

REVIEW ARTICLE

A Comparative In-Vitro Evaluation of Different Irrigating Solutions Against Enterococcus faecalis Present in Biofilm of Root Canal

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ABSTRACT

Background: Enterococcus faecalis is a resistant, biofilm-forming microorganism frequently associated with persistent endodontic infection. Effective chemical irrigation is therefore important for root canal disinfection.

Aim: To evaluate and compare the antimicrobial efficacy of MTAD, 2% chlorhexidine, 5% acetic acid and 5% sodium hypochlorite against E. faecalis in root-canal biofilm.

Methods: Sixty-five freshly extracted human mandibular premolars were collected. Sixty teeth were allocated to four experimental groups (n=15 each) and five teeth served as a positive control. Root canals were prepared to a master apical file size #40, inoculated with E. faecalis ATCC 29212, and irrigated with 5 mL of the assigned solution for 5 min. Bacterial recovery was assessed by colony counting on 5% sheep blood agar. Data were summarized as mean $\pm$ SD and median and analysed using nonparametric rank-based comparisons and z tests; $P < 0.05$ was considered statistically significant.

Results: Mean E. faecalis colony counts were 0.53 ± 2.07 CFU 1/mL for MTAD, 30.60 ± 6.33 for 2% chlorhexidine, 39.40 ± 8.38 for 5% acetic acid and 16.00 ± 5.28 for 5% sodium hypochlorite. MTAD showed significantly lower colony counts than 2% chlorhexidine and 5% acetic acid ($P < 0.001$). The descending order of antimicrobial activity was MTAD > 5% sodium hypochlorite > 2% chlorhexidine > 5% acetic acid.

Conclusion: MTAD demonstrated the greatest antimicrobial efficacy among the tested irrigants under the conditions of this in-vitro study. None of the tested irrigants completely eradicated E. faecalis from the root canal.

Introduction

Endodontic therapy aims to eliminate microorganisms and their by-products from the

root canal system. Although mechanical instrumentation is one of the main steps in root canal treatment, it cannot achieve complete removal of

microorganisms [1]. *Enterococcus faecalis* is a Gram-positive facultative anaerobe that is thought to be one of the most resistant species in infected root canals and is frequently linked to failure of endodontic therapy. Difficulty in its removal may be related to inherent antimicrobial resistance, deep penetration into dentinal tubules and biofilm formation [2]. Owing to complex root canal anatomy, antimicrobial irrigation solutions are critical during cleaning and shaping. They can penetrate dentinal tubules, reach canal ramifications and other difficult areas, and therefore act as an adjunct to mechanical instrumentation [3]. MTAD (mixture of tetracycline isomer, citric acid and detergent) is a comparatively newer irrigating solution used as a final rinse for smear-layer removal and antimicrobial action against resistant microorganisms such as *E. faecalis* [4]. Sodium hypochlorite is widely used because of its antimicrobial activity and ability to dissolve organic matter. Chlorhexidine is used at concentrations of 0.2% to 2%, with 2% commonly preferred for its antimicrobial effect and substantivity. Acetic acid has been studied as a mild chelating and antimicrobial solution [5].

Aims and Objectives

The aim of this study was to evaluate the antimicrobial efficiency of MTAD, 2% chlorhexidine, 5% acetic acid and 5% sodium hypochlorite against *E. faecalis*.

Objectives:

- To evaluate the antimicrobial efficiency of MTAD against *E. faecalis*.
- To evaluate the antimicrobial efficiency of 2% chlorhexidine against *E. faecalis*.
- To evaluate the antimicrobial efficiency of 5% acetic acid against *E. faecalis*.
- To evaluate the antimicrobial efficiency of 5% sodium hypochlorite against *E. faecalis*.
- To compare which irrigating solution provides the highest antibacterial effect against *E. faecalis*.

