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ORIGINAL RESEARCH

Comparison of Antibacterial Effects of Various Root Canal Irrigants on *Enterococcus faecalis*

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ABSTRACT

Aims: To compare the antibacterial effects of various root canal irrigants against *E.faecalis*. Irrigants tested were 5.25% NaOCl, 0.2% chlorhexidine gluconate, and 0.2% cetrimide individually and combined.

Materials and methods: Root canal preparation was performed on 120 extracted permanent maxillary central and lateral incisor teeth. Following root canal preparation, apical foramina were sealed with epoxy resin to prevent bacterial leakage. The root canals were then contaminated with *Enterococcus faecalis*. After incubation, the contaminated roots were divided into four groups of 30 each. 2 mm of irrigant was delivered which remained in the canal for 10 minutes. The canals were then irrigated with 1 ml saline solution and with size 45 sterile paper point bacteria were sampled. The growth of *E. faecalis* which occurred in the tubes was inoculated onto blood agar plates.

Statistical analysis: Difference between the antibacterial efficacies of irrigants was evaluated statistically using chi-square test.

Results: There was no significant difference between the antibacterial efficacies of 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine gluconate with 0.2% cetrimide, but both had a significantly lower antibacterial effect than 5.25% sodium hypochlorite and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate. This difference was statistically significant.

Conclusions: Within limitations of this study, it was concluded that the 5.25% sodium hypochlorite and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate had a higher antibacterial effect than 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine gluconate with cetrimide. There was no significant difference between the antibacterial efficacies of 0.2% chlorhexidine gluconate alone and 0.25% chlorhexidine with certrimide.

Keywords: *Enterococcus faecalis*, Blood agar plates, 5.25% sodium hypochlorite, 0.2% chlorhexidine gluconate, Cetrimide.

INTRODUCTION

A substantial number of bacterial species have been identified as inhabitants of the oral cavity. However, because of bacterial interactions, nutrient availability and low oxygen potentials in root canals with necrotic pulp, the number of bacterial species present in endodontic infections are restricted. These selective conditions lead to the predominance of facultative and strictly anaerobic microorganisms that survive and multiply, causing infections that stimulate periapical bone resorption and are more resistant to endodontic treatment.[1] *Enterococcus faecalis* have long been implemented in persistent root canal infections and more recently has been identified as the species most commonly recovered from root canals of teeth with post-treatment disease.[2]

It is a well-known fact that only the mechanical action of instruments is not capable of promoting satisfactory cleansing due to the complexity of internal dental anatomy and because there is no direct contact of instrument with all walls of the root canal system.[3] When these microorganisms remain within a supporting environment they can proliferate and reinfect the root canal system.[4]

The primary objective of root canal therapy is the retention of the pulpless or pulpally involved tooth with its associated periapical tissues in a healthy state. Achievement of this objective requires that the pulpal spaces and contents be eliminated as sources of infection. Therefore, the introduction of an antimicrobial endodontic irrigant during root canal therapy should be given priority in the hierarchy of root canal treatment.[5]

In consideration of all these factors, a comparative study was carried out to compare the antibacterial effects of 5.25% sodium hypochlorite, 0.2% chlorhexidine gluconate, 0.2% chlorhexidine gluconate with 0.2% cetrimide, and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate on *Enterococcus faecalis*.

MATERIALS AND METHODS

A total of 120 freshly extracted, intact permanent maxillary central and lateral incisor teeth having single root canal were selected. The root lengths ranged between 12 and 16 mm. Conventional access preparations were made with a high-speed airotor handpiece with round tungsten carbide bur and long tapered diamond points. K files of size 15 to 50 numbers were used to biomechanically prepare the root canals, and barbed broaches were used to remove pulp tissue; root canals were instrumented 1mm beyond the apical foramen up to size 50. Irrigation with distilled water was performed during preparation.

