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Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against *Enterococcus faecalis* contaminating root canals: an *in vitro* study

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Abstract: This *in vitro* study was performed to evaluate the effect of a diode laser and common disinfectants used in combination on mono-infected dental canals. One hundred and six single-rooted human premolars were prepared and contaminated with *Enterococcus faecalis*. After two weeks of incubation, samples were divided into two experimental groups ($n = 48$) and two control groups ($n = 5$). In the first group, the teeth were rinsed for 5 min with either sterile saline, 2.5% NaOCl, or MTAD, or for 1 min with 2% chlorhexidine gluconate (CHX). In the other group, samples were additionally irradiated with a 810-nm diode laser at 2 W output for 5×5 s. Intracanal bacterial sampling was done, and the samples were plated to determine the CFU count. In the first group, 2.5% NaOCl was as effective as 2% CHX and significantly more effective than MTAD ($P < 0.008$). In the second group, either MTAD, 2% CHX or 2.5% NaOCl in combination with laser treatment had a similar effect. Absence of growth was seen only

for MTAD plus laser treatment. Complete elimination of *E. faecalis* was seen only for the combination of MTAD with diode laser irradiation. Combination therapy with MTAD irrigation and diode laser irradiation, within the parameters used in this study, can be recommended as an effective treatment option for complete elimination of *E. faecalis* from the root canal system. (J Oral Sci 53, 355-360, 2011)

Keywords: chlorhexidine gluconate; combined effect; diode laser; MTAD, sodium hypochlorite.

Introduction

One of the goals of root canal therapy is to completely disinfect the root canal and its three-dimensional tubular network (1). Use of mechanical instrumentation alone cannot sufficiently debride and clean this complex tubular network (2,3).

Many irrigation solutions have been used for root canal treatment along with mechanical instrumentation to achieve better debridement. However, because these solutions act through direct contact with targets and have a limited penetration depth into irregularities of root canal walls, they are unable to eliminate microorganisms from deeper layers of the dentin (4,5).

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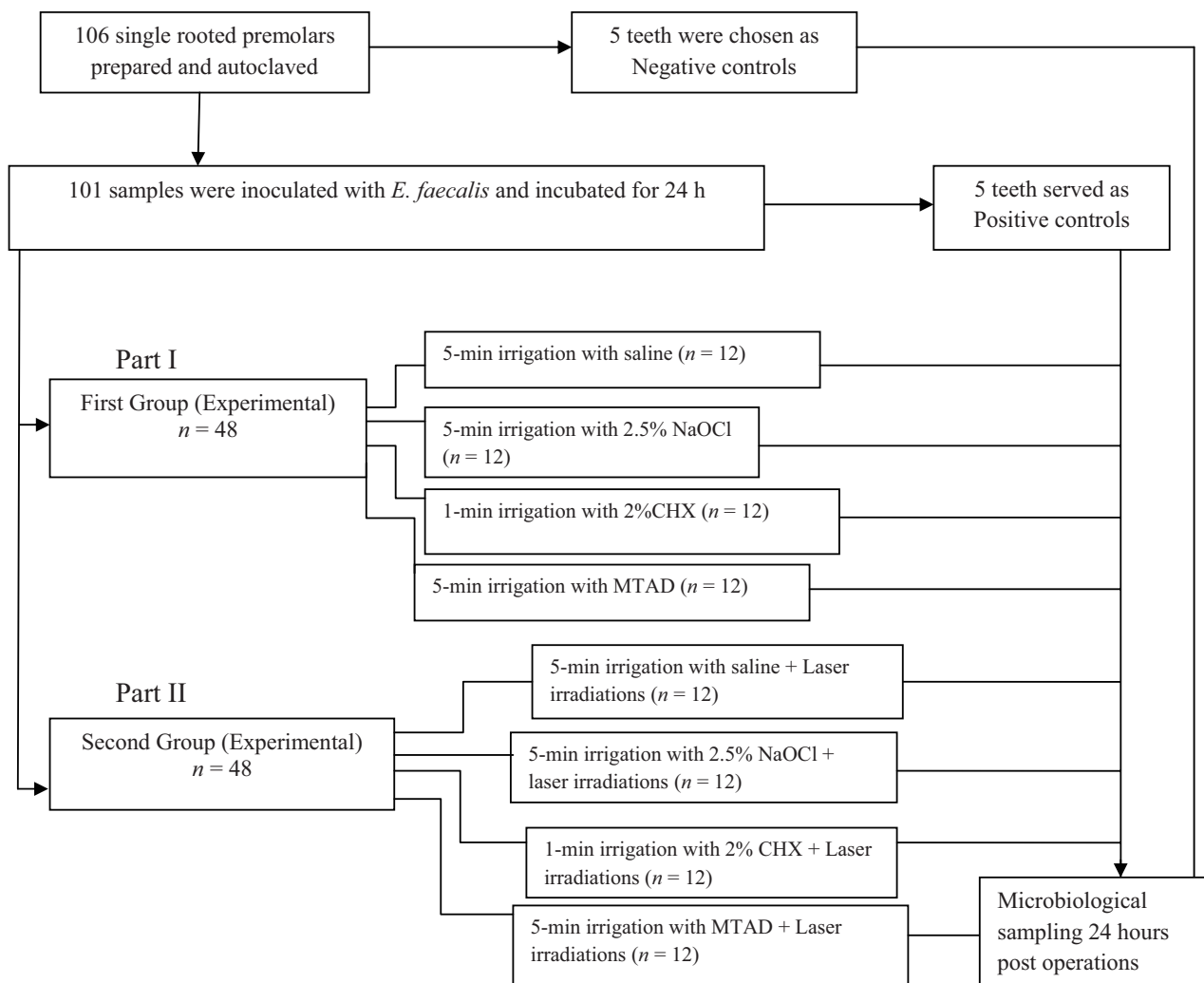


