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Efficiency of Sodium Hypochlorite as Root Canal Disinfectant against *Enterococcus faecalis*: An *In Vitro* Study

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Abstract

Introduction: *Enterococcus faecalis* (*E. faecalis*) is one of the most resistant microorganisms in the root canal system. It should be completely eliminated during endodontic therapy from root canal to prevent reinfection.

Methodology: Eighty human extracted single-rooted teeth were decoronated to a length 14 mm from the apical foramen to the cervical border of the root. The working length for all canals was adjusted to 13 mm. The canals instrumented up to size 45, autoclaved then the samples inoculated with 10 µl a suspension of *E. faecalis* at a concentration 4×10^5 cfu and incubated at 37°C for 24 hours in the incubator. Samples were divided into eight groups each group consisted of 10 prepared roots, sixty samples were irrigated with NaOCl solution at different concentrations (0.5%, 2.5% and 5.25%) at two selected times (2 minutes and 5 minutes) for each concentration. The remainder twenty samples were control positive and negative groups. After disinfection of the root canals by NaOCl, a sample was taken immediately from the root canal using K-type file size 45 which was placed in screw-capped vial containing 1ml of normal saline then 200 µl from it was taken and cultured on the *Enterococcus* agar plate and incubated aerobically at 37°C for 24 hours. Bacterial growth on culture media were counted and multiplied by dilution factor and the data collected, analyzed for statistical analysis.

Results: The results of the experiment showed that all concentrations, and times of NaOCl had significant antimicrobial effect against *E. faecalis*. The results detected that there were non-significant antimicrobial differences between 5.25% at (2 minutes and 5 minutes) and 2.5% at 5 minutes, but there was significant difference between the remaining disinfected groups.

Conclusion: Sodium hypochlorite has bactericidal effect against the most resistant bacteria in root canal and this solution should be used in concentration greater than 0.5% during root canal therapy.

Keywords: *Sodium Hypochlorite; Enterococcus faecalis*

Introduction

It has been demonstrated that bacteria and their products have a vital role in initiation and progression of pulp and periapical diseases. For this reason the principal objective of root canal treatment is to eliminate bacteria and their products from the root canal system [1]. *Enterococcus faecalis* (*E. faecalis*) is one of the most resistant microorganisms in the root canal system [2] and is the most prevalent bacterial strain isolated from teeth with endodontic treatment failure [3]. *It* is able to produce biofilms in different conditions [2], due to virulence factors of this bacteria like aggregation substance, cytolysin, lytic enzymes, can alter host responses and hence able to strive better than other microbes and is the only strain isolated after unsuccessful endodontic treatment [4]. Moreover, they invade dentinal

tubules and adhere to collagen due to their ability to form biofilm and therefore it may act as a nidus for recurrence of infection in failed root canal treated teeth [5].

Irrigation is complementary to instrumentation in facilitating the removal of pulp tissue and/or microorganisms. There are a number of ideal requirements of a root canal irrigant. It should provide a broad spectrum of antimicrobial activity while flushing out debris from the root canal**,** able to sterilize the canal**.** The root canal irrigant should have good lubricating action along with low surface tension to be able to flow into inaccessible areas. Finally, the irrigant should facilitate dentin removal but not weaken the tooth structure [6]. Canal irrigation can disinfect regions that are not accessible to mechanical instrumentation. (NaOCl) is the most commonly used root canal irrigant [7]. However, there is controversy over its best concentration [8]. NaOCl is used in endodontics in concentrations from 0.5 to 5.25%, due to antimicrobial activity [9] and organic tissue dissolution. It dissolves pulpal remnants, organic compounds of dentin, and organic components of the smear layer. The dissolving capacity of NaOCl is significantly better than all other commonly used irrigants [10] but is dependent on the concentration of the solution. NaOCl is effective against *E. faecalis* in all concentrations [11] and it can be used as a lubricant during root canal instrumentation [9]. The main objective of the current *in vitro* study was to detect antimicrobial efficiency of sodium hypochlorite (NaOCl) at different concentrations and times against *Enterococcus faecalis* when used as root canal disinfectant.

