

## EVALUATION OF THE ANTIBACTERIAL SUBSTANTIVITY OF A NEW ENDODONTIC IRRIGATION SOLUTION

### *Avaliação da substantividade antibacteriana de uma nova solução de irrigação endodôntica*

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#### **Abstract**

**OBJECTIVES:** The purpose of this *in vitro* study was to compare the antimicrobial substantivity of 1% MTAD, 2% Chlorhexidine (CHX) and 1.3% sodium hypochlorite (NaOCl) in bovine root dentine. **METHOD:** One hundred and ten dentine tubes prepared from bovine incisors were infected *in vitro* for 14 days with *Enterococcus faecalis*. The specimens were divided in to five groups as follows: CHX; MTAD; and NaOCl; infected dentine tubes (positive control); and sterile dentine tubes (negative control). Dentine chips were collected with round burs into tryptic soy broth (TSB). After culturing the number of colony-forming units (CFU) was counted. **RESULTS:** In all experimental groups CFU was minimum in the first cultures. The results obtained were significantly different from each other at any time period (P<0.05). In the first culture, the NaOCl group and CHX group showed the lowest and highest number of CFU, respectively. In each group, the number of CFUs increased significantly by time-lapse (P<0.05). **CONCLUSION:** In conclusion, the substantivity of MTAD was significantly greater than CHX and NaOCl.

**Keywords:** MTAD; Chlorhexidine; Endodontic solution; *Enterococcus faecalis*.

#### **Resumo**

**OBJETIVOS:** A finalidade deste estudo *in vitro* foi comparar a substantividade de MTD a 1%, Clorexidina 2% (CHX) e hipoclorito de sódio (NaOCl) a 1,3% na dentina da raiz de dentes bovinos. **MÉTODO:** Cento e dez tubos de dentina, preparados utilizando incisivos bovinos, foram infectados *in vitro* por *Enterococcus faecalis*. Os espécimes foram divididos em cinco grupos, como segue: CHX, MTAD e NaOCl; tubos contendo dentina infectada (controle positivo) e tubos com dentina estéril (controle negativo). Raspas de dentina foram coletadas usando brocas esféricas e colocadas em caldo de cultura triptico (soja) (TSB). Após cultura, o número unidades de formação de colônias foi contado. **RESULTADOS:** Em todos os grupos experimentais o CFU foi mínimo nas primeiras culturas. Os resultados obtidos diferiram significativamente entre os grupos em qualquer período de gtempo (P<0.05). Na primeira cultura, o grupo NaOCl e o CHX demonstraram o maior e o menor número de CFU's, respectivamente. Em cada grupo, o número de CFU aumentou significativamente no tempo (P<0.05). **CONCLUSÃO:** A substantividade do MTAD foi significativamente maior do que CHX e NaOCl.

**Palavras-chave:** MTAD; Clorexidina; Solução endodôntica; *Enterococcus faecalis*.

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## Introduction

Viable microorganisms remaining after root canal preparation and disinfection contribute significantly to failure in endodontic therapy (1). Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens and intracanal medicaments (1). In cases with necrotic pulps as well as in retreatment cases, treatment should be performed in two visits, which is more time-consuming than one-visit treatment (2).

Furthermore, some studies have suggested that calcium hydroxide is ineffective against *E. faecalis* (3). To overcome the above mentioned problems, an alternative protocol is to use antimicrobial agents that exhibit substantivity, that is, agents that can have a therapeutic effect for a prolonged period.

NaOCl is the most commonly used root canal irrigant, which has both antimicrobial and tissue dissolving properties (4, 5).

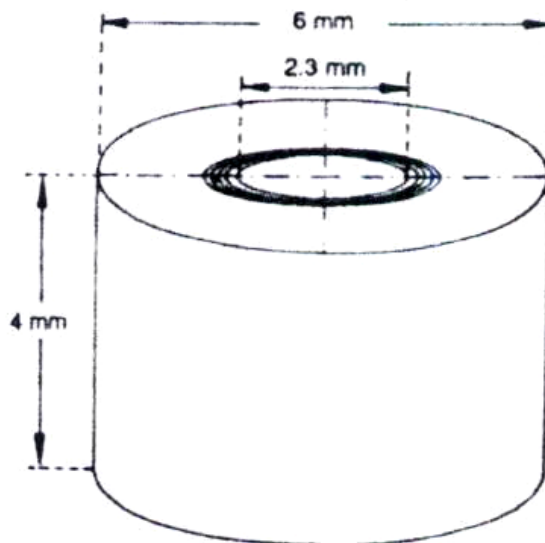
CHX (Sigma Chemicals Co., St. Louis, MO, USA) seems to act by adsorbing onto the cell wall of the microorganism and causing the leakage of intracellular components (6). Antimicrobial substantivity of CHX in the root canal system has been reported from 48 h to 21 days (7, 8, 9). However, Rosenthal et al. (1) indicated that substantivity of CHX was extended for up to 12 weeks.

