

## Comparative evaluation of antimicrobial efficacy of MTAD, DMSA (Di Mercaptosuccinic acid), sodium hypochlorite (NaOCl) and chlorhexidine against *E. faecalis*: An ex-vivo study

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### Abstracts

**Aim:** The aim of this study was to compare the anti-microbial efficacy of four root canal irrigants, MTAD, 10% Di Mercaptosuccinic acid (DMSA), 5.25% Sodium Hypochlorite and 2% Chlorhexidine gluconate against *Enterococcus Faecalis*.

**Materials & Methods:** 75 extracted single rooted teeth with single canal were selected for this study. Conventional access preparations were made and the canals were instrumented 1 mm beyond the apical foramen with K-files up to size 50. The teeth were autoclaved. Pure culture of *E. Faecalis* was grown in brain heart infusion broth and was set to 4 Mac Farland's standard. 5 micro-litre of the broth was used to infect each of the root canal. The teeth were then divided into 5 groups (n=15) according to the irrigant delivered; group 1 was irrigated with MTAD, group 2 with 10% DMSA, group 3 with 5.25% Sodium Hypochlorite, group 4 with 2% Chlorhexidine gluconate and group 5 was irrigated with Normal saline (control group). Samples were collected from the root canals, after 5 minutes and 48 hours of irrigation, with help of absorbent paper points and were immediately transferred to test tubes containing brain heart infusion broth and incubated. Occurrence of broth turbidity was indicative of the viable bacteria remaining in the root canal. The test tubes where turbidity occurred were inoculated onto chocolate Agar plates to check for *E. Faecalis*. Results were analyzed statistically using Chi-Square test and Mann-Whitney test (p<0.05).

**Results:** Viable bacteria were found in all the canals of the control and 10% DMSA group. No viable bacteria were remaining in canals after 5 min of irrigation with MTAD and CHX whereas 67% of the root canals irrigated with NaOCl showed growth. After 48 hours of irrigation only teeth irrigated with CHX were free from viable bacteria.

**Conclusion:** 2% Chlorhexidine has a superior anti-microbial and residual anti-microbial activity against *E. Faecalis* compared to all the irrigants tested.

**Keyword:** MTAD; DMSA, Chlorhexidine, Sodium Hypochlorite, *E. Faecalis*.

### Introduction

Bacteria play the primary aetiological role in the development of necrotic pulps, periapical pathosis and post-treatment disease following root canal treatment.<sup>(1)</sup> One of the crucial factors for the success of the treatment consists of the eradication of microorganisms and their by-products from the root canal system.<sup>(2-3)</sup> Amongst the procedures involved in the control of endodontic infection, instrumentation and irrigation are important agents in eliminating the microorganisms from the root canal system.<sup>(4-5)</sup> However, mechanical debridement alone does not result in total or permanent reduction of bacteria.<sup>(6)</sup> The use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the numbers of microorganisms.<sup>(6,2,7)</sup> The most popular irrigating solution is sodium hypochlorite (NaOCl). It is an effective antimicrobial agent,<sup>(7-8)</sup> and an excellent organic solvent for vital, necrotic and fixed tissues.<sup>(9)</sup> However, it is highly irritating to periapical tissues, especially at high concentrations.<sup>(10)</sup>

Chlorhexidine gluconate has been recommended as a root canal irrigant and medicament.<sup>(11-12)</sup> It is a potent antimicrobial agent,<sup>(13,12,8)</sup> holds substantivity and has a low grade of toxicity.<sup>(14)</sup> However, chlorhexidine is unable to dissolve pulp tissue and debris may remain on canal walls, obstructing the dentinal tubules.

MTAD (a mixture of a tetracycline isomer, an acid and a detergent), has been introduced as a final rinse for disinfection of the root canal system. To rabinejad *et. al.* have shown that MTAD is able to safely remove the smear layer and is effective against *Enterococcus Faecalis*, a microorganism resistant to the action of other antimicrobial medications.<sup>(15,16)</sup>

A study in 1999 was done to evaluate the effects of EDTA in comparison with the effects of two other chelating agents Succimer (Di Mercaptosuccinic acid) and Trientine HCl on the root canals of extracted human teeth and concluded that all three chelating agents removed the smear layer completely and that Succimer widened the dentinal tubules the most followed by Trientine HCl and then EDTA.<sup>(17)</sup>

The search for an irrigating solution with antimicrobial properties, tissue dissolving ability and concomitant biocompatibility with the periapical tissues continues to be the subject of many studies. This study was conducted to compare the antimicrobial efficacy of four root canal irrigants, MTAD, 10% DMSA (Di Mercaptosuccinic acid), 5.25% NaOCl and 2% Chlorhexidine Gluconate, on the strains of *Enterococcus Faecalis* five minutes and 48 hours after irrigation.

