NaOCl and 2% CHX was slightly more efficient at eradicating *E. faecalis* from the root canals than were the five other disinfection regimens that were tested. However, Vianna and Gomes (2009) found no enhancement of the bacterial eradication ability of CHX by using it in combination with NaOCl.³⁹ Baca *et al.* (2011) showed a 100% increase in bactericidal rate when 2.5% NaOCl irrigation was followed by a final rinse with 2% CHX.²⁴ Their study showed that under ideal conditions 2% CHX was able to destroy bacterial biofilm within two minutes, a finding supported by several other investigations.^{24,40}

In contradiction to some literature, the results of the present study indicate that 3% NaOCl is not the best irrigation solution. However, one other paper does report a

poor performance of this solution.⁴¹ The most likely reason for this inefficiency may be that the concentration of 3% NaOCl is not sufficiently potent to completely eradicate *E. faecalis* from infected dentine within the time of exposure to the irrigant.

There are few studies specifically investigating Chlor-XTRA as an irrigation solution. In 2012 Jungbluth *et al.* compared the activity of Chlor-XTRA with that of several brands of household bleach (NaOCl).⁴² The results of this study indicate that Chlor-XTRA (5.25% NaOCl) was significantly better than 3% NaOCl at eradicating *E. faecalis* as also confirmed by several other studies.^{41,43,44}

Souza *et al.* found that PAD applied with either methylene blue (MB) or toluidine blue (TB) did not significantly enhance root canal disinfection compared to chemo-mechanical preparation using NaOCl as an irrigant followed by PAD.⁴⁵ The results of this present investigation, supported Souza's findings whether PAD was used after conventional irrigation with 3% NaOCl or was used alone. In fact, high numbers of *E. faecalis* cells were found in the PAD only treatment group. Several contradictory conclusions have been reported. Soukos *et al.* showed PAD to be 97% effective in reducing *E. faecalis* when applied alone for root canal disinfection⁴⁶ whilst Foschi *et al.* also observed good results with the system, finding that PAD used with a diode laser achieved a bacterial reduction of 77.5%.⁴⁷ In 2010, Schlafer *et al.* demonstrated a 99.7% reduction of the bacteria in suspension and a 95.82% reduction in the quantum of bacteria in adherence to the root canal wall.⁴⁸ The number of viable *E. faecalis* cells in the root canal was reduced to 2.9% when Rios *et al.* treated root canals with PAD for 30 seconds alone and, when PAD was applied after NaOCl, they observed a reduction down to 0.1%.⁴⁹

Irrigation with distilled water had no significant effect on the number of bacteria in the root canals.

Further studies should be carried out to determine the best application of the NaOCl/CHX solution combination regimen for endodontic irrigation. Additional studies need to be carried out to determine whether the additional time, effort and expense needed to apply PAD as a supplementary method of root canal disinfection is justifiable or not.

CONCLUSIONS

Whilst this study failed to identify any regime as offering complete eradication of *E. faecalis* it may be concluded that 3% NaOCl used in combination with 2% CHX will offer the clinician the best option to achieve the desired disinfection of the root canal.

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