Fig. 1: Specimens

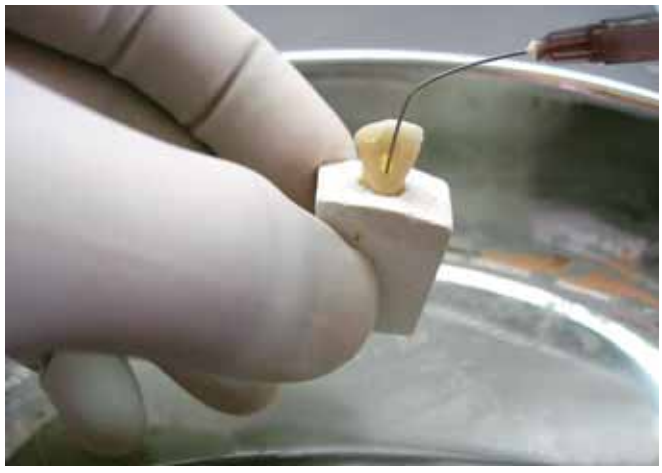


Fig. 3: Irrigant delivery into the root canal

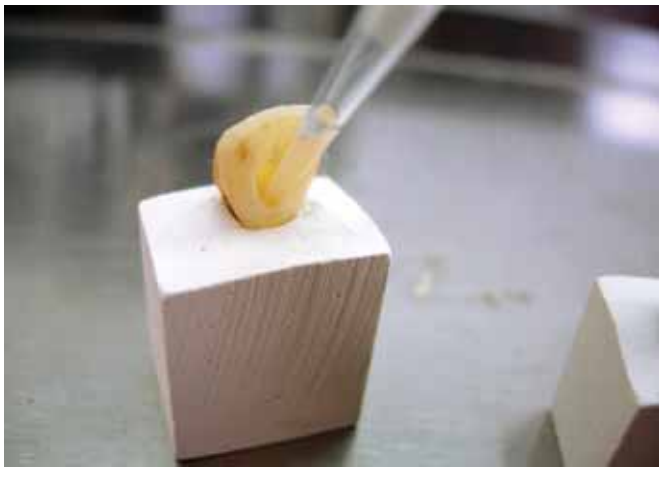


Fig. 2: Inoculation of root canals with *Enterococcus faecalis*

Fig. 4: Sterile paper points left in the wet canal to sample the bacteria from the root canal

Following root canal preparation, the enlarged apical foramina were sealed with epoxy resin (Araldite^{R}) to prevent bacterial leakage. To make both handling and identification easier, the teeth were mounted vertically in plaster blocks and sterilized in an autoclave for 20 minutes at 121°C (Fig. 1).

Pure culture of *Enterococcus faecalis* (ATCC 29212), grown in brain heart infusion broth, was used to contaminate the root canals. The root canals were inoculated with 10 µL of a 1.5×10^{8} cfu/ml^{-1} suspension using sterile 1ml tuberculin syringes (Fig. 2). The plaster blocks were then placed inside stainless steel boxes and incubated at 37°C for 24 hours. After incubation, the contaminated roots were divided into four groups of 30 teeth each according to the irrigation regimen used: 5.25% NaOCl (LobaChemie) (group I), 0.2% chlorhexidine gluconate (Loba Chemie) (group II), 0.2% chlorhexidine gluconate and 0.2% cetrimide (Loba Chemie) (group III), 5.25% NaOCl and 0.2% chlorhexidine gluconate (group IV).

A 24-gauge needle attached to an irrigant-containing sterile 2 ml plastic syringe was inserted into the root canal until it bound, and was then withdrawn, approximately 1 mm, so that it was no longer in contact with the root canal walls. Two milliliters of irrigant was delivered which remained in the canal

Fig. 5: Incubator

for 10 minutes (Fig. 3). The canals were then irrigated with 1 ml sterile saline solution and a size 45 sterile paper point was selected to sample the bacteria from the root canals (Fig. 4). The paper points were left in the wet canal for 1 minute and then transferred to the tubes containing 5 ml of the Mueller-Hinton broth. The tubes were then vortexed for 5 minutes and

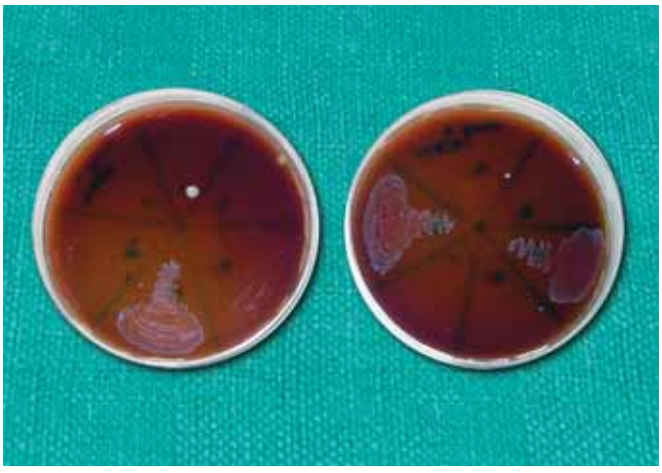


Fig. 6: Blood agar plate showing bacterial colonies

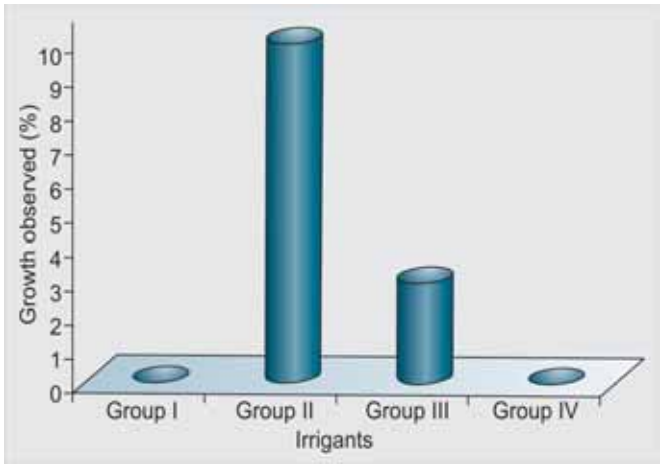


Fig. 7: Antibacterial effects of various root canal irrigants against *Enterococcus faecalis*

