

An In Vitro Comparison of the Antimicrobial Effects of Various Endodontic Medicaments on *Enterococcus faecalis*

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Abstract

The purpose of this in vitro study was to investigate the antimicrobial action of Dermacyn (Oculus Innovative Sciences, Petaluma, CA), BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN), 2% chlorhexidine (CHX; Ultradent, West Jordan, UT), and 5.25% sodium hypochlorite (NaOCl) against *Enterococcus faecalis* (American Type Culture Collection 4082). Eighteen Petri dishes of BHI agar were inoculated with *E faecalis*. Each Petri dish had five saturated paper disks placed. Four of the disks were saturated with a different test solution, and the last paper disk served as the control and was saturated with sterile distilled water. The plates were randomly distributed into two groups. Group one ($n = 9$) was incubated aerobically and group 2 ($n = 9$) was incubated anaerobically for 48 hours at 37°C. The largest diameter of the zones of microbial inhibition was measured in millimeters and recorded. Statistical analysis was performed with repeated-measures analysis of variance. BioPure MTAD showed significantly ($p < 0.05$) more zones of microbial inhibition than 5.25% NaOCl, 2% CHX, and Dermacyn. Sodium hypochlorite and CHX showed significantly ($p < 0.05$) more zones of microbial inhibition than Dermacyn. The zone of inhibition between NaOCl and CHX was not significant ($p > 0.05$). The control group showed no microbial inhibition. (*J Endod* 2007;33:567–569)

Key Words

Enterococcus faecalis, medicaments, superoxidized water

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Intracanal medicaments are used as an antibacterial agent to eliminate residual bacteria in a root canal after instrumentation and irrigation, to render any remaining canal content inert, to dissolve tissue and act as a barrier against leakage or breakdown of the temporary filling, and to control persistent seepage of apical fluids into the root canal system (1). Several antimicrobial rinses are used in endodontics including sodium hypochlorite (NaOCl), BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN), and chlorhexidine (CHX).

Sodium hypochlorite has long been the irrigant of choice for nonsurgical endodontic procedures (2). Sodium hypochlorite dissolves necrotic and vital tissue, has antimicrobial activity, and aids as a lubricant in the canal (2–4). It also has minimal “clinical toxicity” when kept within the confines of the canals (5). However, NaOCl is extremely toxic to the periapical tissues if it is injected beyond the apex of the tooth (5).

BioPure MTAD has been suggested as a final rinse because of its antimicrobial properties and its ability to remove the smear layer (6–10). BioPure MTAD is less cytotoxic than 5.25% NaOCl (11).

Chlorhexidine has also been shown to be a potent broad-spectrum antimicrobial that has the advantage of substantivity (12). CHX has been shown to be more effective against gram-positive organisms than gram-negative organisms (13). Vianna et al. (14) have shown that CHX is able to inactivate many endodontic-resistant organisms in as little as 15 seconds of contact time.

Superoxidized waters have been shown to inactivate many microorganisms as well as inactivate many of the organisms contributing to endodontic infections (15, 16). Recently, Dermacyn (17) (Oculus Innovative Sciences, Petaluma, CA), a super-oxidized water has been introduced in medicine as a wound cleanser that has broad-spectrum antimicrobial properties and is extremely biocompatible. Landa-Solis et al. (17) reported that Dermacyn had efficacy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Candida albicans*. In their study, Dermacyn was also reported to completely inactivate *Bacillus atrophaeus* spores and the human immunodeficiency virus–1 virus. Dermacyn is different from other superoxidized waters in that it is pH neutral and has a shelf-life of over 1 year (17).

Enterococcus faecalis is often found in asymptomatic failing root canal–treated teeth (18). Recently, Gomes et al. (19) showed that *E faecalis* is frequently found in primary necrotic cases. Evans et al. (20) showed that it is resistant to calcium hydroxide ($\text{Ca}(\text{OH})_2$) because of its proton pump. *E faecalis* can also survive by genetic polymorphisms and its ability to bind to dentin, invade dentinal tubules, and survive starvation (18).