MTAD (Dentsply Tulsa Dental, Tulsa, USA), a mixture of doxycycline, citric acid and a detergent (Tween 80), has recently been introduced as a final irrigant for disinfection of the root canal system. Shabahang et al. showed that MTAD was a more effective disinfectant of the root canal system than 5.25% NaOCl. (10). They also found that the combination of 1.3% NaOCl and MTAD as a final treatment eliminated *E. faecalis* from human tooth cementum and dentine (11, 12).

However, its substantivity has not been yet evaluated. The purpose of this study was to compare the antibacterial substantivity of MTAD, CHX, and doxycycline against *E. faecalis* in bovine root dentine *in vitro*.

## Material and methods

The method used was a modification of the previously described by Haapasalo and Orstavik (3). Forty intact bovine central incisors were selected for this study. The specimens were kept in 0.5% NaOCl solution for no longer than seven days. The apical 5 mm and two-thirds of the crown were removed from each tooth with a rotary diamond saw at 1000 rpm (Isomet Plus precision saw, Buehler, IL) under water-cooling. Cementum was removed by using polish paper (Ecomet 3, variable-speed grinder-polisher, Buehler, IL), which resulted in a centre-holed piece of root dentine with 6 mm outer diameter (Figure 1).



**Figure 1** - Schematic view of used dentine tubes (adapted from Gomes et al.)

The roots were cut into 4 mm thick slices with a diamond saw as above. The canals of the 4 mm blocks were enlarged with an ISO 023 round bur using slow speed. All teeth and dentine slices were preserved in vials containing tap water during the procedures to avoid dehydration. The dentin tubes (n=100) were individually treated with 5.25% NaOCl, and 17% EDTA (with pH 7.2) to remove the smear layer. The specimens were then placed in BHI broth (Oxoid, Basingstoke, UK) and autoclaved. They were then kept in an incubator at 37° C for 24 hours to check the efficacy of the sterilization. A total of 110 specimens were randomly divided into five groups as follow: Group 1 (30 specimens): MTAD, Group 2 (30 specimens): 2% CHX, Group 3 (30 specimens): 2.6% NaOCl, Group 4 (10 specimens): positive control (infected dentine tubes), Group 5 (10 specimens): negative control (sterile dentine tubes).

Isolated 24-hour colonies of pure cultures of *E. faecalis* (ATCC 29212) were suspended in 5ml of BHI. The bottles containing each specimen in groups 1, 2, 3 and 4 were opened under laminar flow. Sterile pipettes were used to remove 2 ml of sterile BHI and to replace it with 2 ml of bacterial inoculum. The bottles were closed and kept at 37°C for 14 days, with the replacement of 1 ml of contaminated BHI for 1 ml of freshly prepared BHI every 2 days, to avoid medium saturation. After the contamination period, each specimen was removed from its bottle under aseptic conditions and the canal was irrigated with 5 ml of sterile saline and dried with sterile paper points. The outer surface of the specimens was covered with two layers of nail varnish, in order to prevent contact of the medicament with the external surface. Then, specimens were fixed at the bottom of wells of 24

-well cell culture plates with decontaminated sticky wax, which also obliterated the apical surface of the root canal.

Finally, the irrigation solutions were inserted into the canal lumen with sterile 3-ml plastic syringes and 27-gauge needles until the dentine tubes were totally filled. Five minutes after placement of irrigants, solutions were removed using sterile paper points. The specimens were then incubated at 37° C for a period of 28 days to maintain humidity. Dentine chips were removed from the canals with sequential sterile low - speed round burs with increasing diameters of ISO sizes: 025, 027, 029, 031, and 033 at experimental times of 0, 7, 14, 21, and 28 days. Each bur removed approximately 0.1 mm of dentine around the canal. The powder dentine samples obtained with each bur were immediately collected in separate test tubes containing 3 ml of freshly prepared BHI. Thereafter, 100 microlitres from each test tube was cultured on blood agar. Growing colonies were counted and recorded as CFU. Results were analysed using analysis of variance and covariance with repeated measures (ANOVA) to indicate differences between the experimental groups and the positive control. One-way ANOVA (Tukey's method) was used to indicate differences within each layer.

