Antimicrobial efficacy of nine different root canal irrigation solutions

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INTRODUCTION

Endodontic therapy is a debridement procedure that requires removal of the irritants from the canal and periapical tissues if the treatment is to be successful.[1] It is well established that bacteria are the main etiological factors in the development of dentinal caries and its progression to pulpal and periapical disease.[2] *E. faecalis* is the bacterial species most frequently recovered from root-filled teeth. Studies have shown that *E. faecalis* is able to withstand a high alkaline environment such as the one generated by calcium hydroxide.[3] The resistance appears to be related to a cell proton-pump that is necessary for survival of the bacterium at high pH.[4] Therefore, *E. faecalis* is able to form biofilms even in calcium-hydroxide-medicated root canals.[5] In addition, under starved conditions, this resilient organism shows tolerance to sodium hypochlorite,[6] heat, hydrogen peroxide, acid and ethanol.[7] *E. faecalis* can also survive extended periods of starvation in water,[8,9] within water-filled dentinal tubules,[10] and in human serum.[11]

Current methods available for bacterial reduction in endodontic therapies include mechanical instrumentation to clean and widen the root canal space, and chemical disinfection by irrigation and intracanal medication, known as an antimicrobial dressing.[12] The use of irrigants in conjunction with mechanical instrumentation is essential for loosening and helping to remove debris and bacteria. It is important that the irrigating solution should also provide antibacterial effects which may include the killing of bacteria in the root canal system and provide disinfection in areas of the canal that are inaccessible to mechanical instrumentation.

Numerous irrigating solutions have been recommended for clinical endodontic use.[13] Sodium Hypochlorite (NaOCl) is the most widely used and has aided canal preparation for many years.[14] It is an alkaline solution with a pH of approximately 11 to 12. Many investigators have demonstrated the germicidal and antibacterial properties of NaOCL.[15,16,17,18] Sodium hypochlorite solutions of 5.25% have been shown to be potently bactericidal against Gram-positive and Gram-negative bacteria, spore-producing microorganisms, and are also effective against viruses. The killing efficacy of low concentrations of NaOCl against *E. faecalis* was demonstrated by Siquera et. al. in their 1997 publication.[19]

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ACRONYMS

ECA:	Electrochemically Activated Water
EDTA:	Ethylenediamine Tetra-acetic Acid
FEM:	Flow-through Electrolytic Module
NaOCL:	Sodium Hypochlorite

Chlorhexidine gluconate has been widely used in Periodontics because of its antibacterial activity.[20,21] Its has been proposed both as irrigant and intracanal medicament in Endodontics.[22,23] Chlorhexidine has been considered as an alternative to NaOCL and has been studied for its various properties: antimicrobial activity,[22,23,24,25] residual antimicrobial activity,[25,26] biocompatibility,[22,27] and an action on bacterial lipopolysaccharide (LPS).[28]

Ethylenediamine Tetra-acetic Acid (EDTA) was introduced by Nygaard-Ostby in 1957 to facilitate preparation of root canals, particularly in the case of narrow, calcified canals.[29] EDTA is not irritating to pulpal or periapical tissue, is self-limiting, and is not corrosive to endodontic instruments. As an additional benefit, EDTA has been found to inhibit bacterial growth.[30] Significant antibacterial activity was demonstrated for Smear-Clear (mixture of 17% EDTA, cetrimide, polyoxyethylene (10) iso-octylcyclohexyl ether) with a 78% decrease in bacterial numbers compared with a 27% decrease in bacterial numbers for an irrigating solution containing only 17% EDTA.[31]

