A Comparison of the Antimicrobial Efficacy of NaOCl/Biopure MTAD versus NaOCl/EDTA against *Enterococcus faecalis*

Patricia Kho, DDS, and J. Craig Baumgartner, DDS, PhD

Abstract

The purpose of this investigation was to compare the antimicrobial efficacy of irrigating with 1.3% NaOCl/Biopure MTAD versus irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with *Enterococcus faecalis*. Bilaterally matched human teeth were sterilized and inoculated with *E. faecalis*. After chemomechanical root canal preparation, the root-ends were resected and pulverized in liquid nitrogen to expose *E. faecalis* in dentinal tubules or other recesses away from the main root canal system. The number of colony forming units (CFU) of *E. faecalis* per mg was determined from the pulverized root-ends. No significant differences were seen ($t = 0.70$, $p = 0.495$) between the number of colony forming units of *E. faecalis* for teeth irrigated with 5.25% NaOCl/15% EDTA (mean 131 $\pm$ 291 CFU/mg) versus those teeth irrigated with 1.3% NaOCl/Biopure MTAD (mean 187 $\pm$ 237 CFU/mg). This study demonstrated that there is no difference in antimicrobial efficacy for irrigation with 5.25% NaOCl/15% EDTA versus irrigation with 1.3% NaOCl/Biopure MTAD in the apical 5 mm of roots infected with *E. faecalis*. *(J Endod 2006;32:652–655)*

Key Words

Antibacterial, dentinal tubules, Irrigation, MTAD

From the Oregon Health and Science University, OHSU School of Dentistry, Portland, Oregon.

Address requests for reprint to J. Craig Baumgartner, DDS, PhD, Department of Endodontology, OHSU School of Dentistry, 611 SW Campus Drive, Portland, OR 97201, baumgarc@ohsu.edu.

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Root canal morphology is complex and contains numerous ramifications and anatomical irregularities (1). The microorganisms in root canals not only invade the anatomic irregularities of the root canal system but are also present in the dentinal tubules (2). Persistent endodontic disease after root canal therapy may be caused by bacteria in dentinal tubules (3). Concern has been expressed about the consequence of bacteria left in the root canal system and those that remain in the dentinal tubules (4, 5). It is believed that the bacteria may not survive treatment, are subsequently killed, are entombed and die from lack of nutrition, or remain viable in sufficient numbers to be a potential cause of pathosis (5).

Current techniques of root canal debridement may leave areas of the root canal system completely untouched by the instruments (6). It has also been shown that mechanical instrumentation without irrigation reduces but does not predictably eliminate bacteria in the canal (7). Thus, a root canal irrigant is needed to aid in the debridement of the canals.

Various concentrations of sodium hypochlorite (NaOCl) have been used as root canal irrigants for many decades. The main advantages of NaOCl are its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms. The combined use of EDTA and NaOCl has been recommended for smear layer removal (8, 9) and has been shown to be more effective at killing bacteria than NaOCl alone (10).

Biopure MTAD (Dentsply, Tulsa OK) is a mixture of tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween 80). When used as a root canal irrigant, MTAD has been reported to safely remove the smear layer (11) and effectively eliminate *Enterococcus faecalis* (12). Studies have found *E. faecalis* to be a commonly recovered microbe in failing root canals (13–15). It has been reported that MTAD as a final rinse, when used in combination with 1.3% NaOCl as a root canal irrigant, is significantly more effective than 5.25% NaOCl with 17% EDTA in disinfecting root canals contaminated with whole saliva (16) or with *E. faecalis* (17).

The purpose of this investigation was to compare the antimicrobial efficacy of irrigating with 1.3% NaOCl/Biopure MTAD versus irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with *E. faecalis*.

Materials and Methods

Twenty-five bilaterally matched pairs of extracted human teeth with mature apices were obtained and stored in saline. Each tooth was radiographed to confirm the presence of a single canal. The infected dentin model used in this experiment was modified from that developed by Haapasalo and Orstavik (18).

The teeth were soaked in 5.25% NaOCl for 30 minutes to remove residual tissue and debris from the root surfaces. The incisal/occlusal surfaces were reduced approximately 2 mm and adjusted so that the length of each tooth within a matched pair was the same. An access preparation was made with a high speed round bur, pulp tissue was removed with a barbed broach and patency was confirmed with a #10 K-file.

A customized model was assembled for each tooth for the subsequent instrumentation and irrigation procedures. Polyvinyl siloxane impression material (Reprosil Heavy Body, Dentsply/Caulk) was expressed into surgical tubing that was cut into 2 inch segments. The teeth were embedded in impression material up to their CEJ and removed when the material was set.

