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Antimicrobial efficacy of various irrigating solutions on *E. faecalis* in root canals: an *in-vitro* study

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Abstract

This study was done to test the antimicrobial efficiency of four irrigating solutions such as chlorhexidine (CHX), Oxum, Ozonated water and normal saline, against the *E. Faecalis* microorganisms in root canals. Forty freshly extracted single-rooted human teeth were selected. 20 µL of the suspension was inoculated into each of 40 root canals. The specimens were incubated at 37 °C for 7 days. After 48 h of incubation at 37 °C, microbial growth was verified, confirming the contamination of the root canals. They were enlarged to a size 80 K-file (Maillefer). Instrumentation was followed by irrigation with 3 mL of the above irrigating solutions for each file used. After the instrumentation, the first sampling was done immediately followed by second sampling on 3rd and third sampling on 7th day from the root canals. Within the limitations of this study it was concluded that chlorhexidine significantly reduced the number of *E. faecalis* followed by oxum and ozonated water was observed.

Keywords: Antimicrobial efficacy, various, *E. faecalis*, root canals

Introduction

Microorganisms and their by products are the main cause of pulpal and periapical infections, and their elimination during endodontic treatment is of utmost importance for promoting a favorable environment for periapical lesion healing. Among the microorganisms commonly isolated from root canals, *E. faecalis* is a gram positive facultative anaerobic bacterium frequently isolated from persistent root canal infections. It has the ability to penetrate the dentinal tubules, exhibits strong adhesion to collagen, and shows resistance to irrigation solutions usually used during the instrumentation of root canals. The remaining microorganisms and their by products might induce or maintain periapical lesions, predisposing to unsuccessful endodontic treatment.

Root canal irrigants are used during chemico mechanical procedures not only as antimicrobial agents but also to flush out loose debris, lubricate the dentinal walls and to dissolve organic compounds in the canal. Several irrigants, such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), hydrogen peroxide (H₂O₂), and normal saline, have been proposed as endodontic irrigants of the root canal system.

Chlorhexidine gluconate, a cationic biguanide has been used as an endodontic irrigant due to its broad spectrum antimicrobial action, substantivity, and low grade of toxicity. Ozone is a very reactive gas that shows important antimicrobial properties. It is found in natural form in the atmosphere or it can be produced by generators. This gas oxidates bacterial cell walls and cytoplasmic membranes and acts on fungi, protozoa, and viruses. It forms oxidated radicals in the presence of water that penetrate and act on cell membranes, affecting the osmotic stability, promoting the oxidation of aminoacids and nucleic acids, and causing cellular lysis depending on the reaction's extension. The uses of ozone in various areas of dentistry is periodontal pocket disinfection, remineralization, accelerated healing, prevention of caries.

Super oxidized water has been widely studied in the endodontic practice as an effective antimicrobial means for irrigating the root canal. Microcyn (oxum) is a superoxidized solution with a neutral pH and a longer shelf life. This is a hypotonic solution with an osmolarity of 13 mOsm/kg containing Hypochlorous acid, Sodium hypochlorite, Chlorine dioxide, Ozone, Hydrogen peroxide, and Sodium chloride.

Aim of the study

To determine the antimicrobial efficacy of chlorhexidine, Oxum, Ozonated water in root canals infected by *Enterococcus faecalis*.

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Material and Methods

Ozonated water preparation

For the preparation of ozonated water, a generator was used. The ozone was generated by an electrical discharge on high-purity oxygen molecules. Ozonation of the water was performed by bubbling ozone through sterile distilled water (O₃ concentration 24 mg/L).

Selection and standardization of teeth

Forty freshly extracted single-rooted human teeth were used. Root surfaces were cleaned using a Gracey curette, and all teeth were stored in physiologic saline solution (NaCl 0.85%) until use.

Crowns were sectioned with a diamond disk and the root lengths were standardized to 14 mm. Gates-Glidden drill #3 were used to prepare the root canal orifices (3 mm). The specimens with apical diameters smaller than 10 K-file or greater than 20 K-file were discharged.