incubated at 37°C for 4 days (Fig. 5). The occurrence of the broth turbidity was indicative of bacteria remaining in the root canal.

In order to check the growth of *E.faecalis* in the tubes, in which broth turbidity occurred, samples were inoculated onto blood agar plates (Colombia agar base). Tubes, where *E. faecalis* growth was observed, were counted and then analyzed statistically (Fig. 6).

STATISTICAL ANALYSIS

Comparisons between the groups were made using Kruskal-Wallis test. Proportion of antibacterial efficacies between the groups was compared using Chi-square test. Differences at $p < 0.05$ were considered to be statistically significant.

RESULTS

A total of 120 teeth were studied. This included four groups of 30 teeth each. Group I was irrigated with 5.25% sodium hypochlorite, group II was irrigated with 0.2% chlorhexidine gluconate, group III was irrigated with 0.2% chlorhexidine gluconate and 0.2% cetrimide, and the group IV was irrigated with 5.25% sodium hypochlorite and 0.2% chlorhexidine gluconate. Table 1 outlines that the most effective irrigants with zero growth in all the samples were 5.25% sodium hypochlorite (group I) and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate (group IV), and the least effective irrigants were 0.2% chlorhexidine gluconate (group II) and 0.2% chlorhexidine gluconate with 0.2% cetrimide (group III).

There was no significant difference between the antibacterial efficacies of 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine gluconate with 0.2% cetrimide, but both had a significantly lower antibacterial effect than 5.25% sodium hypochlorite and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate. This difference was statistically significant ($p = 0.045$). However, median loading when comparing all the irrigants tested was statistically not significant (Fig. 7).

Table 1: Number (percent) of canals with growth at 24 hrs, 48 hrs and 72 hrs after irrigation

Groups	After 24 hrs	After 48 hrs	After 72 hrs
I	No growth	No growth	No growth
II	No growth	No growth	Growth seen in one sample 1st sample—7×10^4 (3%)
III	—	—	Growth seen in three samples 1st sample—1×10^5 cfu/ml 2nd sample—3×10^4 cfu/ml 3rd sample—8×10^4 cfu/ml (10%)
IV	—	—	No growth

DISCUSSION

Root canal irrigation plays a key role in the success of endodontic treatment, because it helps in the progressive removal of the smear layer and neutralizes the root canal microbic flora.[6] Results of the present study reinforced this concept.

This *in vitro* study was designed to assess the antibacterial effects of various root canal irrigants to *Enterococcus faecalis*. Various root canal irrigants tested were:

- 5.25% sodium hypochlorite
- 0.2% chlorhexidine gluconate
- 0.2% chlorhexidine gluconate and 0.2% cetrimide
- 5.25% sodium hypochlorite and 0.2% chlorhexidine gluconate.

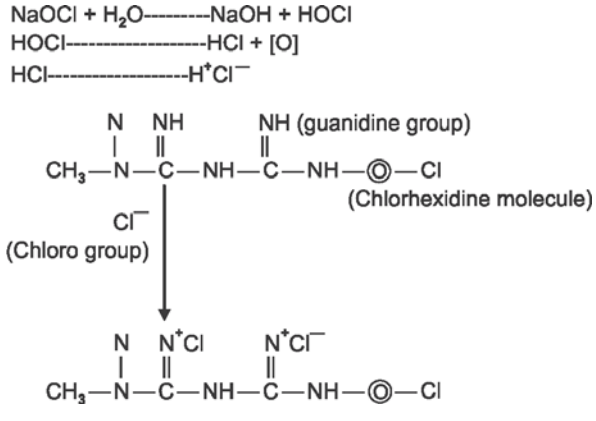
Enterococcus faecalis, a facultative anaerobic Gram-positive coccus, has been implicated in persistent root canal infections and has been used in several previous studies on the efficacy of endodontic irrigants, especially for its high level of resistance to a wide range of antimicrobial agents. In the present study, we used ATCC strain because it was also utilized in previous *in vitro* studies, investigating the antibacterial effects of endodontic irrigants.[7]