Electrochemically Activated Water (ECA) uses various electrode systems to electrically charge or activate water or watery solutions such as saline. ECA is produced from salt solutions of low concentration in a special unit that houses a unique flow-through electrolytic module (FEM). The FEM is capable of producing special solutions that have bactericidal and sporicidal activity, yet are odourless, safe to human tissue and essentially non-corrosive to metal surfaces.[32,33] The ECA devices have been in widespread commercial use in Russia and the Commonwealth of Independent States for a

number of years, mainly in the areas of hospital disinfection, sterilization, and in agricultural and industrial processes.[34] Van der Merwe, Marais and Botha compared the antimicrobial efficacy and irrigating potential of different solutions in the removal of *E. faecalis* from infected canals. ECA gave the best results in removing the smear layer and eliminating the bacterium from the root canals.[35]

Citric acid has been used in periodontal procedures as an aid in connective tissue reattachment by exposing collagen fibres on the root surface. Citric acid also exhibits antibacterial properties, as well as inhibition of bacterial growth.[36] Like EDTA, this demineralizing agent has been recommended as an adjuvant in root canal therapy.[37]

A more recent root canal irrigation solution called MTAD has been proposed.[38]

Biopure MTAD irrigation solution contains:
- Tetracycline (150mg/5ml Doxycycline, Sigma-Aldrich Co., St Louis, MO, USA)
- Acid (Citric acid, Sigma-Aldrich)
- Detergent (Tween 80, Sigma-Aldrich)

The citric acid and tetracycline remove the smear layer and allows the antibiotic molecule to enter into the dentinal tubules. Thereafter, the detergent has the function of reducing the surface tension and increasing the penetrability of the irrigation solution into the tubules.[38]

The use of ozonated water for treatment of endodontic infections has been suggested.[39,40] Ozone has also been used in the water industry to eliminate bacteria[41] and its properties could be useful in dentistry.[42] Ozone is a blue gas, containing three oxygen atoms. It is an irritant, toxic, unstable and also very reactive.[43] The antimicrobial effect from ozone results from oxidation of microbial cellular components. Ozone is generated by passing oxygen through a high-voltage environment.[44] In a study by Nagayoshi *et al.*, the authors observed that ozonated water had nearly the same antimicrobial activity as did 2.5% NaOCl during irrigation, especially when combined with ultrasonification.[39]

It is clear that several options are available to the clinician. The objective of this *in vitro* study was to establish the antimicrobial efficacy of nine different root canal irrigation solutions.

MATERIALS AND METHODS

The antimicrobial activity of the following nine different irrigation solutions against *E. faecalis* were determined using the disc diffusion test:
- 3.5% Sodium Hypochlorite Liquid (NaOCL) (Rekitt Benckiser South Africa (Pty) Ltd., Elandsfontein, Gauteng, South Africa) (Batch no: 0055366);
- EDTA 18% Root Canal Irrigating Solution (Ultradent Products, Inc., South Jordan, Utah, USA.) (Batch no: B0FVZ);
- Sterilox Electrolyte Solution activated in Sterilox Machine (Optident, International Development Centre, West Yorkshire, UK) (Batch no: MM17604);
- TopClear Solution (mixture of 0.008% Cetrimide and 17% EDTA) (Dental Discounts CC, Paulshof, Sandton, South Africa) (Batch no: 10557);
- Vista CHX 2% Chlorhexidine Gluconate Solution (Vista Dental Products, Racine, Wisconsin, USA) (Batch no: 090905);
- Citric Acid 10% Root Canal Irrigating Solution (Ultradent Products, Inc., South Jordan, Utah, USA.) (Batch no: B0C3F);
- BioPure MTAD Antibacterial Root Canal Cleanser (mixture of 150mg/5ml Doxycycline, Citric Acid and Tween 80) (Dentsply Tulsa Dental, Johnson City, Tulsa, USA) (Batch no: 040920);
- Ozonated Water produced in OH DENT Generator (Unique Dental, Centurion, South Africa) (Batch no: 0702021); and,
- SmearClear (mixture of 17% EDTA and Tween 80) (Sybron-Endo, Glendora, California, USA) (Batch no: 450788).

Sterile water served as a tenth and control solution.