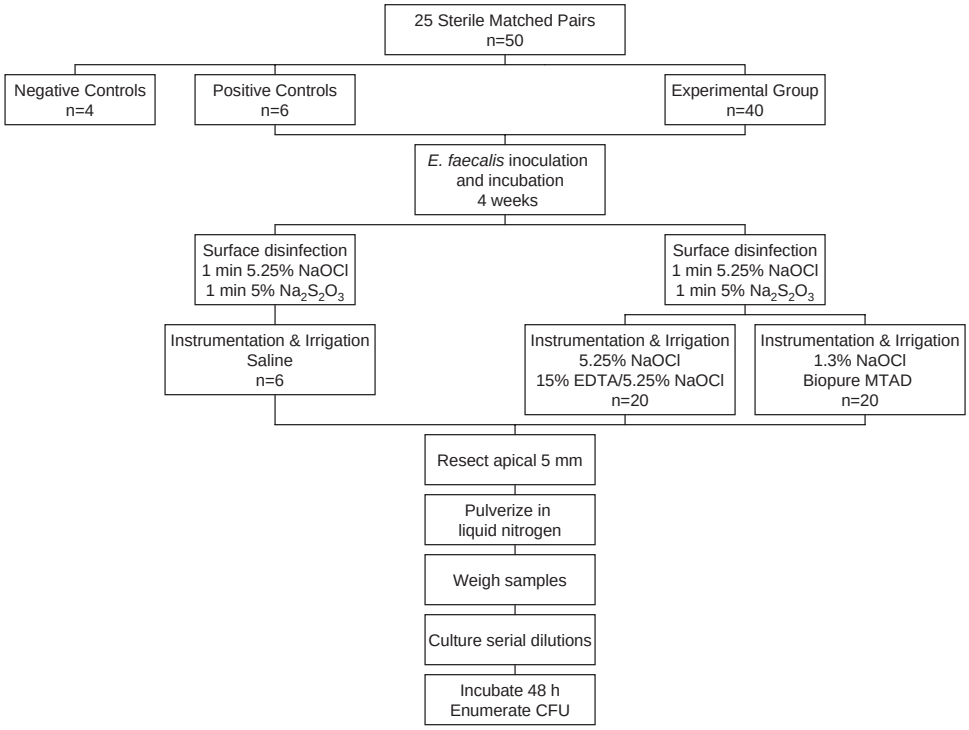

Figure 1. Flowchart of the methodology.

The teeth, customized models and 20 ml scintillation vials were separately steam autoclaved at 121°C for 30 minutes. Each tooth was individually placed into a sterile scintillation vial, and 5 ml of sterile Brain Heart Infusion (BHI) broth was added. The samples were incubated in their sealed vials for 48 hours at 37°C and inspected daily to ensure that the BHI broth showed no signs of turbidity. Five matched pairs of teeth were randomly selected as controls. These were further divided into two negative control pairs and three positive control pairs. Negative control teeth were cultured to ascertain the effectiveness of the sterilization procedures. The methodology for each group is summarized in Fig. 1.

An inoculum of a 24 hours pure culture suspension of *E. faecalis* (ATCC 19433) grown in BHI broth was prepared. The 20 experimental and the three positive controls pairs were inoculated with *E. faecalis* and cultured for 4 weeks under aerobic conditions at 37°C. The media was replenished every 7 days. When replacing intracanal media, random samples from the root canals were cultured to confirm growth of *E. faecalis*. Colonies of *E. faecalis* in pure culture were detected as white pinpoint colonies on the agar media that microscopically consisted of gram-positive cocci arranged in a cross-chain pattern.

Before working length determination, the surfaces of the experimental and positive control teeth were disinfected by immersion in 5.25% NaOCl for 1 minute followed by inactivation of the NaOCl with sodium thiosulphate. Efficacy of the surface disinfection was tested by sampling the root surface with paper points. The paper points were placed into a test tube containing 1 ml reduced transport fluid, vortexed for 10 seconds, plated onto BHI agar, incubated at 37°C for 48 hours and examined for growth.

Teeth were randomly assigned to either group A or group B within each experimental pair. To establish working length, a #10 K-file was inserted into each root canal until it appeared at the minor diameter under 10× magnification. With the file in place, the apical foramen was sealed with cyanoacrylate (Super Glue Corporation, Rancho Cucamonga, CA) and each tooth was placed into their respective sterile customized models for instrumentation. A coronal seal between the tooth and the model was achieved with cyanoacrylate. Both teeth within each matched pair were instrumented to the same master apical file size and the same volume of irrigants was used during the instrumentation process.

The instrumentation sequence for both teeth within each matched pair was the same except for the irrigation regimen. Coronal flaring was accomplished using size 2-4 Gates Glidden drills. The canals were prepared to working length in a crown-down manner using ProFile Series 29 .04 taper rotary files (Dentsply, Tulsa OK). The master apical file size was a ProFile Series 29 #6 file (ISO .360). Irrigation of the root canals was completed as follows.

Group A

During chemomechanical root canal preparation, each file was followed by irrigation with 1 ml of 5.25% NaOCl. Irrigation was accomplished using a 30 gauge ProRinse needle (Dentsply) placed 1 to 2 mm short of the working length. Final irrigation was completed by using 5 ml of 15% EDTA followed by 5 ml of 5.25% NaOCl. The total irrigation time for the final irrigation sequence was 2 minutes. The canals were then dried with sterile paper points.

