

Comparative Evaluation of Antimicrobial Efficacy of Three Irrigating Solutions for Root Canal Treatment - An *In Vitro* Study

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Abstract

Background: Complete elimination of microorganisms from root canal system is one of the most important objectives in endodontic therapy. This cannot be achieved until all the pulp tissue, and bacteria's present in dentinal tubules have been removed. Use of irrigating solution plays an important role in cleaning and shaping of the root canal. Sodium hypochlorite along with various other irritants has been used successfully in the removal of debris and killing microorganisms.

Aims and Objectives: To compare the antimicrobial efficacy of three different type of irrigating solutions.

Materials and Methods: Twenty-three pairs of extracted single-rooted teeth were taken for the study. Crown was removed till cemento-enamel junction. Each set was stored in a sterilized vial containing brain heart infusion broth. *Enterococcus faecalis* was cultured in this vial for 4 weeks. After 4 weeks, cleaning and shaping of the root canals were done followed by irrigation of 20 teeth by 3% NaOCl/BioPure MTAD and 20 teeth by 5.25% NaOCl/hydrogen peroxide. 3 pairs were kept as control. Sampling of the teeth was done and was placed in BHI agar culture where the growth of bacteria was studied.

Results/Conclusion: About 5.25% NaOCl/hydrogen peroxide had a better antimicrobial action than 3% NaOCl/BioPure MTAD.

Key words: Chemomechanical preparation, *Enterococcus faecalis*, Irrigants, MTAD

INTRODUCTION

The most important objective of endodontic therapy is the complete eradication of microbes from the root canal system.¹ Only chemomechanical preparation of root canal cannot eliminate all the microorganisms from root canal system; it may leave some areas of root canal system completely untouched by the instrument which are located deep in dentinal tubules.² According to Grossman, a thorough instrumentation and biomechanical preparation is the most important part of root canal therapy.³ Use of irrigants helps effectively in disinfecting the root canal. Many of the compounds used for irrigation have

been chemically modified for the effective disinfection of root canal. Enterococci are Gram-positive cocci which are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. *Enterococcus faecalis* is associated with different forms of pulpal and periapical diseases.⁴ Various concentrations of NaOCl have been used as root canal irrigants for many decades because of its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms. Its main disadvantages are its unpleasant taste and its toxicity if extruded beyond the apex. BioPure MTAD- a mixture of doxycycline, citric acid and a detergent is less toxic than NaOCl, and it effectively removes a smear layer present in the root canal. Hydrogen peroxide (H₂O₂) is an irrigating solution which degrades to form H₂O and O₂. It is effective against viruses, bacteria and bacterial spores and yeasts through hydroxyl free radicals. The purpose of this study was to compare the antimicrobial efficacy of irrigating solutions which includes 3% NaOCl/MTAD and 5% NaOCl/3% H₂O₂.

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MATERIALS AND METHODS

Materials used in the study are:

1. 3% NaOCL
2. BioPure MTAD
3. 5.25% NaOCL
4. 3% H₂O₂.

Twenty-three pairs of mature human premolar teeth with closed apices, extracted for orthodontic treatment were collected. Teeth were soaked in 0.5% NaOCl for 24 h to remove residual loose tissue and debris. Crowns of teeth were reduced to cemento-enamel junction. Apex of each tooth was sealed with cyanoacrylate and mounted vertically in dental stone blocks. Teeth were then subjected to endodontic therapy. Access opening was prepared, and pulp was removed with barbed broach. Biomechanical preparation was done using standardized technique to a master apical file size of #40. Each tooth was steam autoclaved for 30 min under 15 psi pressure at 121°C. Each tooth was placed in sterilized vial, immersed in sterile brain heart infusion broth in which pure culture of *E. faecalis* was grown and sealed to contaminate the root canals. 3 pairs of teeth served as a negative control. 5 ml of the culture was added to the 20 ml vial containing BHI broth and sterilized tooth. The teeth were incubated with *E. faecalis* for 4 weeks under aerobic conditions at 37°. Fresh media was added every 7th day. After 4 weeks teeth were removed from the broth. Microbial samplings were taken. Aliquots of 0.1 ml suspension were plated on to the BHI agar plates and incubated at 37°C for 48 h. Colony-forming units (CFUs) per 1ml were enumerated.

RESULTS

All samples in negative control showed absence of turbidity throughout the experimental period while all the samples in positive control showed the presence of bacterial growth. The study showed a significant difference ($P < 0.01$) between total number of CFUs. Teeth treated with 5.25% of NaOCL/hydrogen peroxide produced 2-3 CFU/ml, while those treated with 3% NaOCl/BioPure MTAD produced a mean of 19 ± 8 CFU/ml. 3 of the 20 samples in Group A produced growth while 8 of 20 samples in Group B yielded growth (Table 1).