## Materials & Methods

Seventy five extracted intact single rooted teeth with single canal, which had not received root canal treatment earlier, were selected for this study. After cleaning the teeth, of all calculus and extraneous soft tissue, the teeth were stored in 10% buffered formalin solution. Teeth with incompletely formed apices, root caries, fractures and resorption were not included.

Conventional access preparations were made in all the sample teeth with a high speed air-rotor. The working length was measured visually and the teeth were instrumented 1mm beyond the apical foramen. K-files (Dentsply Maillefer, Switzerland) were used to instrument the canals beyond the apex upto size 50. During the instrumentation tap water was used for irrigation of the canal. After the root canal preparation was completed the enlarged apical foramen was sealed with epoxy resin to prevent bacterial leakage. The teeth were then autoclaved to kill all the microorganisms and spores present inside the canals. The teeth were kept in sterile environment and were later intentionally contaminated with pure culture of *E. Faecalis* (ATCC 29212).

### Method of contamination of the root canal

Pure culture of *E. Faecalis* was obtained by culturing the bacteria in Brain Heart Infusion broth (BHI). The turbidity of the BHI broth containing the microorganism was adjusted to 4 Mac Farland standards which contains 900 million bacteria per milliliter of the solution. Each root canal was contaminated with 5 micro-liters, after which all the samples were incubated at 37°C for 24 hours.

After 24 hours of incubation, the contaminated teeth were randomly divided into four groups (n=15) according to the irrigant delivered.

Group 1 MTAD (Dentsply, Tulsa Dental, USA)

Group 2 10% DMSA (Sigma Alderich Chemicals, India)

Group 3 5.25% NaOCl solution

Group 4 2% Chlorhexidine Gluconate (Safe Gronet, India)

Group 5 Normal saline (control)

The root canals were irrigated with their respective irrigants; 2ml of the irrigant was delivered with side vented syringe (Dentsply, Tulsa Dental, USA) into each root canal and allowed to overflow. The irrigant was allowed to stay inside the canals for five minutes, following which the root canals were irrigated with 1 ml sterile saline solution. Size 35 sterile paper points were then kept inside the root canals for 1 minute for the collection of samples. On removal the paper points were immediately transferred to test tubes containing 5ml of BHI broth. The test tubes with the paper points inside were then vortexed for five minutes and incubated at 37°C for four days. The sample teeth were then again incubated at 37°C for further 48hrs without any other treatment. During this period of incubation the root canals of the sample teeth were left empty. After completion of 48hrs fresh samples with help of paper points were collected, vortexed with BHI broth and incubated. This was done to test and compare the long term antibacterial efficacy of the irrigants used. Change in the Broth turbidity was indicative that viable bacteria were still present in the root canal.

In order to check the growth for *E. Faecalis* in the tubes in which broth turbidity occurred, samples from these tubes were inoculated onto Chocolate Agar plates and incubated.

## Results

Table 1 shows the distribution of the number of canals with/without growth after 5 min of irrigation. Teeth irrigated with MTAD and CHX showed no growth. All the samples irrigated with DMSA and saline showed growth. 15 (33%) teeth irrigated with NaOCl showed growth and 10 (67%) teeth showed no growth.

**Table 1: Number (percent) of canals with growth 5 mins. after irrigation**

	MTAD	10% DMSA	5.25% NaOCl	2% CHX	Saline (control group)
No growth	15 (100 %)	0	5 (33%)	15 (100%)	0
Growth	0	15 (100%)	10 (67%)	0	3(100%)

Table 2 shows the distribution of the number of canals with/without growth after 48hrs of irrigation. 2 (13%) and 6 (40%) teeth irrigated with MTAD and NaOCl respectively showed growth. All the samples irrigated with DMSA and saline showed growth. No sample irrigated with CHX showed growth.