The purpose of this in vitro study was to compare the antimicrobial action of Dermacyn, BioPure MTAD, 2% CHX (Ultradent, West Jordan, UT), and 5.25% NaOCl against *E faecalis*.

Materials and Methods

Eighteen agar plates were prepared by using brain-heart infusion (BHI) agar (Difco, Sparks, MD). Agar was mixed according to the manufacturer’s directions, and enough agar was poured to cover the surface of a 125-mm diameter Petri dish. The BHI agar dishes were then stored at room temperature for 2 days before use to verify that they had remained sterile. BHI broth (Becton, Dickinson, and Co., Sparks, MD) was also prepared and stored in 5-mL vials for 2 days.

E faecalis (American Type Culture Collection 4082) was obtained from the American Type Culture Collection (Manassas, VA) and maintained by using BHI broth and

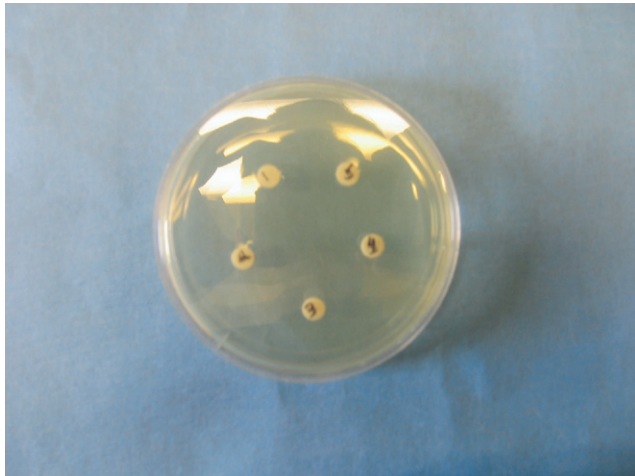


Figure 1. Model for Petri dish setup.

agar. The culture on receipt was grown, and cultures were frozen (−20°C) in vials with glycerol from which new stock cultures were periodically established. Cultures of *E faecalis* were grown overnight at 37°C in BHI broth and bacterial growth was checked by changes in turbidity at 24 hours.

An unpublished pilot study was run to determine the amount of medicament needed to saturate a 6-mm paper test disk (Becton, Dickinson, and Co., Sparks, MD). It was found that 20 µL of Dermacyn, 15 µL of BioPure MTAD, 20 µL of 2% CHX, and 15 µL of 5.25% NaOCl was needed to completely saturate the paper disks. Twenty microliters of sterile distilled water was determined to be the correct amount in order to saturate the control disk. The pilot study also determined the optimum incubation time for *E faecalis* both anaerobically and aerobically was 48 hours.

The BHI broth was inoculated with *E faecalis* from a freshly grown culture on an agar plate. The broth culture was incubated at 37°C for 24 hours aerobically on a rotary shaker (150 rpm). The broth was then used to inoculate 18 of the BHI agar plates by using sterile cotton swabs to provide an even lawn of cells. After inoculation, five saturated paper disks were placed on each agar plate. Four of the disks were saturated with one of the four test solutions, and the last paper disk served as the control and was saturated with sterile distilled water (Fig. 1). The Petri dishes were marked to identify the medicament on the bottom of the plate. The plates were randomly divided into two groups. Group one ($n = 9$) was incubated aerobically at 37°C for 48 hours. Group two ($n = 9$) was incubated anaerobically at 37°C for 48 hours. GasPaks (Becton, Dickinson, and Co.) with catalyst were used to consume the oxygen and produce carbon dioxide in an anaerobic jar to create the anaerobic environment for growth.

GasPak anaerobic indicator strips (Becton, Dickinson, and Co.) were used to verify anaerobic environment.