Fig. 1 Flow chart of the methodology employed.

Many researchers have discussed the advantages of intracanal medications such as Ca(OH)₂ between treatment sessions (6,7). However, careful packing of Ca(OH)₂ is required for dealing with microbial targets, and it is difficult to maintain a high pH throughout the root canal (6,8). Moreover, certain resistant bacterial species such as *Enterococcus faecalis* are able to survive in areas of high pH produced by Ca(OH)₂, due to a proton-pump mechanism (9) and ability to form intra- and extra-radicular biofilms, making eradication difficult (10).

Accordingly, many researchers have tried to disinfect the complex tubular network using various laser devices (11-13). These devices have been shown to be effective against microbial agents; however there is some controversy regarding the antibacterial effects of different types of lasers within the dental canal. Although most of these previous studies have demonstrated an improved degree

of disinfection of dentinal tubules, complete eradication of bacterial species, especially endodontic pathogens that grow as biofilms, has not been achieved (13,14).

Combination therapy using various medicaments or irrigation solutions together or in succession has been reported to be more effective for canal disinfection. The use of MTAD (a mixture of a tetracycline isomer, an acid and a detergent) as a final rinse after using 1.3% NaOCl during instrumentation has been advocated to be effective against *E. faecalis* (15). Furthermore, laser-assisted endodontics using high-power diode laser irradiation followed by canal irrigation with 0.5% NaOCl and 17% EDTA-T is able to eliminate *E. faecalis* completely (16).

In this *in vitro* study, we investigated the application of diode laser irradiation after use of disinfecting irrigation solutions in *E. faecalis*-contaminated root canals. The hypothesis tested was that combination therapy involving laser treatment and irrigation solutions would eliminate

E. faecalis completely from dentinal tubules.

Materials and Methods

This study was approved by the Research Ethics Committee of Tehran Azad University, Tehran, Iran.