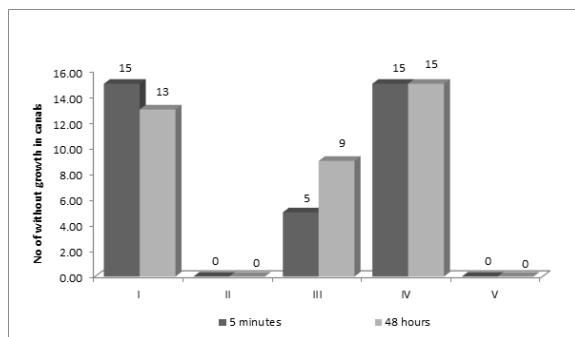
**Table 2: Number (percent) of canals with growth 48hrs after irrigation**

	MTAD	10% DMSA	5.25% NaOCl	2% CHX	Saline (control group)
No growth	13 (86 %)	0	9 (60%)	15 (100%)	0
Growth	2 (14%)	15 (100 %)	6(40%)	0	3(100%)

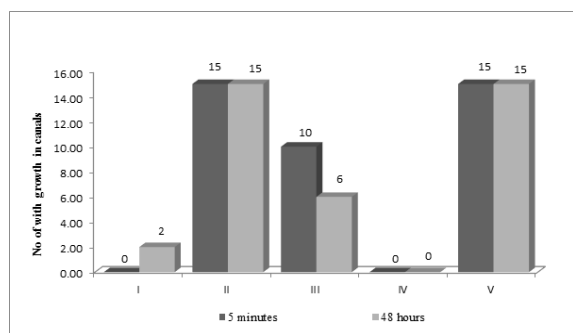
There was no statistically significant difference between the antibacterial activities of MTAD and 2%

chlorhexidine ( $p > 0.005$ ), but both had significantly higher antibacterial effect than 5.25% NaOCl, after 5 minutes of irrigation ( $p = 0.0001$ ).

However, after 48 hours of irrigation there was no significant difference between MTAD and 5.25% NaOCl ( $p = 0.09$ ), but chlorhexidine showed significant antibacterial activity than NaOCl ( $p = 0.006$ ) (Fig 1a, 1b).



**Fig 1a: Comparison of canals without growth in five groups**



**Fig 1b: comparison of canals without growth in five groups**

## Discussion

Root canals of extracted teeth were used in this study rather than Agar diffusion tests or test tube broths because previous studies has shown that there is inactivation of root canal medicaments by dentin of the tooth and that the magnitude of the inhibition was dependent on both the concentration of the medicament and the time of contact between the medicament and dentin. It is postulated that binding to dentin powder had resulted in a reduced, but not abolished, activity, of 0.05% Chlorhexidine.<sup>(18,19)</sup>

In this study *Enterococcus Faecalis* was taken as the test microorganism because it is part of the human normal flora and an important pathogen in opportunistic infections in humans. It is ecologically tolerant and has the ability to survive in harsh conditions. In stationary phase it shows resistance against sodium hypochlorite 900-fold higher than that of a growing cell.<sup>(20)</sup> *E. Faecalis* is small enough to invade and live within dentinal tubules. It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. *E. Faecalis* is rarely present in

primary apical periodontitis, but it is the dominant microorganism in root-filled teeth presenting with post-treatment apical periodontitis. Eradication of *E. Faecalis* from the root canals remains a challenge.<sup>(21)</sup>

Although an irrigant can penetrate into the dentinal tubules, it does not mean that the concentration is sufficient to kill all types of bacteria present. It has been shown that bacteria may remain viable in tubules at great distances from the pulp. Studies have shown that disinfection of root dentin is not achieved by chemo-mechanical preparation alone. Bacteria deep in dentinal tubules are apparently protected from instrumentations and irrigation, making their removal or eradication difficult. It has been demonstrated that after three weeks incubation of root canals inoculated with *E. Faecalis* a dense infection reaching 300 to 400 micro-meters into the dentinal tubules was found. Prolonged incubation leads to more tubules being infected, whereas the average depth of penetration of the tubules by bacteria has been found to increase slowly with time.<sup>(22)</sup>

Numerous solutions have been used in endodontics to achieve a desired chemical effect. In this study 5.25% NaOCl solution is used, because 5.25% sodium hypochlorite has shown in previous studies to eliminate *E. Faecalis* in Agar diffusion tests in just 30 seconds.<sup>(7)</sup> In another study, 5.25% NaOCl took 2 min in reducing the viable count to zero in Agar diffusion test. It was also stated that interaction of NaOCl with tissue fluids, blood, dentin and smear layer reduces its effectiveness.<sup>(23)</sup>

In this study the results obtained were different from above studies as after 5 min irrigation with 5.25% NaOCl in infected root canals of extracted teeth 67% of the teeth showed growth and after 48 hours of irrigation 40% of the teeth showed growth. Shahrokh & Torabinejad demonstrated somewhat similar results in their study, where after irrigation with 5.25% Sodium Hypochlorite for 5 minutes six out of the fifteen teeth showed growth. The microorganism used was the same in both the studies that is *E. Faecalis*.