- Smallest of value is 6, Wilcoxon T statistic is 6
- Calculate: $T = n(n+1)/4 = 20(20+1)/4 = 105$.

Observed Wilcoxon T (i.e., 6) differs significantly from T (105).

DISCUSSION

Studies have demonstrated conclusively that mechanical instrumentation cannot sufficiently disinfect root

Table 1: Growth of Microorganism

I (5.25% of NaOCL/ hydrogen peroxide)	II (3% NaOCl/BioPure MTAD)	Difference (I-II)	Rank
0	0	0	0
0	0	0	0
0	17	-17	-6.5
0	12	-12	-4.5
0	0	0	0
0	19	-19	-10
0	0	0	0
3	0	+3	3
0	21	-21	-11
0	0	0	0
2	0	+2	1.5
0	0	0	0
0	18	-18	-8.5
0	17	-17	-6.5
0	12	-12	-4.5
0	0	0	0
2	0	+2	1.5
0	26	-26	-12
0	0	0	0
0	18	-18	-8.5

Total number of negative values=72

Total number of positive values=6

canals, regardless of whether stainless steel⁵ or NiTi Instrumentation.⁶ Irrigation solutions are required to eradicate microbiota, and over time, a variety of chemicals have been promoted for this purpose.

The ideal irrigant or combination of irrigants kills bacteria, dissolves necrotic tissue, lubricates the canal, removes smear layer and does not irritate healthy tissues.¹ It is well established that failure of endodontic therapy and pulp and periapical diseases are due to the presence of microbes. The intent of the study was to determine whether irrigation with 3% NaOCl/BioPure MTAD was more effective than 5.25% NaOCl/Hydrogen peroxide.

E. faecalis was chosen as test organism because it has been associated with persistent apical inflammation and has found suitable for experimental penetration into the dentinal tubules. It is an unrelating organism that, despite making up a small fraction of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesion after orthograde endodontic therapy. They have shown resistance to calcium hydroxide and number of intracanal medicaments. Thus an irrigant effective against it is desirable. 4 weeks of incubation was done to ensure adequate penetration of bacteria into the dentinal tubules.

NaOCl used in the study have been commonly used for irrigation of root canal in different concentrations. These are effective against bacterial spores and viruses and have higher tissue dissolving effect on necrotic than on vital tissues. However, it has various disadvantages including its tendency to induce an inflammatory reaction

in tissues when extruded beyond apex of the root. It may also damage the patient's clothing, a foul odor, and an unpleasant taste. Due to these biocompatibility issues, different irrigants have been tried.

BioPure MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA) was introduced by Torabinejad and Johnson in 2003.⁷ This solution contains 3% doxycycline hyclate which helps to remove smear layer, tetracycline for sustainability, 4.25% citric acid which acts as a chelating agent and a detergent 0.5% polysorbate 80 which reduces the surface tension and increases its wettability.

About 3% H₂O₂ is a clear, odorless liquid. It is easily decomposed by heat and light and rapidly dissociates into H₂O and [O]. This nascent oxygen produces a bactericidal effect by interfering with bacterial metabolism. Its action is more effective when used in combination with 5.2% NaOCl.

Lack of turbidity of BHI by negative control groups demonstrated sterilization procedures were effective; positive control group confirm the presence of *E. faecalis* within the root canal.

The apices of all samples were sealed with cyanoacrylate to prevent any contamination from outer tooth surface during the sampling procedure.

The results of this study showed that 5.25% NaOCl/ Hydrogen peroxide significantly reduced intra canal bacterial levels when compared to 3% NaOCl/BioPure MTAD.

2 of the 20 samples in group A showed a growth, while 8 of the 20 samples in group B yielded growth.

It has been shown that invasion of dentinal tubules by *E. faecalis* usually occurs to a depth of 50-100 µm.

Culturing the color contents at a greater depth allowed determination of the efficacy to the test irrigant at penetrating and disinfecting deeper layers of dentin. The results of the study showed that 5.25% NaOCl/hydrogen peroxide significantly reduced intra canal bacterial levels when compared to 3% NaOCl/BioPure MTAD.⁸⁻¹¹

CONCLUSION

The results of this *in vitro* investigation showed consistent disinfection of infected root canals with 5.25% NaOCl/hydrogen peroxide. The combination of 3% NaOCl/BioPure MTAD left nearly 50% of the canals contaminated with *E. faecalis*.

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