For standardization of root canal diameter, initial enlargement was accomplished with a 35 K-file (Maillefer, dentsply) followed by irrigation with 3 mL 1% sodium hypochlorite solution for each file used. After initial preparation, root canals were filled with 17% EDTA for 3 min, followed by final irrigation with 5 mL physiologic saline solution.

Canals were dried with sterile paper points (Dentsply) and apical region was sealed with light-cured resin composites (Z100, 3M Dental Products, St. Paul, MN). The outer surfaces of the specimens were covered with 2 layers of epoxy adhesive resin except for the cervical opening. All the specimens were sterilized by autoclaving (15 min at 121 °C) and divided into 4 groups with 10 teeth each. The teeth were stabilized with chemically activated acrylic resin.

Inoculation of E. faecalis in root canals

All the microbiologic procedures were performed under aseptic conditions. Standardized suspensions (1.5 × 10⁸ cells/mL) of *E. faecalis* ATCC 29212 were spectrophotometrically obtained. Then, 20 µL of the suspension was inoculated into each of 40 root canals with the aid of an automatic micropipette (Gison). A sterile cotton ball was soaked in the microbial suspension and placed in the

cervical third of the canals. Cervical access was sealed with gutta-percha stick. The specimens were incubated at 37 °C for 7 days. During this period, tryptic soy broth (Difco, Detroit, MI) was added to the canals with the aid of a 2ml of syringe for every 3 days, followed by sealing the cervical opening with gutta-percha stick.

Seven days after the inoculation, contamination confirmation sampling was performed using #35 sterile paper points (Dentsply) was placed inside the root canal for 1 min and then transferred to test tubes (Eppendorf) containing 1 mL sterile physiologic solution. The tubes were submitted to agitation for 30 s (Vortex App). Aliquots of 0.1 mL were plated in duplicate on Sabouraud dextrose or *Mitis salivarius* agar (Difco). After 48 h of incubation at 37 °C, microbial growth was verified, confirming the contamination of the root canals.

Root canals were enlarged to a size 80 K-file (Maillefer). Instrumentation was followed by irrigation with 3 mL of the above irrigating solutions for each file used. The irrigation was performed with the aid of apyrogenic syringe with apyrogenic needle with a gauge of

Immediately after the instrumentation, the first sampling of the root canals was performed as described earlier. After the first microbial sampling, roots were filled with irrigating solutions. Specimens were sealed with temporary cement and incubated for 7 days at 37 °C. After this period, the second sampling was performed on 7th day. Third sampling was done on 10th day. The number of viable *E. faecalis* cells was obtained (number of colony-forming units per mL; CFU/mL).

Results were analyzed statistically by using One-way Anova and F-test.

Groups

Experimental group	Irrigation solution	No of roots
I	Ozonated water	10
II	Oxum	10
III	2% Chlorhexidine	10
IV	Saline(control)	10

Results

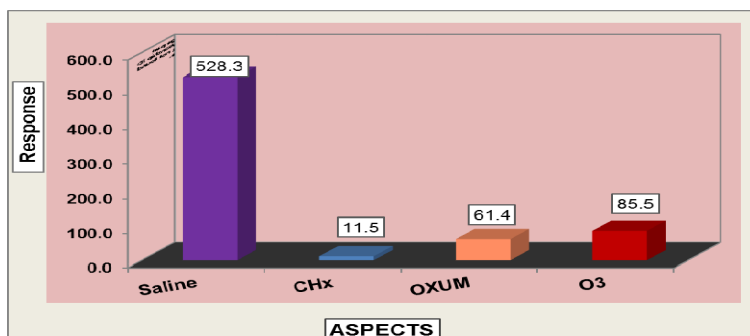
Table 1: Response of various irrigating solutions over duration

Duration	Saline Response (10 ⁶)		CHx Response (10 ⁶)		Oxum Response (10 ⁶)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre test	554.8 ^a	4.57	552.6 ^a	3.24	552.6 ^a	4.88	557.7 ^a	6.27
7 days	541.5 ^b	4.38	10.4 ^b	5.15	292.3 ^b	6.48	426.7 ^b	6.95
10 days	551.9 ^c	6.5	111.0 ^b	3.06	440.2 ^c	6.75	544.4 ^c	4.74
Post test	528.3 ^d	3.50	11.5 ^b	4.25	61.4 ^d	2.95	85.5 ^d	6.40
F-Test	*							
SEm±	1.536		1.265		1.732		1.949	
CD at 5% level	4.258		3.506		4.801		5.402	