One hundred six straight, single-rooted human premolars were collected. After caries removal, a conventional access cavity was prepared in each case. A #10 K-type file (K-Files, Mani Inc., Utsunomiya, Japan) was used to confirm patency and to establish a clinical working length 1 mm short of the apical foramen. Root canals were prepared using the crown-down technique with ProTaper rotary instruments (Maillefer-Dentsply, Baillagues, Switzerland) to an apical size equal to #40. Between instrumentations, 2 ml of 2.5% NaOCl was applied. The smear layer was removed using 17% EDTA (Pulpdent EDTA Solution, Pulpdent, Watertown, MA, USA) in an ultrasonic bath for 4 min, followed by another 4-min rinse with 5.25% NaOCl, in accordance with Trisba et al. (17). The apical foramen was sealed using light-cured restorative glass ionomer cement (Densell MPLC, Industria, Buenos Aires, Argentina). The other surfaces of the roots were covered with two layers of nail varnish. Each sample was transferred to a plastic cryo-tube (Cryo. S, PP Greiner Bio-One GmbH., Frickenhausen, Germany) containing sterile brain heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) and autoclaved under a pressure of 15 psi at 121°C for 30 min. Samples were incubated in their sealed tubes for 48 h at 37°C. Daily inspection revealed no signs of turbidity. Five teeth were selected randomly to serve as negative controls.

An overnight pure culture of *E. faecalis* (ATCC 29212) in BHI broth at a concentration of 1.5×10^8 CFU/ml was used for inoculation. The bacterial suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 scale. A 0.01-ml aliquot of the suspension was inoculated into each canal using a sterile insulin syringe. Then the samples were incubated for two weeks under aerobic conditions at 37°C. The inoculum inside the canal was replaced with 0.01 ml of fresh bacterial suspension every other day. Every seven days, random sampling was done for gram staining to confirm the purity and viability of the *E. faecalis* cultures. After the incubation period, five inoculated teeth were chosen to serve as positive controls, while the rest were divided into two experimental groups of 48 each. Each group was further subdivided into four subgroups ($n = 12$). The BHI broth inside the canal was dried out using sterile paper points before irrigating each canal. All irrigations were done using 28-gauge Pro Rinse needles (Dentsply Tulsa Dental, Johnson City, TN, USA) (Fig. 1).

Part I

In the first group, 12 teeth were irrigated with 5 ml of normal saline (Darou pakhsh Inc., Tehran, Iran) for 5 min in subgroup A. In subgroup B, teeth were irrigated with 5 ml of 2.5% NaOCl (Shimi Tajzie Pars Inc., Tehran, Iran) for 5 min. In subgroup C, samples were irrigated with 1.2 ml of 2% chlorhexidine gluconate (Consepsis type, Ultradent products, South Jordan, UT, USA) for 1 min in accordance with the manufacturer's instructions, and finally 12 teeth were irrigated with 5 ml of Biopure MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA) for 5 min in accordance with the manufacturer's instructions. The canals were dried with sterile paper points. Sterile BHI broth, according to the volume of each canal (average 10 μ l), was then added into the canals, followed by transfer of each tooth to another sterile cryo-tube and incubation at 37°C for 24 h.

Part II

In the second group, irrigations similar to those in the first group were done. After irrigation, the dental canals were dried out using sterile paper points, and subsequently laser irradiation was performed 5 times for 5 s each time, with a 15-s interval between irradiations. Laser treatments were carried out with a GaAlAs diode laser (TwiLite™, Biolase Technology, San Clemente, CA, USA), at a wavelength of 810 nm and output power of 2 W with the repeated pulse mode, using a pulse duration of 20 ms and a pulse interval of 40 ms. The laser irradiation was delivered into the canal up to 1 mm short of the working length via a fiber tip 400 μ m in diameter. The handpiece was held to form an angle of approximately 10 degrees between the fiber and the root canal wall. Irradiation was performed with circling movements from the apical part toward the coronal part (step-back technique) without any water spray or air cooling. After laser irradiation, samples in the second group were treated in a similar way to the first group.