Methodology

Sample selection and preparation

A total of Eighty freshly extracted human non-carious, single-rooted teeth (upper central, upper lateral and lower second premolar) were placed in 1.3% NaOCl for primary surface disinfection. Each tooth was decoronated at the level of cemento-enamel junction. Patency was confirmed with No.15 K-type file and pulp tissue was removed with a barbed broach. The working length for each root canal was determined to13 mm and prepared up to the size 45. Teeth were irrigated until 1.3% NaOCl and then left in sterile distilled water for 24 hrs. The apical foramen for each root was sealed with acrylic resin and the external root surface was coated with two layers of nail polish. The roots embedded in silicone impression material, then covered with aluminum foil and adapted in the stainless steel boxes for autoclave sterilization.

Sample groups

Teeth were divided into eight groups 10 teeth for each. Six groups for NaOCl treatment, and two control groups (positive and negative).

Microbial isolation and identification

Enterococcus faecalis were isolated and identified on the *Enterococcus* Agar. Then prepared to final concentration of (4×10^7 cfu/ml) [12]. Each root canal was inoculated with 10 µl of bacterial suspension and incubated at 37°C for 24 hrs.

Disinfection of the infected root canals

Each infected root canal injected with 10 µl of freshly diluted solution of NaOCl via micropipette for the selected time, then each canal was dried with sterilized absorbed endodontic paper point.

Microbiological sampling from treated root canals

Sample was taken from each root canal using K-type file size 45 which was inserted inside the root canal to full working length. Then the file is rotated 360° in clock wise direction for engagement in dentine. The sample was transferred immediately to screw-capped vial containing 1 ml normal saline. The solution was agitated for 30 seconds, then 200 µl was cultured on agar plate [12], culturing was done by spreading using swab on *Enterococcus* agar and incubated aerobically at 37°C for 24 hrs. Then the number of bacterial colonies were counted.

Results

A comparison was done between the effect of NaOCl as root canal disinfectant against *E. faecalis* and control group using unpaired t-test at level ($p < 0.001$). The results indicated that all treated groups with NaOCl at different concentrations and times had significantly

high antimicrobial effect compared with the untreated control group. The results are shown in table 1 and figure 1. When the comparison was made between different concentrations at the selected time using ANOVA for each time at level ($p < 0.001$), the results revealed that at 2 minutes there was significant antimicrobial difference among 0.5%, 2.5% and 5.25% concentrations of NaOCl, while at 5 minutes the antimicrobial effect of 5.25% and 2.5% was the same in which both of them was significantly different from 0.5%. The results are shown in table 2. Non-significant difference was obtained when the exposure period of the infected root canal to 5.25% concentration of NaOCl at both 2 minutes and 5 minutes. However, at both concentrations (0.5% and 2.5%), the antimicrobial effect at 5 minutes exposure to NaOCl solution was higher than 2 minutes, using unpaired t-test for each concentration at level ($p < 0.001$) as in table 3.

Conc.	Time/min	Mean	SD	p-value
0.5	2	2157.50	140.78	0.000*
0.5	5	269.5	79.81	0.000*
2.5	2	146.5	29.06	0.000*
2.5	5	8	10.06	0.000*
5.25	2	0	0	0.000*
5.25	5	0	0	0.000*
Control		2823.5	71.69	

Table 1: *A comparison between the antimicrobial effect of NaOCl as root canal disinfectant against E. faecalis compared with control group.*

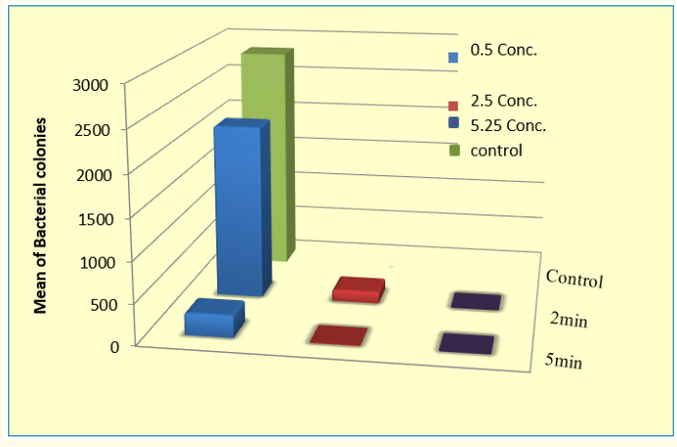

Figure 1: *A histogram showing the antimicrobial activity of different NaOCl treated groups against E. faecalis infected root canals and the control group.*