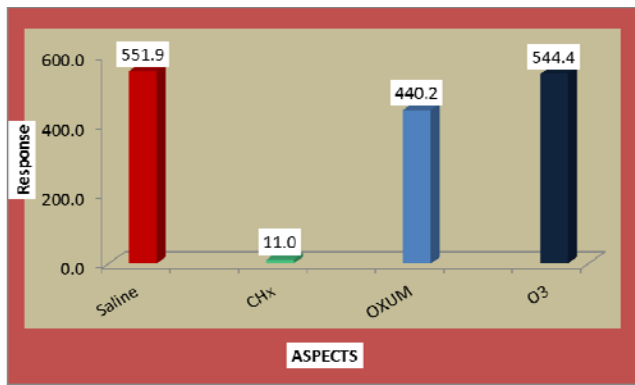
Note: Different letter indicate significant difference,

* Significant at 5% level

Graphs



Graph 1: Comparative Response of Factors during Post test



Graph 2: Comparative Response of Factors during 10th day

Discussion

The primary endodontic treatment goal must thus be to optimize root canal disinfection and to prevent reinfection. Bacteria have long been recognized as the primary etiologic factors in the development of pulp and periapical lesions. Success root canal therapy depends on thorough chemo-mechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. Irrigants can augment mechanical debridement by flushing out debris, dissolving tissue, and disinfecting the root canal system. Chemical debridement is especially needed for teeth with complex internal anatomy such as fins or other irregularities that might be missed by instrumentation. An ideal irrigating solution should possess maximal antimicrobial and tissue dissolving properties and minimal toxic effects.

Chlorhexidine digluconate is widely used in disinfection because of its excellent antimicrobial activity, its antibacterial and antifungal activity, its effect on biofilm, its substantivity (residual antibacterial activity), its tissue solvent ability, its interaction with calcium hydroxide and sodium hypochlorite, its anticollagenolytic activity, its effect on coronal and apical leakage of bacteria, its toxicity and allergenicity and the modulating effect of dentine and root canal components on its antimicrobial activity.

Superoxidized Solutions have shown to be both safe and efficient as a wound care product that moistens, lubricates, debrides and reduces the microbial load of various types of lesions. They are electrochemically processed aqueous solutions manufactured from pure solutions which is rich in reactive oxygen species with neutral pH and longer half life (>12 months). Oxum is a stable, non-flammable and non-corrosive bactericidal, virucidal, fungicidal and sporicidal solution that is ready to use with no further dilution or mixing. During the last decade it has been widely studied in the endodontic practice as an effective antimicrobial means for irrigating the root canal. Super Oxidized Solution is an electrochemically processed aqueous solution manufactured from pure water and sodium chloride. During this electrolysis process reactive species of oxygen and chlorine are formed. These released reactive species creates an unbalanced osmolarity, so that it damages the integrity of the cell membrane, then reacts and denatures the lipids & proteins of single cell organisms. This is because of a direct result of the osmolarity difference between the ion concentrations of the solution and single cell organism. Multicellular organisms are not prone to such osmolarity changes.

Ozone presents antimicrobial properties that are used in food industry and treatment of diseases such as arthritis, otitis and ulcers. The ozone (O₃) produced by an electrical discharge on high-purity oxygen molecules with a generator. Since gaseous ozone has been established to get toxic action if inhaled into

the respiratory system, therefore, ozonated water might be beneficial to management oral infections and varied pathogens. Gaining ozone in water was made by bubbling ozone through sterile distilled water (O₃ concentration 24 mg/L). The selection of the ozonated water concentration (24 mg/L) was according to the higher concentration, which the generator can produce. Ozonated water usage for therapy of endodontic infections has been recommended.