The test plates were inoculated with *E. faecalis* and the zones of inhibition effected by the various test substances were measured. A MacFarland Standard 1 suspension[45] was prepared from an overnight culture of *E. faecalis* (ATCC 49474) and spread with a sterile glass rod onto each of 50 Casein-peptone-Soymeal-peptone Agar (CASO-Agar) plates (Merck SA (Pty) Ltd., Halfway House, South Africa).

The prepared agar plates were randomly divided into ten groups of five plates each. Five replicates were prepared for each sample solution. The following concentrations of each irrigation solution were prepared: 100% (undiluted), 1/10, and 1/100. Ten microlitres of each concentration of each test solution were severally dispensed onto standardized, sterilized, 5mm-diameter filter paper discs and left to dry. Control discs were prepared for each plate using sterile water, and left to dry. The Agar plates were divided into quadrants. To each Agar plate three paper discs were assigned, representing the three concentrations of the test solution, and one of each was placed onto one of the quadrants of the plate. To the fourth quadrant in each plate an identifying label was assigned. Plates were incubated anaerobically (37°C for 24 hours) using an Anaerocult A® (Merck SA (Pty) Ltd.) to increase CO_2 concentration because *E. faecalis* grows better under facultative anaerobic conditions. The antibacterial activity of the test materials was apparent from the circular clear inhibition zones which formed around the filtration paper discs. The diameters of these inhibition zones were measured using a calliper, at three different positions for each paper disc. An average was calculated for the 15 measurements that were obtained for each test solution.

The One-way ANOVA test using Statistix 8 Software (Analytical Software) was used to determine whether there were statistically significant differences between the inhibition zones obtained by the different irrigation solutions.

RESULTS

The means and standard deviations of the measurements of the zones of inhibition for all the test solutions are presented in Tables 1 to 3. Figures 1 to 3 show the inhibition zones obtained for selected irrigation solutions in the undiluted, 1/10 diluted and 1/100 diluted concentrations.

Undiluted Solutions (100%)

No antibacterial activity was observed adjacent to the filter paper discs saturated with sterile water (control), nor with Sterilox and Ozonated water (Table 1). The average zones of inhibition for 3.5% NaOCl, 18% EDTA, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, BioPure MTAD and SmearClear were 9.2mm, 8.3mm, 8.8mm, 6.4mm, 0.7mm, 11.5mm and 10mm respectively. Figure 1 shows the comparison of the average areas of inhibition for the undiluted irrigation solutions.

Table 4 shows the statistical comparisons between the different inhibition zones for the undiluted irrigation solutions. Statistical analysis using the One-Way ANOVA test showed a statistically significant difference between the inhibition zones obtained for BioPure MTAD compared with all the other

Table 1: Comparison of *in vitro* antimicrobial activity against *E. faecalis* of the undiluted irrigation solutions.

100% Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H_2O control	0	0	0
3.5% NaOCl	9.20	± 2.51	27.3
EDTA 18%	8.25	± 0.23	2.8
Sterilox	0	0	0
TopClear	8.84	± 0.11	1.2
CHX	6.44	± 0.09	1.4
Citric acid	0.70	± 0.21	30.0
MTAD	11.53	± 0.35	3.0
Ozonated water	0	0	0
SmearClear	10.08	± 0.31	3.1

Table 2: Comparison of *in vitro* antimicrobial activity against *E. faecalis* of 1/10 diluted irrigation solutions.

1/10 Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H_2O control	0	0	0
3.5% NaOCl	0	0	0
EDTA 18%	0.54	± 0.02	3.7
Sterilox	0	0	0
TopClear	2.22	± 0.19	8.6
CHX	1.26	± 0.22	17.5
Citric acid	0	0	0
MTAD	9.38	± 0.29	3.1
Ozonated water	0	0	0
SmearClear	6.31	± 0.67	10.6

Table 3: Comparison of *in vitro* antimicrobial activity against *E. faecalis* of 1/100 diluted diluted irrigation solutions.