Group B

During chemomechanical root canal preparation, each file was followed by irrigation with 1 ml of 1.3% NaOCl. Irrigation was accomplished using a 30 gauge ProRinse needle placed 1 to 2 mm short of the working length. Biopure MTAD was prepared and utilized according to the manufacturer's instructions. One millimeter Biopure MTAD was placed into the canal for 5 minutes. This was followed by a 4 ml irrigation of the canals with Biopure MTAD. The canals were then dried with sterile paper points.

The positive control teeth were instrumented as described earlier except teeth were irrigated with 1 ml saline after each instrument. The

TABLE 1. Mean CFU/mg

Irrigation	Mean CFU/mg	SD
5.25% NaOCl/15% EDTA	1.31×10^2	2.91×10^2
1.3% NaOCl/Biopure MTAD	1.87×10^2	2.37×10^2
Saline	3.77×10^2	3.02×10^2

No significant differences between all groups.

final irrigation of the canals was accomplished with 5 ml of saline. The canals were then dried with sterile paper points.

To test for bacterial survival in the apical 5 mm of the root canal system and dentinal tubules, sterile multipurpose burs in a rear exhaust surgical handpiece (Impact Air 45, Palisades Dental, NJ) were used to resect the apical 5 mm of all experimental and positive control teeth. The samples were pulverized for 30 seconds in liquid nitrogen using a sterile mortar and pestle. The crushed samples were collected and weighed on sterile aluminum foil. The samples were then suspended in 1 ml of reduced transport fluid. Ten-fold dilutions were prepared and 0.1 ml aliquots of the suspensions were spread onto BHI agar media. They were incubated at 37°C for 48 hours and the colony forming units (CFU) enumerated. Using the weight of the pulverized root-end, the number of CFU/mg was determined. Specimens from the control pairs were sampled and cultured using the same techniques. The purity of the positive cultures was also confirmed.

A repeated measures *t* test was used to determine if there was a significant difference in CFU/mg between groups A and B. Independent *t* tests were used to compare group A to the positive controls and group B to the positive controls.

Results

The results are summarized in Table 1. Mean CFU/mg for group A was 131 ± 291 and 187 ± 237 for group B. The mean CFU/mg for the positive control group was 377 ± 301. No significant differences were found between groups A and B ($t = 0.70$, $p = 0.495$). Independent *t* tests also showed no significant differences between group A versus the positive controls ($t = 1.80$, $p = 0.0857$) and no significant differences between group B and the positive controls ($t = 1.61$, $p = 0.121$). Negative control teeth showed no growth throughout the experiment. No bacterial growth was seen from the root surface samples that had been disinfected with 5.25% NaOCl.

Discussion

Three weeks incubation of root canals inoculated with *E. faecalis* has been shown to produce a dense infection reaching 300 to 400 μm into the dentinal tubules (18). Prolonged incubation leads to more tubules being infected, whereas the average depth of penetration of the tubules by bacteria has been found to increase only slowly with time (18).

Pulverizing teeth for bacterial sampling has been previously used (18, 20). Baker et al. (20) used liquid nitrogen to freeze bovine teeth and then pulverized them for evaluation of the antibacterial action of medicaments. By crushing teeth with a mortar and pestle, it is possible to determine CFU in dentinal tubules and other morphological recesses in addition to the main root canal system. Bacteriological sampling with paper points is limited because only the microorganisms collected on the paper points can be cultivated.

Previous studies on the antimicrobial efficacy of MTAD have shown conflicting results. Shabahang and Torabinejad (17) found the combination of 1.3% NaOCl as a root canal irrigant with MTAD as a final rinse was more effective against *E. faecalis* than 5.25% NaOCl with 17% EDTA. However, Dunavant et al. (21) who compared the efficacy of irrigants against *E. faecalis* biofilms in vitro, found 6% and 1% NaOCl were significantly more efficient in eliminating *E. faecalis* biofilms than Smear Clear, REDTA, and Biopure MTAD. A study by Johal et al. (22) found no growth of *E. faecalis* in root canals irrigated with 5.25% NaOCl/15% EDTA, while 50% of the canals irrigated with 1.3% NaOCl/Biopure MTAD demonstrated growth of *E. faecalis*. The difference was statistically significant.

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 (23). It has been previously shown that bacteria may remain viable in tubules at great distances from the pulp (24). This study and previous ones (25) have shown that disinfection of root dentin is not achieved by chemomechanical preparation alone. Bacteria deep in dentinal tubules are apparently protected from instrumentation and irrigation, making their removal or eradication difficult.

Microorganisms may be eliminated or rendered harmless by entombing them through complete obturation of the canal space after chemomechanical root canal preparation (14). Although the consequences of microbes remaining in the dentinal tubules after root canal treatment is not clear (5), the main goal of root canal treatment is still the elimination of microorganisms. The efficacy of other irrigants and more effective irrigant delivery systems needs further research.

In conclusion, the results of this study showed no significant differences in the antimicrobial efficacy of irrigating with 1.3% NaOCl/Biopure MTAD versus irrigation with 5.25% NaOCl/15% EDTA in the apical 5 mm of roots infected with *E. faecalis*. The efficacy of saline irrigation was also not statistically different than the experimental irrigation groups.

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