

## An ex vivo comparative study determining the bactericidal activity of 3 different irrigants against *Enterococcus faecalis*

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### Abstract

**Aim:** To evaluate and compare the antimicrobial activity of 3% sodium hypochlorite, 2% chlorhexidine gluconate and freshclor (stabilized chlorine dioxide) against *Enterococcus faecalis*.

**Materials and Method:** 3% sodium hypochlorite, 2% chlorhexidine gluconate and freshclor were the materials used in this study.

**Agar diffusion test:** *E. faecalis* isolated from root canal infected samples were included in the study. Cultures were maintained on brain heart infusion (BHI) broth and agar. Cultures grown overnight at 37 degree Celsius in BHI broth on a rotary shaker 150 rpm and bacterial growth checked by changes in turbidity at 24 hours. BHI agar plates were prepared and cultures (200ul) were spread onto agar plates. Wells of 6mm diameter were made in the agar surfaces. Samples were divided into 3 groups. Group 1- 3% Sodium hypochlorite, Group 2- 2% Chlorhexidine gluconate, Group 3- Freshclor(stabilized chlorine dioxide), Control-distilled water. 50ul each was added to the respective wells and the plates were incubated for 24 hours in an incubator. After incubation period, plates were removed and zones of inhibition were recorded. Experiment was performed 3 times and mean zone of inhibition was recorded in mm.

**Statistical Analysis:** The data were analysed using One-way analysis of variance & Tukeys post hoc test.

**Results:** Significant difference between chlorhexidine and stabilized chlorine dioxide was found as well as between sodium hypochlorite and stabilized chlorine dioxide.

**Conclusion:** 0.1% stabilised chlorine dioxide had bactericidal activity against *E. faecalis*, but less than the other two groups. As chlorine dioxide has minimal toxicity and tissue dissolving property as well, it can be considered as an alternative endodontic irrigant at higher concentration.

**Keywords:** 3% sodium hypochlorite, 2% chlorhexidine gluconate, Freshclor(0.1% stabilized chlorine dioxide)

### Introduction

The removal of pulpal and dentinal debris with the elimination of viable microorganisms from the root canal system are of paramount importance during endodontic therapy. Bacteria are the main factor in pulpal and periapical inflammation, and failure to effectively eliminate them and their by-products could result in persistent irritation and impaired healing.<sup>(1)</sup>

Instrumentation and irrigation with 5.25% sodium hypochlorite can reduce bacterial concentrations, but cannot eliminate *Enterococcus faecalis* from the canal system.<sup>(1,2)</sup>

*E. faecalis* is an extensively evaluated biological indicator. It has demonstrated a high resistance and ability to inactivate antimicrobial agents, survival capacity in harsh environments, with scarce nutrient supply and extreme alkaline pH as well as the capacity for growth as a biofilm on root canal walls. Therefore, several laboratory studies have been conducted in order to test the susceptibility of *E. faecalis* to endodontic procedures.<sup>(3,4)</sup>

Sodium hypochlorite has been widely recommended as an irrigant for chemomechanical debridement of root canals because of its tissue dissolution and antimicrobial activity.<sup>(5)</sup>

Chlorhexidine gluconate, a less malodorous and toxic agent, has been suggested as an irrigant based on its antibacterial effects, substantivity and lower cytotoxicity than sodium hypochlorite, whilst demonstrating efficient clinical performance.<sup>(6)</sup>

Chlorine dioxide, much like sodium hypochlorite, is used to eliminate contaminants from drinking water. Its disinfectant properties have been recognized since the early 1900s, and it was registered with the EPA in a liquid form for use as a disinfectant and sanitizer in 1967. Its powerful oxidizing properties enables it to kill bacteria by disrupting the transport of nutrients across the cell wall. This strong antibacterial activity makes it a potentially useful endodontic irrigant.<sup>(1)</sup>

The purpose of this study is to evaluate and compare the antimicrobial activity of 3% sodium hypochlorite, 2% chlorhexidine gluconate and freshclor (stabilized chlorine dioxide) against *Enterococcus faecalis*.

### Materials and Method

In this study, 3% sodium hypochlorite, 2% chlorhexidine gluconate and freshclor (stabilized chlorine dioxide) were the materials used (Fig. 1, 2, 3).



Fig. 1



Fig. 2



Fig. 3

Fig. 1, 2, 3: Materials Used

**Agar diffusion test:** *E. faecalis* isolated from root canal infected samples were included in the study. Cultures were maintained on brain heart infusion (BHI) broth and agar (Fig. 4, 5)

Fig. 4: *E. faecalis* (ATCC) 51299Fig. 5: 1ml of a pure culture of *E. faecalis* grown in brain-heart infusion broth for 24hours

Cultures grown overnight at 37°C in BHI broth on a rotary shaker 150 rpm and bacterial growth checked by changes in turbidity at 24 hours.

To check the antimicrobial efficacy of freshclor, 3% sodium hypochlorite, 2% chlorhexidine gluconate, agar well diffusion method was performed. BHI agar plates were prepared and cultures (200ul) were spread onto agar plates. Wells of 6mm diameter were made in the agar surfaces (Fig. 6, 7). Samples were divided into 3 groups.