Time (min)	Conc.	Mean	SD	F-value	P-value	Duncan
2	0.5	2157.50	140.78	1880.331	0.000	C
	2.5	146.50	29.06			B
	5.25	0	0			A
5	0.5	269.5	79.81	109.019	0.000	
	2.5	8	10.06			A
	5.25	0	0			A

Table 2: *A comparison between the antimicrobial effect of NaOCl at specific time among different concentrations against E. faecalis.*

According to ANOVA $p < 0.001$ for each time, means with different letters vertically for each level of time alone have significant difference at $p < 0.001$ according to Duncan test.

SD: Standard deviation.

Conc.	Time (min)	Mean	SD	p-value	F-value	P-value	Duncan
0.5	2	2157.5	140.78	0.000	1609.0	0.000	D
	5	269.5	79.81				C
2.5	2	146.5	29.06	0.000			B
	5	8	10.06				A
5.25	2	0	0	N.S			A
	5	0	0				A

Table 3: *A comparison between the antimicrobial effect of NaOCl at specific concentration between different times and among all the NaOCl treated groups.*

Further comparison among different concentrations and times using ANOVA at level ($P < 0.001$), the results indicated that there were non-significant antimicrobial differences between 5.25%/(2 minutes and 5 minutes) and 2.5%/5 minutes, but there was significant difference between the remaining disinfected groups as in table 3.

Discussion

E. faecalis was chosen for the current study because of its high resistance to a wide range of antimicrobial agents than other microorganisms, associated with persistent endodontic infections [13]. In addition, this bacteria seem to be the best organism to penetrate dentinal tubules leading to gross infection [14]. as well as its character of faster growth than other bacterial strains, the potential to colonize, dentinal tubule invasion, physical properties of the biofilm structure and interaction between composing cells [15]. Therefore, endodontic infection with *E. faecalis* usually constitutes a problem with treatment because this bacteria is difficult to be eliminated from the root canal with the use of chemomechanical procedures [16]. A culture dependent approach was used in the present investigation. It is one of the most reliable methods of detecting viable bacteria, particularly when samples are taken immediately after antimicrobial treatment [17]. However, this method is a particularly difficult, time consuming technique and it requires microbiological facilities. It is probably an effective method of evaluating root canals disinfection [18]. The microbiological growth of bacteria used was analysis by culturing on *Enterococcus* agar to avoid any contamination which may result from growth of other bacteria.

Root canal infection different from the infection in other human sites. This type of infection cannot be eliminated by host defense mechanism or by systemic antibiotic therapy. This is due to the absence of the blood supply in the necrotic pulp that impedes the transport of defense cell and systemically administrated antibiotic to the infected site. Therefore, endodontic infection treated locally by both chemical and mechanical procedure [19]. Sodium hypochlorite was selected in this study because of its well-known bactericidal action against all microorganisms upon direct contact and unique effect on the organic tissue dissolution. However, no general agreement exists regarding its optimal concentration, which ranges from 0.5% to 5.25% [20]. A bactericidal effect of NaOCl against *E. faecalis* was detected in the present experiment, which varied with different concentrations and times. A significant bactericidal reduction was observed in all concentrations and times compared with untreated control group.