Materials

Study Sample

The present in-vitro analysis compared the

antimicrobial efficacy of four irrigating solutions against *E. faecalis* in root-canal biofilm. Sixty-five freshly extracted human mandibular premolars with fully developed root apices were collected after ethical approval. The teeth were cleaned of calculus and soft-tissue debris and disinfected with 0.5% sodium hypochlorite for 24 h followed by 0.9% normal saline until instrumentation.

Inclusion Criteria

- Mandibular premolars with straight roots • Single canal
- Non-carious tooth
- Completely formed apex

Exclusion Criteria

- Mutilated teeth
- Fractured teeth
- Teeth with root resorption
- Teeth with more than one canal

Armamentarium and Materials

- Airotor handpiece (Waldent) and carbide disc for decoronation.
 - K-file (Waldent), Endoblock, side-vented needle, 5.25% sodium hypochlorite, 17% EDTA (Waldent) and paper points.
 - Autoclave, centrifugal machine, biosafety cabinet, laminar airflow, thermocol plates and glass tubes.
 - 5% sheep blood agar plates, micropipette, inoculating wire and incubator.
 - Digital colony counter for colony-count analysis.
- ### Irrigating Solutions
- MTAD (mixture of tetracycline isomer, citric acid and detergent)
 - 2% chlorhexidine
 - 5% acetic acid
 - 5% sodium hypochlorite

Sample Size

The sample size was based on the expected mean difference and standard deviation in antimicrobial efficacy between groups, with $\alpha = 0.05$, 80% power and a 1:1 ratio. The calculated minimum sample size was 60 specimens, corresponding to 15 samples per experimental group.

Methodology

Sixty-five freshly extracted mandibular premolars were decoronated at the cemento-enamel junction to obtain a standardized root length of 14 mm. Working length was determined using a size 10 K-file, and the apices were sealed with adhesive to prevent extrusion of irrigating solutions. Working length was confirmed by subtracting 1 mm from the length of the apical foramen. Root canals were prepared by hand instrumentation up to master apical file #40. Seventeen percent EDTA and normal saline were used with disposable side-vented needles for smear layer removal. After sterilization, all specimens were transported and manipulated under aseptic conditions using sterile instruments and equipment. Prepared root specimens were autoclaved in glass tubes containing 3 mL BHI broth at 121°C for 20 min and incubated for 24 h at 37°C to confirm sterility. Pure culture of *E. faecalis* (ATCC 29212) grown in BHI broth for 24 h at 37°C was used to contaminate the root canals. A 10- μ L inoculum was introduced into the prepared canals, and the teeth were incubated for 48 h. The teeth were randomly divided into four experimental groups of 15 samples each and one positive-control group of five samples. Experimental groups were irrigated with 5 mL of the respective solution for 5 min. The positive-control group was irrigated with 5 mL sterile normal saline for 5 min. Size 35 paper points were inserted to 1 mm short of working length for 10 s and then transferred into tubes containing 10 μ L sterile normal saline and vortexed for 1 min. Samples were cultured using a standard loop technique on 5% sheep blood agar and incubated at 37°C for 48 h under aerobic conditions. Colony counts were obtained using a digital colony counter.

Observation and Results

The *E. faecalis* colony count of four groups was summarized in Mean $\pm$ SD, median, sum of ranks and mean sum of ranks. (Table 1) The *E. faecalis* colony count of Group I, Group II, Group III and Group IV ranged from 0-8, 20-40, 30-55 and 10-25 (CFU 1/ml) respectively with mean ($\pm$ SD) 0.53 ± 2.07 , 30.60 ± 6.33 , 39.40 ± 8.38 and 16.00 ± 5.28 (CFU 1/ml) respectively and median 0, 30, 41 and 15 (CFU 1/ml) respectively. The mean (or median) of *E. faecalis* colony count of Group III was the maximum followed by Group II, Group IV and Group I, the minimum (Group I < Group IV < Group II < Group III).