1/100 Solution	Mean (mm) Inhibition Zones	Standard Deviation	Coefficient of variance (%)
H_2O control	0	0	0
3.5% NaOCl	0	0	0
EDTA 18%	0	0	0
Sterilox	0	0	0
TopClear	0	0	0
CHX	0	0	0
Citric acid	0	0	0
MTAD	2.39	± 0.17	7.1
Ozonated water	0	0	0
SmearClear	0	0	0

products ($p<0.05$) except SmearClear ($p>0.05$). The zones of inhibition for these two products were significantly larger than those seen around the filter papers saturated with Sterilox, 10% Citric acid, Ozonated water, 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine. There was no significant difference between the inhibition zones of SmearClear and 3.5% NaOCl ($p>0.05$), TopClear and 3.5% NaOCl ($p>0.05$), and neither between 3.5% NaOCl and 18% EDTA ($p>0.05$). There was a statistically significant difference between the inhibition zones of 2% Chlorhexidine and 3.5% NaOCl ($p<0.05$).

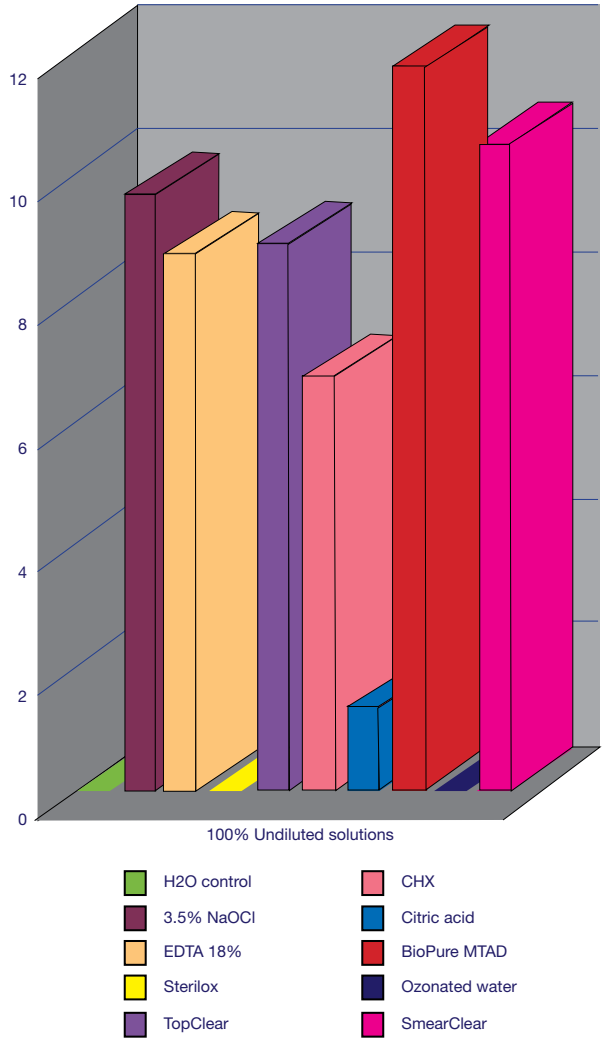

Figure 1: The average zones of inhibition recorded by the undiluted irrigation solutions.

1/10 Diluted Solutions

No antibacterial activity was observed adjacent to the filter papers saturated with sterile water (control), 3.5% NaOCl, Sterilox, 10% Citric acid and Ozonated water (Table 2). The average zones of inhibition for 18% EDTA, TopClear 17% EDTA, 2% Chlorhexidine, BioPure MTAD and SmearClear were 0.5mm, 2.2mm, 1.3mm, 9.4mm and 6.3mm respectively. Figure 2 depicts the comparison of the average areas of inhibition for the 1/10 diluted irrigation solutions.