**Results**

The CFUs represent a close estimate of the number of viable bacteria that penetrated into the dentinal tubules at different layer depths. The number of CFU obtained from five consecutive dentinal layers is presented in Table 1.

**Table 1** - Means of the CFU and the Standard Deviation of *E. faecalis* in experimental groups

	Day 0	Day 7	Day 14	Day 21	Day 28
NaOCl	0.40±0.69	16.11±8.05	37.40±8.99	61.80±11.11	88.55±5.50
CHX	4.72±3.68	9.10±3.74	15.43±4.55	37.30±5.33	57.66±5.36
MTAD	0.50±3.97	4.46±2.34	8.70±2.75	19.20±3.22	42.44±5.52

The number of CFU in all three experimental groups was minimum in the first cultures. The positive control group showed viable bacteria at all experimental times, which indicated

the efficiency of the method. In contrast, negative control group showed no viable bacteria at all experimental times. At Day 0, the NaOCl group demonstrated the most effective antibacterial action.

However, at days 7, 14, 21, and 28, MTAD demonstrated the most effective antibacterial action ( $p < 0.05$ ).

## Discussion

Current techniques of debridement leave many areas of the root canal completely untouched by the instruments (13). Thus, a root canal irrigant is needed to aid in the debridement of the canals. *E. faecalis* was chosen for inoculum in this study because it is resistant to intracanal medication with calcium hydroxide (6). In the present study, the antibacterial substantivity of NaOCl, CHX, and MTAD was monitored for 28 days.

The 2.6% NaOCl solution had the most effective antibacterial action at the first culture, but its antibacterial action dropped rapidly. This indicates that NaOCl has little to no antibacterial substantivity. The probable reason is that NaOCl has an antimicrobial effect as long as free chlorine is available in the solution. The mean numbers of CFU were statistically lower in MTAD compared to other solutions at all experimental periods except for the first time, thus stressing the ability of MTAD to adsorb to hydroxyapatite with prolonged gradual release at therapeutic levels.

Shabahang and Torabinejad (12) compared the antibacterial effect of MTAD with that of NaOCl with and without EDTA. Their findings showed that the combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse was significantly more effective than the other regimens.

Kho and Baumgartner (14) compared the antimicrobial efficacy of irrigating with 1.3% NaOCl/MTAD versus irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with *E. faecalis*. Their results demonstrated that there was no difference in antimicrobial efficacy for irrigation with 5.25% NaOCl/15% EDTA versus irrigation with 1.3% NaOCl/MTAD.

In another study Krause et al. (15) compared the antibacterial effect of MTAD, NaOCl, doxycycline, and citric acid on *E. faecalis*. Their findings showed that NaOCl was more effective than other solutions. Khademi et al. (16) compared the antibacterial substantivity of 2% CHX, 100 mg/ml doxycycline, and 2.6% NaOCl in bovine root dentin *in vitro*. They found that substantivity of

CHX was significantly greater than doxycycline, and NaOCl, which is in contrast to the findings of the present study. It seems that the presence of a detergent (Tween 80) in MTAD increases the depth of penetration of this material into dentinal tubules by decreasing surface tension. Rosenthal et al. (1) found that treatment with a 2% solution of CHX induced substantivity for up to 12 weeks, which is in contrast to the findings of the present study. However, White et al. (7) concluded that antimicrobial activity of 2% CHX as a canal irrigant lasted 72 hours.

In an *in vivo* study to evaluate the substantivity of 2% CHX as root canal irrigating solution, Leonardo et al. (8) found that CHX prevents microbial activity with residual effects in the root canal system up to 48h, whereas the present study showed that substantivity of 2% CHX was remained for 28 days.

Komorowski et al. (17) reported that for induction of substantivity, dentine should be treated with CHX for 7 days and 5 min. treatment with CHX did not induce substantivity, which is in contrast to our findings. Lin et al. (18) attributed the limited antibacterial effect of CHX irrigation to absorb the medication to dentine during the first hour and stated that only after the saturation point after the first hour that the antibacterial capability of CHX increase with time.

Stabholz et al. (19) found that the antimicrobial substantivity of CHX was significantly lesser than tetracycline HC1 50 mg/ml for 12 days.

## Conclusion

Under the conditions of the present study, the substantivity of MTAD was significantly higher than CHX and retained in root canal dentine for at least 28 days. Furthermore, NaOCl displayed no substantivity.

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