Fig. 6: Wells of 6mm diameter were made in the BHI agar plates



Fig. 7: 200ul of the suspension were spread onto agar plates using swab in a laminar airflow cabinet

Group 1- 3% Sodium hypochlorite  
Group 2- 2% Chlorhexidine gluconate  
Group 3- Freshclor(0.1% stabilized chlorine dioxide)  
Control-distilled water

50ul each was added to the respective wells and the plates were incubated for 24 hours at 37°C in an incubator. After incubation period, plates were removed and zones of inhibition were recorded. Experiment was

performed 3 times and mean zone of inhibition was recorded in mm.

**Statistical Analysis:** The data were analysed using One-way analysis of variance & Tukeys post hoc

test. The level of statistical significance was set at  $P < 0.05$ .

## Results

The results were tabulated and statistically analysed.

**Table 1**

Groups	Standard Deviation	ANOVA	p value	Tukeys post hoc
2% CHX	0.57	38.400	0.000	Significant difference between CHX & Stabilized chlorine dioxide and NaOCL & Stabilized chlorine dioxide
3% NaOCL	1.52		Highly significant	
Freshclor (0.1% stabilized chlorine dioxide)	1.52			

Table 1 depicts significant difference between chlorhexidine and stabilized chlorine dioxide was found as well as between sodium hypochlorite and stabilized chlorine dioxide.

## Discussion

Very few studies have been conducted to investigate the antibacterial activity of stabilized chlorine dioxide against *Enterococcus faecalis*. *E. faecalis* was selected for this experiment because it is the most commonly isolated bacteria in failed endodontic cases. It can survive chemomechanical preparation and remains viable even after calcium hydroxide therapy. McHugh et al, found that pH 10.5 to 11.0 retards growth of *E. faecalis*, whereas at pH 11.5 or greater *E. faecalis* is destroyed.<sup>(1,7)</sup>

Numerous studies have attempted to identify an endodontic irrigant that combines both high antibacterial activity with low tissue toxicity. Sodium hypochlorite is currently one of the most commonly used irrigants in endodontic therapy, and its antimicrobial and tissue dissolving properties have been widely reported. However, it reacts with natural organic matter to produce trihalomethanes and haloacetic acids both of which are animal carcinogens and suspected human carcinogens.<sup>(5)</sup>

Chlorhexidine gluconate is a synthetic cationic bisguanide that consists of two symmetric 4-chlorophenyl rings and two bisguanide groups, connected by a central hexamethylene chain. According to the results of this study, the antibacterial property of 2% chlorhexidine gluconate could be because, it is a positively charged hydrophobic and lipophilic molecule that interacts with the negatively charged phospholipids and lipopolysaccharides on the cell membrane of bacteria, thereby altering the cells osmotic equilibrium. This increases the permeability of the cell wall, which allows the molecule to penetrate into the bacteria. Chlorhexidine gluconate at low concentrations (0.2%) causes the leakage of potassium and phosphorous out of

the cell and act as a bacteristatic agent but at higher concentration (2%) it is a bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death and confirms with the results of this study.

The use of Chlorhexidine gluconate as an endodontic irrigant is generally restricted because it can discolor teeth and some patients might have side effects such as loss of taste, burning sensation of the oral mucosa, subjective dryness of the oral cavity, and discoloration of the tongue. Even the presence of inflammatory exudates and killed microorganisms can inhibit the action of chlorhexidine in root canals.<sup>(8)</sup>

The potential side effects, and safety concerns, suggests a need to find a substance which can be used to reduce or eradicate *Enterococcus faecalis* with least amount of side effects.

Stabilised chlorine dioxide Current uses include food processing, water treatment, veterinary care, surface disinfection, and dental waterline treatment. Its powerful oxidizing properties enable it to kill bacteria by disrupting the transport of nutrients across the cell wall. This strong antibacterial activity makes it a potentially useful endodontic irrigant.<sup>(9)</sup>

Chlorine dioxide is used as a disinfectant at concentrations of 0.1-15mM, but an effective clinical concentration has not been determined. If a lower effective concentration can be determined it can be proved to be a relatively non toxic irrigant.<sup>(10)</sup>

Chlorine dioxide produces little or no trihalomethanes, and may be a better dental disinfectant than NaOCl. The recent detection of Cytomegalovirus and Epstein Barr virus associated with periradicular lesions may promote the use of chlorine dioxide, which kills both enveloped and non enveloped viruses, at the same time by adsorbing onto and penetrating the protein coat of the viral capsid.<sup>(11)</sup>

Chlorine dioxide is capable of dissolving human pulp tissue but is not as efficacious as sodium hypochlorite. Further research needs to be done on determining optimum concentration of aqueous solution of chlorine dioxide, its effect on dentin, smear layer

removing capability when used at a lower pH and its compatibility with various obturating and restorative materials used in Endodontics.

### Conclusion

Based on the results of this study, 3% sodium hypochlorite, 2% chlorhexidine gluconate had almost similar bactericidal activity. Even 0.1% stabilised chlorine dioxide had bactericidal activity against *E. faecalis*, but less than the other two groups. As chlorine dioxide has minimal toxicity and tissue dissolving property as well, it can be considered as an alternative endodontic irrigant at higher concentration.

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