Table 5 shows the statistical comparisons between the different inhibition zones for the diluted 1/10 irrigation solutions. Statistical analysis using the One-Way ANOVA test showed a statistically significant difference between the inhibition zones obtained for BioPure MTAD and SmearClear ($p<0.05$). The zones of inhibition for these two products were significantly larger than those seen around the filter papers saturated with Sterilox, 10% Citric acid, Ozonated water, 3.5% NaOCl NaOCl, 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine. TopClear created a zone of inhibition that was significantly larger in diameter than that seen with 2% Chlorhexidine and 18% EDTA ($p<0.05$). There was also a significant difference between the inhibition zones associated with 2% Chlorhexidine and 18% EDTA ($p<0.05$).

1/100 Diluted Solutions

No antibacterial activity was observed adjacent to the filter papers saturated with sterile water (control), 3.5% NaOCl,

Table 4: Significance of difference between the mean values (Table 1) of the *in vitro* antimicrobial activity of the undiluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

	3.5% NaOCl	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
Sterile water	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
3.5% NaOCl			p<0.05		p<0.05	p<0.05	p<0.05	p<0.05	
EDTA 18%			p<0.05			p<0.05	p<0.05	p<0.05	p<0.05
Sterilox	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
TopClear			p<0.05			p<0.05	p<0.05		p<0.05
CHX	p<0.05		p<0.05			p<0.05	p<0.05	p<0.05	p<0.05
Citric acid	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
MTAD	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	
Ozonated water	p<0.05	p<0.05		p<0.05	p<0.05		p<0.05		p<0.05
SmearClear		p<0.05	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	

Table 5: Significance of difference between the mean values (Table 2) of the *in vitro* antimicrobial activity of the 1/10 diluted irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

	3.5% NaOCl	EDTA 18%	Sterilox	TopClear	CHX	Citric acid	MTAD	Ozonated water	SmearClear
Sterile water				p<0.05	p<0.05		p<0.05		p<0.05
3.5% NaOCl				p<0.05	p<0.05		p<0.05		p<0.05
EDTA 18%				p<0.05	p<0.05		p<0.05		p<0.05
Sterilox				p<0.05	p<0.05		p<0.05		p<0.05
TopClear	p<0.05	p<0.05	p<0.05		p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
CHX	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	p<0.05	p<0.05	p<0.05
Citric acid				p<0.05	p<0.05		p<0.05		p<0.05
MTAD	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05		p<0.05	p<0.05
Ozonated water				p<0.05	p<0.05		p<0.05		p<0.05
SmearClear	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	