Microbial zones of inhibition were measured in millimeters, and the largest diameter was recorded. Results were recorded by group and were statistically analyzed with repeated measures analysis of variance (ANOVA).

Results

BioPure MTAD showed a larger zone of microbial inhibition for both aerobic and anaerobic samples when compared with 2% CHX and 5.25% NaOCl ($p < 0.05$, ANOVA; Fig. 2). The zones of inhibition for 2% CHX and 5.25% NaOCl were not different from each other ($p > 0.05$, ANOVA), but they both resulted in larger zones of inhibition than Dermacyn and the control ($p < 0.05$, ANOVA; Fig. 2). Dermacyn and the control showed zones of microbial inhibition that were not different from each other ($p > 0.05$, ANOVA).

Discussion

The results of this in vitro study showed that BioPure MTAD is a viable medicament against *E faecalis* in vitro. Although 2% CHX and 5.25% NaOCl showed less antimicrobial effect on *E faecalis* than BioPure MTAD ($p < 0.05$, Fig. 2), both medicaments still had an observable effectiveness against this bacterium. Dermacyn was ineffective against *E faecalis*, despite the Landa-Solis (17) study’s findings of its broad-spectrum antibiotic activity.

The results of this study support the findings of Shabahang and Torbinejad (8) in which they showed the efficacy of BioPure MTAD against *E faecalis*. Within the parameters of this study, BioPure MTAD had twice the effective inhibitory zone of 2% CHX or 5.25% NaOCl. Further clinical trials are needed to determine if BioPure MTAD is as effective against *E faecalis* as reported in this in vitro study; Portenier et al. (21) showed that both BioPure MTAD and CHX were inhibited by dentine powder and bovine serum albumin.

In this study, CHX (2%) did not perform any better than the 5.25% NaOCl on its antimicrobial effect on *E faecalis*. It is important to note that this study did not address the property of substantivity of the medicament, only the ability to inhibit the growth of the microorganism. Therefore, CHX may still clinically have a longer antimicrobial effect on *E faecalis* than NaOCl.

Dermacyn showed no ability to prevent the growth of *E faecalis*. Landa-Solis et al. (17) tested the ability of Dermacyn to reduce the number of colony-forming units (CFUs) in aliquots of broth and found that it effectively reduced the number of CFUs. Dermacyn may have some value as a cold sterile solution, open wound irrigant, or surface disinfectant as the Land-Solis et al. (17) study suggests, but further studies are needed to verify these claims. The study design presented in this article is more consistent with other studies testing ability of antimicrobial action (22).

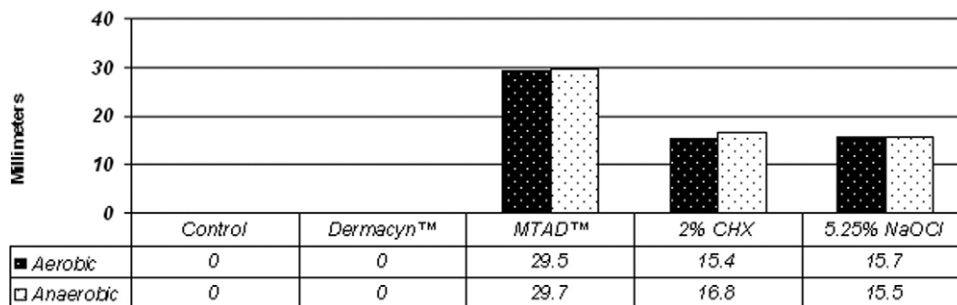


Figure 2. Mean zones of microbial inhibition.

Summary

BioPure MTAD showed significantly ($p < 0.05$) more zone of microbial inhibition than NaOCl, CHX, and Dermacyn. NaOCl and CHX showed significantly ($p < 0.05$) larger zones of microbial inhibition than Dermacyn. The size of the zone of inhibition between NaOCl and CHX was not different ($p > 0.05$). Dermacyn and the control group showed no microbial inhibition.

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