The antibacterial effect of 1% NaOCl on *E. Faecalis* was reduced but not totally eliminated by the presence of dentin.<sup>(18)</sup> According to a previously published work Sodium Hypochlorite reacts with organic debris in the root canal and in that way facilitates cleaning but this reaction also inactivates the Hypochlorite and reduces its antibacterial capacity and also that in spite of frequent application of 5% Sodium Hypochlorite solution all the bacteria was not eliminated from the infected root canals.<sup>(24)</sup>

The results of this study, that 2% Chlorhexidine has marked antimicrobial effect along with substantivity, are in agreement with previous studies conducted on the anti-bacterial effect of 2% Chlorhexidine.<sup>(25,26)</sup> Gomes *et al.* 2001 tested the antimicrobial efficacy of CHX and sodium hypochlorite and demonstrated that growth inhibition haloes produced were larger in diameter for Chlorhexidine than for sodium hypochlorite.<sup>(7)</sup>

In agreement to the results of this study a previously conducted study also demonstrated that antimicrobial activity of Chlorhexidine was superior to that of Sodium hypochlorite 5.25% except for 0.2% Chlorhexidine gel.<sup>(27)</sup> Carol *et al.* demonstrated that residual antimicrobial activity of 2% Chlorhexidine was significantly superior to 5.25% Sodium Hypochlorite solution<sup>(28)</sup> which is also in accordance with this study. Chlorhexidine is cationic bisguanide that seems to act by adsorbing onto the cell wall of microorganism and causing the leakage of intracellular components.

At low concentrations of CHX, small molecular weight substances will leak out, especially, potassium and phosphorous, resulting in a bacteriostatic effect. At higher concentrations, CHX has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross linking.<sup>(29)</sup> This could be the reason for CHX performance in this study.

MTAD (a mixture of a tetracycline isomer, an acid and a detergent) was introduced as a final rinse for disinfection of the root canal system. Torabinejad *et al.* 2003 have shown that MTAD is able to safely remove the smear layer and is effective against *E. Faecalis*. Shahrokh & Torabinejad 2003 demonstrated that 1.3% NaOCl followed by MTAD was more effective against *E. Faecalis* when compared to 5.25% NaOCl alone. The efficacy of MTAD in disinfecting the internal and external surfaces of roots is a result of the presence of the antibacterial effect of doxycycline. Its ability to remove organic and inorganic substances from the surfaces of the roots, which is facilitated by the presence of citric acid and the presence of detergent aids its propensity to diffuse into the root canal and the dentinal tubules.<sup>(30)</sup> Reduction of the surface tension by detergents, has been shown to improve the antimicrobial properties.<sup>(31)</sup>

Another previous study showed that MTAD is more effective in disinfecting root canals in extracted human teeth than 5.25% NaOCl which is in accordance with this study.<sup>(30)</sup>

DMSA is a chelating agent used in mercury poisoning. It was used by Hottel *et al.* 1999 as a root canal chelating agent and they demonstrated that it removed the smear layer completely and that it widened the dentinal tubules much more than EDTA.<sup>(17)</sup> DMSA was used in this study to check if it possessed any antimicrobial properties against *E. Faecalis*.

According to the results of this study and the previous published literature a regimen for irrigation should be outlined. The regimen will include irrigant to be used throughout the biomechanical preparation, an irrigant for smear layer removal and for final wash.

Further in-vitro and in-vivo studies are required, as we cannot draw conclusions based on in-vitro studies with isolated bacteria.

## Conclusions

Within the limitations of this study we can conclude that

- i. 2% Chlorhexidine has a superior anti-microbial and residual anti-microbial activity against the resistant microorganism *E. Faecalis* when compared to all the irrigants tested.
- ii. MTAD has a similar anti-microbial activity as 2% Chlorhexidine but lower residual anti-microbial activity.
- iii. 5.25% Sodium Hypochlorite irrigation, for 5 minutes and after 48 hours of irrigation, is not sufficiently effective against *E. Faecalis*.
- iv. DMSA has no anti-microbial activity against *E. Faecalis*.

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