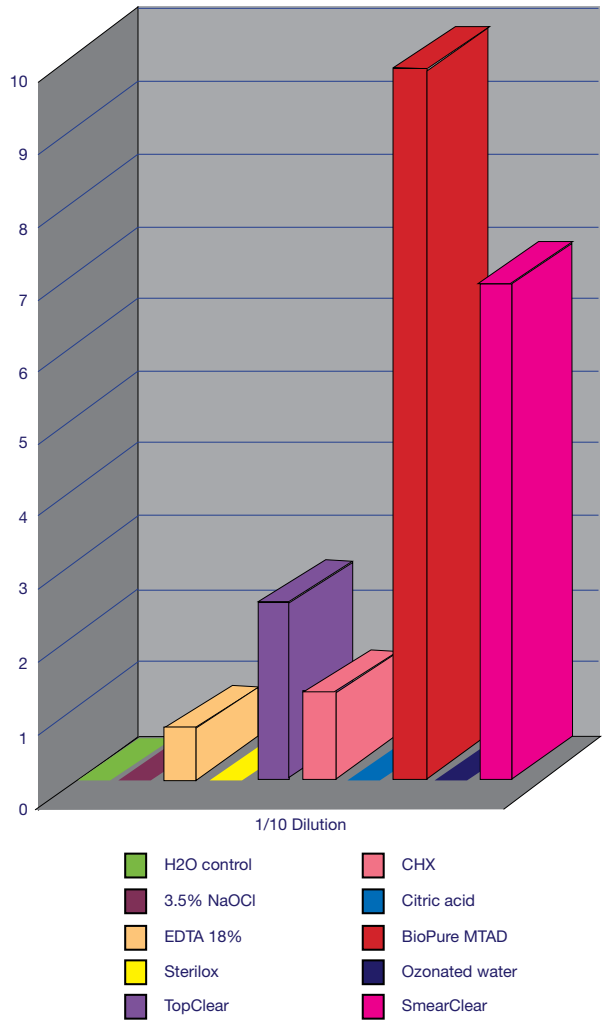

Figure 2: The average zones of inhibition recorded by the 1/10 diluted irrigation solutions.

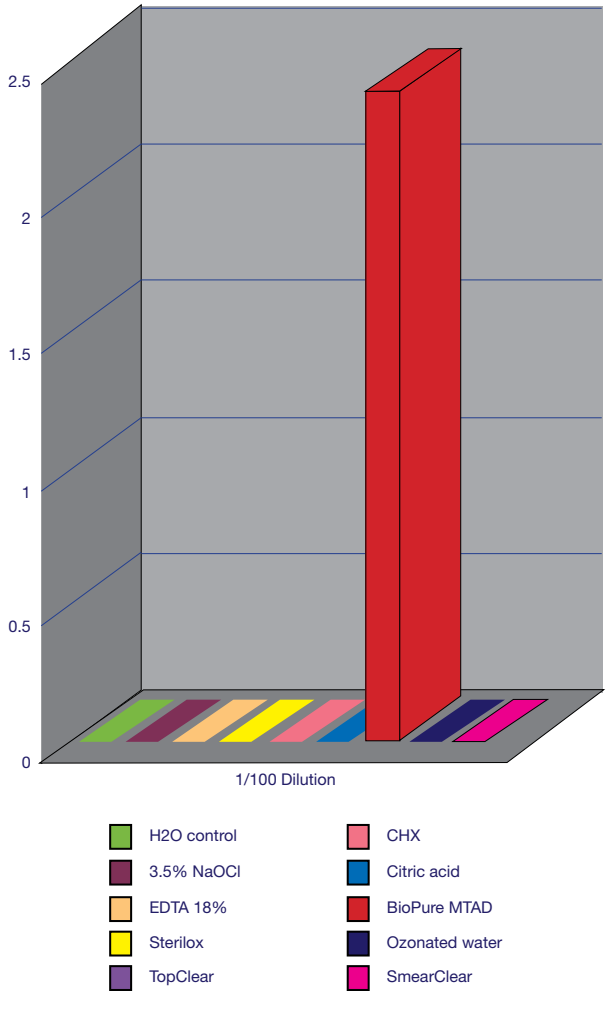

Figure 3: The average zones of inhibition recorded by the 1/100 diluted irrigation solutions.

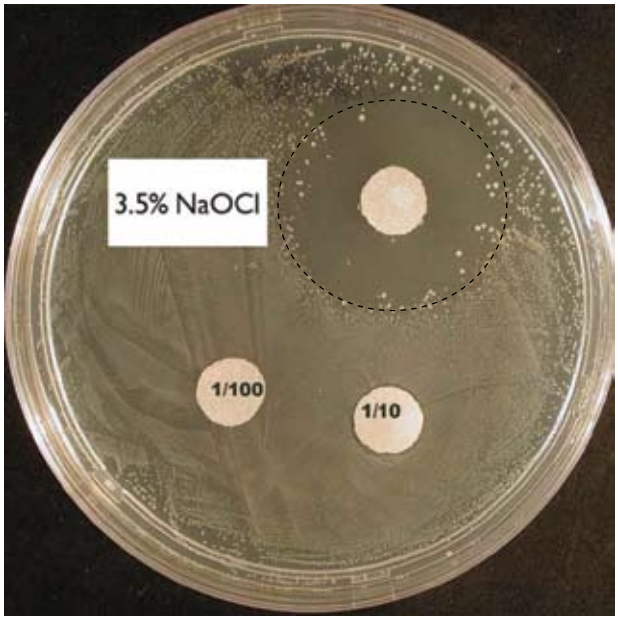

Figure 4: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Paper discs carrying NaOCl solutions. The average zone of inhibition of the undiluted 3.5% NaOCL was 9.2mm. No zone of inhibition was observed adjacent to the filter papers saturated with the 1/10 and 1/100 solutions.