Randomised controlled clinical trials have assessed the role of probiotics as an adjunct to mechanical debridement in the treatment of peri-implant mucositis. One notable study found that the addition of *Lactobacillus reuteri* lozenges led to significant reductions in bleeding on probing and peri-implant inflammation compared to a placebo [6]. Subsequent clinical investigations reported improvements in plaque index, bleeding on probing, and peri-implant probing depths following short-term probiotic supplementation, suggesting a notable enhancement in the resolution of soft tissue inflammation [7].

Discussion

This study evaluated whether MTAD offers superior antimicrobial efficacy compared with conventional irrigants against a resistant, biofilm-forming organism such as *E. faecalis* [6]. At an exposure time of 5 min, antimicrobial activity decreased in the order Group I > Group IV > Group II > Group III > Group V. The superior antimicrobial performance of MTAD observed in the present study may be attributed to its combination of doxycycline/tetracycline isomer, citric acid and detergent, which facilitates penetration and biofilm disruption [7]. 5% sodium hypochlorite demonstrated the second-highest antimicrobial efficacy after MTAD [8]. Sodium hypochlorite is widely used because of its antimicrobial activity, tissue dissolving capacity and ability to disrupt organic biofilms [9]. 2% chlorhexidine showed moderate antimicrobial efficacy and performed better than 5% acetic acid but was inferior to MTAD and sodium hypochlorite [10]. Chlorhexidine has broad spectrum antimicrobial activity and substantivity, although it lacks tissue-dissolving capability [11]. Acetic acid

showed the least antimicrobial efficacy among the experimental groups. Although it has mild chelating properties and may contribute to smear-layer removal, its antibacterial effect appeared insufficient for complete root-canal disinfection under the conditions of the present study [12]. The findings support the importance of combining mechanical preparation with effective chemical irrigation. The descending order of antimicrobial efficacy was MTAD > 5% sodium hypochlorite > 2% chlorhexidine > 5% acetic acid > positive control [13].

Conclusion

MTAD (Group I) was the most effective antimicrobial agent among the experimental groups. 5% sodium hypochlorite (Group IV) was less efficacious than MTAD but more effective than 2% chlorhexidine and 5% acetic acid. 2% chlorhexidine (Group II) was less effective than 5% sodium hypochlorite but more effective than 5% acetic acid. 5% acetic acid was the least effective experimental irrigant but demonstrated antibacterial activity against *E. faecalis*. The positive-control group showed no antimicrobial activity because no antimicrobial irrigant was used. Overall antimicrobial action was Group I > Group IV > Group II > Group III > Group V. None of the tested irrigating solutions completely eradicated *E. faecalis* from the root canal.

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Tables

Group	n	Mean $\pm$ SD	Median	Sum of ranks	Mean sum of ranks
Group I	15	0.53 $\pm$ 2.07	0	120	8.00
Group II	15	30.60 $\pm$ 6.33	30	601	40.07
Group III	15	39.40 $\pm$ 8.38	41	751	50.10
Group IV	15	16.00 $\pm$ 5.28	15	357	23.83

Table 1- This table shows the summary statistics of E. faecalis colony count (CFU 1/ml) of four groups

Comparison	Mean sum of ranks	Mean sum of ranks difference	z value	P value
Group I vs. Group II	8.00 vs. 40.07	32.07	5.03	< 0.001
Group I vs. Group III	8.00 vs. 50.10	42.10	6.60	< 0.001
Group I vs. Group IV	8.00 vs. 23.83	15.83	2.48	0.078
Group II vs. Group III	40.07 vs. 50.10	10.03	1.57	0.694
Group II vs. Group IV	40.07 vs. 23.83	16.23	2.55	0.065
Group III vs. Group IV	50.10 vs. 23.83	26.27	4.12	< 0.001

Table 2. This table shows the comparison of the mean sum of ranks of E. faecalis colony count (CFU 1/ml) between groups by z-test