Twenty four hours later, the BHI broth was dried out of the root canals, and the canals were refilled with normal saline as a transfer fluid. Sampling from inside the canals was done using a sterile #25 H-file (H-Type Files, Mani Inc.), and circumferential filing was performed for 20 s to collect dentin chips, mostly from the coronal and mid-parts of the canal. A sterile #35 K-file (Mani Inc.) was used for sampling from the apical part by reaming for 20 s. Sterile paper points were used to collect the transfer fluid and dentin chips. Sterile paper points and sampling H- and K-files had been placed into a test tube containing 10 ml of sterile saline and vortexed for 20 s. Fifty microliters of the vortexed saline was applied to Bile

Table 1 Mean and standard deviation of CFU/ml and degree of disinfection in the experimental groups and subgroups, relative to the positive controls

Experimental groups	Sub group	Disinfection (%)	CFU/ml (\pm SD)
First group (Without laser)	Saline	38.59	$1.13 \times 10^4 (\pm 8.1 \times 10^3)$
	2.5 %NaOCl	99.91	$1.66 \times 10 (\pm 5.77 \times 10)$
	2 % CHX	99.91	$1.66 \times 10 (\pm 5.77 \times 10)$
	MTAD	97.56	$4.5 \times 10^2 (\pm 5.33 \times 10^2)$
Second group (With laser)	Saline	80	$3.68 \times 10^3 (\pm 1.34 \times 10^3)$
	2.5 %NaOCl	99.91	$3.33 \times 10 (\pm 1.15 \times 10^2)$
	2 % CHX	99.82	$1.66 \times 10 (\pm 5.77 \times 10)$
	MTAD	100	0

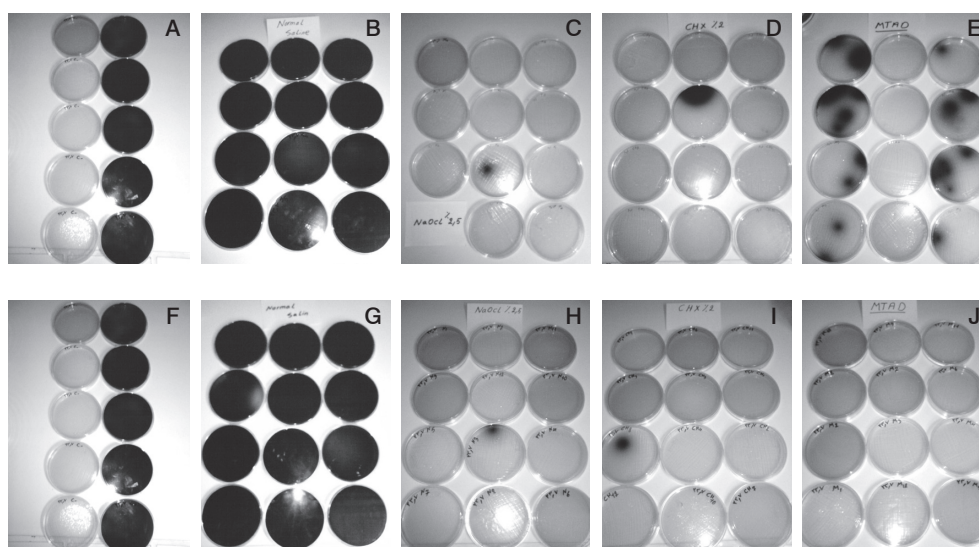


Fig. 2 First group (unirradiated samples): A, Positive and negative controls; B, Normal saline; C, 2.5% NaOCl; D, 2% CHX; E, MTAD. Second group (irradiated samples): F, Positive and negative controls; G, Normal saline plus laser irradiation; H, 2.5% NaOCl plus laser irradiation; I, 2% CHX plus laser irradiation; J, MTAD plus laser irradiation.

Esculin agar culture plates (Merck KGaA, Germany) and incubated at 37°C for 48 h. All procedures were carried out under sterile and aseptic conditions.

The CFU/ml for each plate was calculated using a bacterial colony counter (Colony Star, Funke Gerber Product, Gebr Liebsch, Germany).

The mean and standard deviation of CFU values were calculated for the samples. Degrees of disinfection in the experimental subgroups were calculated in relation to the positive controls. The CFU values were analyzed by Kruskal-Wallis test. Mann-Whitney *U*-test was used for subgroup comparisons.