Mueller-Hinton medium was preferred in this study, as it supports the growth of most obligate anaerobes. This medium contains beef infusion, Bacto casamino acids, starch and Bacto agar.[8]

The irrigating solutions used in this study have been accepted as root canal irrigants for endodontic usage.[5] Sodium hypochlorite has been used for a long time in endodontics as a root canal irrigating solution at different concentrations ranging from 0.5 to 5%.[9] About 5% of sodium hypochlorite has been shown to dissolve necrotic pulpal tissue faster than 2.5% sodium hypochlorite or lower concentrations. In addition, other studies have shown a decrease in the tissue dissolving capability of sodium hypochlorite with a decrease in concentration. To obtain maximum benefit in necrotic tissue dissolution, full strength sodium hypochlorite would be ideal.[10] Its established clinical use results from its ability to dissolve necrotic tissue and organic remnants, its antimicrobial activity, and its low toxicity at low concentrations. On the other hand, sodium hypochlorite presents negative properties, such as corrosion of endodontic instruments, ineffectiveness for some microorganisms when used at low concentrations, and not differentiating between necrotic and vital tissue when in contact with apical and periapical tissue.[9]

Chlorhexidine is a cationic bisbiguanide with optimal antimicrobial action ranging from pH 5.55 to 7.0. It is active against a wide range of microorganisms, such as Gram-positive and Gram-negative bacteria, bacterial spores, lipophilic virus, yeast and dermatophytes being bacteriostatic at low concentrations and bactericidal at high concentrations. Furthermore, chlorhexidine adsorbs to surfaces covered with acidic proteins, such as hydroxyapatite, and is gradually released in the form of an active cation (substantivity), justifying its clinical use as a root canal irrigating solution at different concentrations *in vitro* and *in vivo*.[9] The efficacy of chlorhexidine as root canal irrigant was proved for the first time by parson et al. In that study, chlorhexidine was absorbed and released from dentin and enamel even after 48 to 72 hours of instrumentation.[6]

The results of present study confirms that the use of sodium hypochlorite and chlorhexidine gluconate combined within the root canal result in the greatest percentage reduction of postirrigant positive culture, which is consistent with previous study.[5] Chlorhexidine is a base, in itself capable of forming salts with a number of organic acids. Sodium hypochlorite is an oxidizing agent that may be capable of oxidizing the gluconate part of chlorhexidine gluconate to gluconic acid. The chloro groups might get added onto the guanidine component of the chlorhexidine molecule, thereby forming "chlorhexidine chloride". This reaction may be depicted as follows:



If this was to happen, it would increase the ionizing capacity of the chlorhexidine molecule, and the solution would incline toward an alkaline pH. This was evident when the pH of sodium hypochlorite solution, chlorhexidine gluconate solution, and their combination was recorded using pH meter. The pH was 2.5% for NaOCl—9, 0.2% chlorhexidine gluconate—6.5, and combination—10. It is a known fact that the ionized species exert better antibacterial action than the unionized species. Further studies to validate the above findings would be of interest.[5]

Cetrimide is good tensioactive agent with consistent bactericidal properties.[6] Cetrimide added to chlorhexidine can easily and thoroughly penetrate into the root canals and dentine tubules. The results obtained from present study demonstrated that there was no significance between the antibacterial effects of cetrexidine (0.2% clorhexidine gluconate and 0.2% cetrimide) and 0.2% chlorhexidine gluconate, which are consistent with previous studies.[1]

The results obtained from this study indicate that there was significant difference between the antibacterial effects of 5.25% sodium hypochlorite, 5.25% sodium hypochlorite and 0.2% chlorhexidine gluconate, and 0.25% chlorhexidine gluconate alone and 0.2% chlorhexidine glulconate and 0.2% cetrimide. This study showed that 5.25% sodium hypochlorite, and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate had a greater antibacterial effect than 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine glulconate with 0.2% cetrimide. This difference could be due to the use of more dilute (0.2%) chlorhexidine gluconate and the cetrimide substance (0.2%) in cetrixidine.[1] The results of this study are in confirmation with various *in vivo* and *in vitro* studies.[1,5,10]

The antibacterial effectiveness of irrigants in root canal treatment may be different from the results of *in vitro* studies because of the dynamic biological environment with mixed bacterial cultures that usually occurs *in vivo*. Further *in vivo* (clinical) tests will be necessary to determine whether the results *in vitro* will be validated.

CONCLUSIONS

Following conclusions were derived from this study:

1. 5.25% sodium hypochlorite and 5.25% sodium hypochlorite with 0.2% chlorhexidine gluconate had a higher antibacterial effect than 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine gluconate with 0.2% cetrimide
2. There was no significant difference between the antibacterial efficacies of 0.2% chlorhexidine gluconate alone and 0.2% chlorhexidine with 0.2% cetrimide.

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