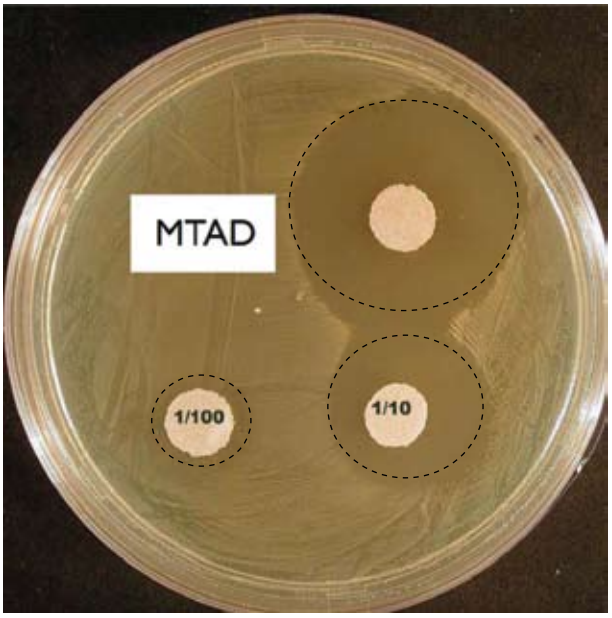

Figure 5: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours – Paper discs carrying BioPure MTAD Antibacterial Root Canal Cleanser solutions. The zone of inhibition of the undiluted MTAD solution was 11.5mm, for the 1/10 solution 9.4mm and for the 1/100 solution 2.4mm.

Sterilox, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, Ozonated water and SmearClear (Table 3). The average zone of inhibition for BioPure MTAD was 2.4mm. Figure 3 shows the comparison of the average areas of inhibition for the 1/100 diluted irrigation solutions.

Statistical analysis using the One-Way ANOVA test showed a statistically significant difference between the mean inhibition zones obtained for BioPure MTAD compared with all the other irrigation solutions ($p<0.05$).

Figures 4 and 5 are representative photographs of *E. faecalis*-seeded CASO Agar plates, which show the zones of inhibition for the different concentrations of 3.5% NaOCl and MTAD solutions, respectively.

DISCUSSION

The intention of this *in vitro* study was to establish the antimicrobial efficacy of nine different irrigation solutions against *E. faecalis*.

Bacteria play the primary etiological role in the development of necrotic pulps and periapical disease following root canal treatment.[46] One of the crucial factors for success of the treatment is eradication of microorganisms and their by-products from the root canal system.[47,48,49] In the control of endodontic infection, irrigation and instrumentation are essential factors in eliminating microorganisms from the root canal system.[50,51] Mechanical debridement alone does not result in total or permanent reduction of bacteria.[15] The use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the number of microorganisms.[15,47,48]

E. faecalis was chosen as the test organism in this study because it has been associated with persistent apical inflammation in clinical situations,[52,53] and also because a recent study claimed that Biopure MTAD may not be effective against *E. faecalis* biofilms.[31] Love investigated a possible mechanism that would explain how *E. faecalis* could survive and grow within dentinal tubules and then reinfect canals.[11]

The author postulated that a virulence factor of *E. faecalis* in root-filled teeth with post-treatment disease may be related to the fact that *E. faecalis* cells maintain the capability to invade dentinal tubules and to adhere to collagen in the presence of human serum.