In the present study we found a significant decrease in the number of CFU of *E. faecalis* in the first when treated with CHX followed by Oxum, Ozonated water and saline. These results are in accordance with the result of some other investigators. Accor Onçag *et al.* reported that 5 min 2% CHX had a faster and more effective action on *E. faecalis* compared NaOCl. Satish *et al.* concluded that Oxum is Bactericidal, Fungicidal, Virucidal and Sporicidal During the electrolysis process, solutions molecules are broken, ions and free radicals are formed. They rapidly react and denature proteins of bacterial cell wall, have anti-inflammatory effect, produce an environment with an unbalanced osmolarity that damages single cell organism. Nagayoshi *et al.* concluded that ozonated water had almost the equal antimicrobial effectiveness as 2.5% NaOCl for endodontic irrigation. They also showed that a lower grade of toxicity against bacterial cells. Among the current irrigating solutions, ozone has some interesting features; debriding action, bactericidal effect, angiogenesis stimulation capability and high oxidizing power. In addition, as far as we know, ozonated water has not been studied as endodontic irrigation agent.

In the second sampling and third sampling the CFU of *E. faecalis* was significantly reduced in CHX. White *et al.* claimed that, after instrumentation, CHX continues to be released while 48–72 hrs. CHX preserved antibacterial effect. The stability of antibacterial efficacy of endodontic irrigants, especially in prolonged periods of treatment, is very important. Leonardo *et al.* concluded that CHX gluconate has been recommended as an irrigation solution because of its antibacterial effectiveness, substantivity and lower cytotoxicity compared with other irrigating solutions. Satish *et al.* claimed Oxum causing damage is a direct result of the osmolarity difference between the concentrations of the ions in the solution versus the concentration of the same ions in the cell. Multicellular organisms are not prone to such osmolarity changes, therefore host tissues are spared. Once the single cell membrane is damaged, the ions in the product denature the bacterial proteins as well. However O₃ groups' showed decreased antimicrobial effectiveness rapidly compared with other groups. We concluded that the ozonated water had no residual effect during the study. This may be correlated to poor diffusion ability of these substances to deeper areas of the dentinal tubules. Rapid deterioration of the ozone just after contact with organic compounds which is one of its environmental disadvantages, may cause a decrease in antimicrobial effectiveness of ozonated water. Furthermore, Cardoso *et al.* reported that the ozonated water was efficient against *E. faecalis* but had limited residual effect. Haas and Kaymak reported that the antimicrobial effect of ozone depends on varied factors, such as ozone concentration and quantity of bacteria, exposure period, and variables in the bacterial permeability that verified the occurrence of diverse effects.

Conclusion

Within the limitations of this study it was concluded that chlorhexidine significantly reduced the number of *E. faecalis*

followed by oxum and ozonated water immediately was observed. Although there was a significant increase in bacterial count in both oxum and ozonated groups in the second and third sampling.



Fig 1: Samples after inoculation

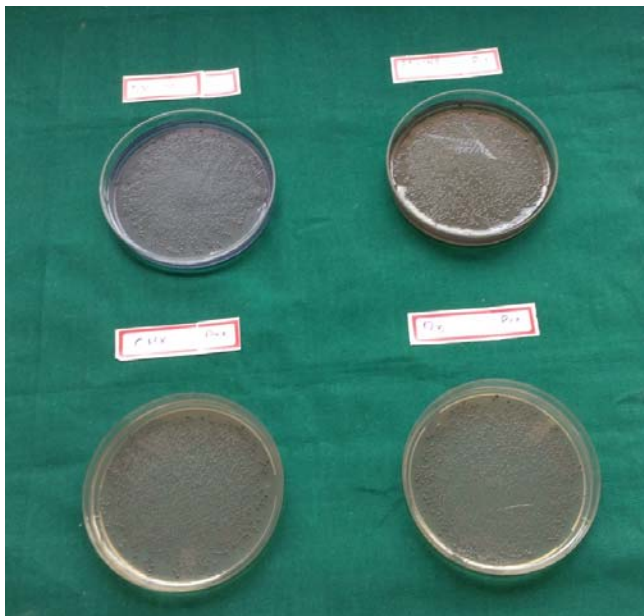


Fig 2: Growth of E. faecalis on the culture plates of various groups of irrigating solution



Fig 3: Armamentarium

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