Results

Absence of growth was seen in the negative control agar plates. All positive control plates showed exten-

sive bacterial growth ($1.84 \times 10^4 \pm 8.08 \times 10^3$). In the first group, significant differences were noted between subgroup A and subgroups B, C, and D ($P < 0.0001$). In subgroups B and C, the lowest mean CFU/ml ($1.66 \times 10 \pm 5.77 \times 10$) was detected, being significantly different from subgroup D ($P < 0.008$). (Table 1)

In the second group, the mean CFU/ml for all subgroups was significantly different from the positive controls. Absence of bacterial growth was seen only in the plates of subgroup D (Biopure MTAD plus laser), being significantly different from subgroup A (saline plus laser) ($P < 0.0001$), but not from subgroups B and C (2.5% NaOCl and 2% chlorhexidine gluconate plus laser). A significant reduction in CFU/ml was seen in subgroups B and C, relative to subgroup A ($P < 0.0001$).

Also, significant differences were noted only between

the Biopure MTAD and MTAD plus laser subgroups, and between saline- and saline plus laser-treated samples ($P < 0.0001$). Photos of the agar plates in the first and second groups after one week of incubation are shown in Fig. 2.

Discussion

This *in vitro* study was conducted to clarify whether intracanal irradiation with a diode laser in combination with 2.5% NaOCl, 2% chlorhexidine gluconate and Biopure MTAD would be able to eradicate *E. faecalis* contaminating root canals. The teeth employed were not decoronated in order to simulate clinical conditions and limitations during root canal irrigation and laser irradiation.

E. faecalis, as a biofilm-forming pathogen, was chosen for this investigation since it has been used for evaluation of the antibactericidal effects of several irrigation solutions and various laser devices (13,14,17,18).

The results of microbiological cultures in the first group indicated that 2.5% NaOCl and 2% chlorhexidine gluconate were more effective than Biopure MTAD, in agreement with the results reported by Baumgartner et al. (18) and Trisba et al. (17); however, they conflicted with the results of some other studies (15,19). This difference may have been due to the fact that these previous workers soaked the teeth in MTAD, which is not practical in a clinical situation. Furthermore, the carryover effect of doxycycline in MTAD may have been contributory, since complete removal of binding between doxycycline and dentin requires over 160 min of elution with water (20).

It is more than a decade since the diode laser was introduced to the field of laser-assisted endodontics as an effective and safe tool for clinical endodontic treatment (12). Some studies have indicated that diode lasers are unable to completely eliminate *E. faecalis* (13,21,22). Moritz et al. (12) showed that complete eradication of bacteria was achieved only when teeth were treated using higher-power irradiation that produced higher temperatures on the root surface. In the present study, the results of laser treatment after sterile saline irrigation revealed that the diode laser beam was only able to disinfect root canals up to 80% at 2 W. It has been reported that a diode laser has an antibacterial effect of more than 95% on dentin slices with a thickness of 100 μm (21,22), in contrast to our results. These differences may be due to the fact that they used dentin slices, and performed sampling for microbiological analysis immediately after irradiation, whereas in our study human teeth were used, and intra-canal sampling was performed 24 h later, which would have provided time for growth of possibly surviving bacteria.

Our results suggest that diode laser irradiation in combination with Biopure MTAD is able to eliminate *E. faecalis*. This result was similar to a previous study indicating that high-power diode laser irradiation (830 nm, at a power of 3 W) followed by irrigation with 0.5% NaOCl and 17% EDTA-T was able to provide increased disinfection of deep radicular dentin (16). Also, the absence of bacterial growth might have been related to insufficient sensitivity of the methodology used for detecting possible viable bacterial cells at lower concentrations (16). This might have accounted for the apparent absence of growth seen in the agar plates after treatment with Biopure MTAD plus the laser. On the other hand, it should be noted that the citric acid component of the Biopure MTAD in our study and the EDTA-T used in the previous study are capable of removing the smear layer (23). This additional smear layer removal may allow deeper penetration of the laser beam into dentinal tubules, which act as optical fibers, resulting in better laser propagation through the peripheral dentin (13). Along with the prolonged action of doxycycline bound to the dentin (20), these phenomena might account for the better outcomes obtained with MTAD plus laser irradiation than for MTAD alone.