The result of this experiment demonstrated the ability of NaOCl solution to eliminate *E. faecalis* when used as root canal irrigants during endodontic therapy. The antimicrobial effectiveness of NaOCl due to its high pH (hydroxyl ion action) that interferes with bacterial cytoplasmic membrane and also this solution contains active chlorine that exerts its antibacterial effect by oxidation of bacterial enzyme and disrupting the metabolic function of bacterial cell. Chlorine may also combine with bacterial cytoplasmic component to form toxic complex which destroys the microorganism [21]. The result of this study showed significant difference among concentrations (0.5%, 2.5% and 5.25%) at 2 minutes contact time. This is disagreed with the results of other studies that did not demonstrate any significant antimicrobial difference between concentrations ranging from 0.5% to 5% NaOCl in killing *E. faecalis* in the root canals [22,23].

In this experiment the result showed that NaOCl at 5.25% was the most efficient concentration that eliminated *E. faecalis* completely at both times (2 minutes and 5 minutes) therefore it needs not to wait more than 2 minutes at this concentration to eliminate this bacteria completely. This result in agreement with Sheykhrezaei., *et al.* [24], who found that 5.25% NaOCl eliminated *E. faecalis* completely from root canal at 5 minutes. Gomes., *et al.* [25], found that 5.25% NaOCl eliminated *E. faecalis* completely at a time less than 30 seconds *in vitro*. In the present experiment the result demonstrated that bacterial reduction of *E. faecalis* by NaOCl at low concentrations (0.5%/2 min, 5 min and 2.5%/2 min) had statistically significant difference when compared with 5.25%/2 min. This means that 0.5%/2 min, 5 min and 2.5%/2 min need more time to eliminate *E. faecalis* completely. When 2.5% NaOCl/5 min compared with 5.25% NaOCl/2 min, the result showed no significant difference and 2.5% NaOCl/5 min did not completely eliminate bacteria from the root canal compared with 5.25%/2 min. This result is in agreement with Giardino., *et al.* [26], who found that 5.25% NaOCl is the most efficient concentration capable in removing *E. faecalis* and 2.5% NaOCl/5 min have the same effect, but disagreement with the result obtained by Radcliffe., *et al.* [13], who found that 2.5% NaOCl/5 min eliminated *E. faecalis* completely.

Also the results of this study are in agreement with the result carried by Ayhan., *et al.* [27], who reported the antimicrobial effect of various endodontic irrigants on this selected microorganism and observed that 5.25% NaOCl was superior and the reduced concentration to 0.5% resulted in significantly decreased antimicrobial effectiveness. Radcliffe., *et al*. [13], found that *E. faecalis* was resistant to 0.5% NaOCl at a time less than 30 minutes. Thus, the optimum concentration for NaOCl is 2.5%/5 min which gives less reduction in microhardness of dentine and toxicity to periapical tissue compared with 5.25%/2 min, this concentration reduces microhardness of dentine more than 2.5%/5 min but 5.25%/2 min had optimal chair side time. So we have two choices either reducing concentration but with 5 minutes or we can increase concentration that leading to increased reduction in microhardness of dentine and toxicity but with advantage of less time (2 minutes).

The results of the study demonstrated that disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution which contains active chlorine [28]. When the original concentration of NaOCl diluted with distilled water, this may reduce the amount of active chlorine in the solution which leads to limit the antimicrobial effect. Also this experiment showed that the antimicrobial effect of NaOCl is time dependent. One possible explanation, probably the increased time could enable NaOCl to diffuse inside the root canal. From over all, the bactericidal effect of sodium hypochlorite within the root canal relays on its concentration and duration of time retained in the canal as the concentration increased the time required to eliminate this bacteria decreased.

Conclusions

Under the limitation of this study it can be concluded that:

1. The bactericidal effect of NaOCl at all concentrations and selected times in root canals infected with *E. faecalis* have been significantly different from control group.
2. The time required to eliminate *E. faecalis* depends on the concentration of the solution, less time required for NaOCl to eliminate *E. faecalis* in the root canal at high concentration.
3. *E. faecalis* is resistant to 0.5% NaOCl, while 2.5% had moderate effect against this bacteria but its effect depends on the time. Thus, at 5 minutes this concentration has no significant differences from 5.25% and at the concentration 5.25% *E. faecalis* is eliminated completely at 2 minutes and no need to wait more.
4. NaOCl solution should be used in concentration greater than 0.5% during root canal therapy.

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