On the basis of the laser parameters used in this *in vitro* study, it can be concluded that combination therapy consisting of irrigation and laser irradiation, especially when using Biopure MTAD, is an effective treatment option for eliminating *E. faecalis* from the root canal system. Due to limitations and complications during treatment procedures in a clinical situation, further studies are required to investigate the clinical effectiveness of this approach.

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References

1. Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G (1990) Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. *J Endod* 16, 580-588.
2. Byström A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 89, 321-328.
3. Dalton BC, Orstavik D, Phillips C, Pettiette M, Trope M (1998) Bacterial reduction with nickel-

- titanium rotary instrumentation. *J Endod* 24, 763-767.
4. Kouchi Y, Ninomiya J, Yasuda H, Fukui K, Moriyama T, Okamoto H (1980) Location of streptococcus mutans in the dentinal tubules of open infected root canals. *J Dent Res* 59, 2038-2046.
 5. Berutti E, Marini R, Angeretti A (1997) Penetration ability of different irrigants into dentinal tubules. *J Endod* 23, 725-727.
 6. Byström A, Claesson R, Sundqvist G (1985) The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol* 1, 170-175.
 7. Shuping GB, Ørstavik D, Sigurdsson A, Trope M (2000) Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J Endod* 26, 751-755.
 8. Nerwich A, Figdor D, Messer HH (1993) pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. *J Endod* 19, 302-306.
 9. Evans M, Davies JK, Sundqvist G, Figdor D (2002) Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J* 35, 221-228.
 10. Spratt DA, Pratten J, Wilson M, Gulabivala K (2001) An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *Int Endod J* 34, 300-307.
 11. Klinker T, Klimm W, Gutknecht N (1997) Antibacterial effects of Nd:YAG laser irradiation within root canal dentin. *J Clin Laser Med Surg* 15, 29-31.
 12. Moritz A, Gutknecht N, Goharkhay, Schoop U, Wernisch J, Sperr W (1997) In vitro irradiation of infected root canals with a diode laser: results of microbiologic, infrared spectrometric, and stain penetration examinations. *Quintessence Int* 28, 205-209.
 13. Schoop U, Kluger W, Moritz A, Nedjelic N, Georgopoulos A, Sperr W (2004) Bactericidal effect of different laser systems in the deep layers of dentin. *Lasers Surg Med* 35, 111-116.
 14. Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P (2006) Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. *Int Endod J* 39, 547-557.
 15. Shabahang S, Torabinejad M (2003) Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. *J Endod* 29, 576-579.
 16. de Souza EB, Cai S, Simionato MR, Lage-Marques JL (2008) High-power diode laser in the disinfection in depth of the root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 106, 68-72.
 17. Krause TA, Liewehr FR, Hahn CL (2007) The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. *J Endod* 33, 28-30.
 18. Johal S, Baumgartner JC, Marshall JG (2007) Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. *J Endod* 33, 48-51.
 19. Torabinejad M, Shabahang S, Apereo RM, Kettering JD (2003) The antimicrobial effect of MTAD: an in vitro investigation. *J Endod* 29, 400-403.
 20. Ciarlone AE, Johnson RD, Tomaselli DL Jr, Sue Seale N, Pashley DH (1988) The quantitative binding of tetracycline to dentin. *J Endod* 14, 494-496.
 21. Gutknecht N, van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F (2000) Diode laser radiation and its bactericidal effect in root canal wall dentin. *J Clin Laser Med Surg* 18, 57-60.
 22. Gutknecht N, Franzen R, Schippers M, Lampert F (2004) Bactericidal effect of a 980-nm diode laser in the root canal wall dentin of bovine teeth. *J Clin Laser Med Surg* 22, 9-13.
 23. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S (2003) A new solution for the removal of the smear layer. *J Endod* 29, 170-175.