An ideal intracanal irrigant or medication should be able to disinfect the dentine and its tubules in one visit. In addition, it should have a sustained antimicrobial effect after its application.[54]

The most popular irrigating solution is NaOCL. It is an effective antimicrobial agent[15,55] and an excellent organic solvent for vital, necrotic and fixated tissues.[56] However, it should be noted that it is intensely irritating to periapical tissues, especially in high concentrations.[57,58] In the present study the undiluted 3.5% NaOCl demonstrated excellent antimicrobial properties against *E. faecalis*. This is in agreement with the findings of Harrison and Hand who showed that NaOCl is an effective bacterial agent when it is used undiluted. However, when diluted, it was shown to be completely ineffective against *E. faecalis*.[59]

Some authors recommend the use of a chelating agent as an irrigation solution together with NaOCl.[29] In the present study four different chelator solutions were tested: EDTA 18% Root Canal Irrigating Solution, TopClear Solution (mixture of 0.008% cetremide and 17% EDTA), SmearClear (mixture of 17% EDTA, cetrimide, polyoxyethylene (10) iso-octylcyclohexyl ether) and Citric Acid 10% Root Canal Irrigating Solution. Three of these solutions (EDTA 18%, TopClear and SmearClear) demonstrated antimicrobial properties against *E. faecalis*.

TopClear and SmearClear, used in the present study, are combination solutions of EDTA and cetrimide. These solutions demonstrated similar antimicrobial properties against *E. faecalis* compared with the 18% EDTA solution in the undiluted form. It should be noted that SmearClear was the only EDTA-containing solution that showed antimicrobial properties to *E. faecalis* after the solutions were diluted to a 1/10 dilution. The SmearClear results found in the present study conform to those of a recent investigation carried out

by Dunavant *et al*.[31] That study demonstrated significant antibacterial activity, with a 78% decrease in bacterial numbers with SmearClear as compared with a 27% decrease in bacterial numbers for an irrigating solution containing only 17% EDTA. The authors attributed the increase in antimicrobial activity of SmearClear to the addition of the surfactant Cetrimide.

The 10% Citric Acid solution failed to show any antimicrobial properties against *E. faecalis*. It must be noted that only a low concentration of citric acid (10%) was used in comparison to the average concentration of EDTA-containing solutions.

Another group of antiseptic agents that can be added to Citric Acid irrigants to increase their antimicrobial capacity are tetracycline antibiotics.[60] BioPure MTAD is an example of such a product. This endodontic irrigant contains 3% doxycline hyclate, 4.25% Citric acid and 0.5% polysorbate 80 detergent.[61] BioPure MTAD represents an innovative approach to approach simultaneous removal of the smear layer and disinfection of root canals.[61] The results of the present study confirm the antimicrobial properties against *E. faecalis* (undiluted and 1/10 dilution).

Chlorhexidine is a potent antiseptic and its use in Endodontics has been proposed both as irrigant and intracanal medicament.[22,23] The undiluted, 1/10 and 1/100 diluted solutions of 2% Chlorhexidine solutions used in the present study demonstrated some antimicrobial properties against *E. faecalis*. Despite its use as a root canal irrigant, it cannot be advocated as the main irrigant because chlorhexidine is unable to dissolve necrotic tissue remnants[62] and is also less effective on Gram-negative- than on Gram-positive bacteria.[63,64]

The undiluted as well the diluted ozonated water that was used in the present study failed to demonstrate any antimicrobial properties against *E. faecalis*.

The antimicrobial results of our study are in agreement with Hems *et al*., who showed that biofilms incubated for four minutes with ozonated water showed no significant reduction in cell viability attributable to ozone alone, whereas no viable cells were detected when NaOCl was used for the same time period.[40] The results of this study differ from those of another study where ozonated water combined with ultrasonification had almost the same antimicrobial activity as 2.5% NaOCl during irrigation.[39] However, ultrasonification alone can cause cell death by lysis.[39]

In a preliminary report Marais has asserted that the cleansing efficacy of electrochemically activated water (Steds, Radical Waters, Johannesburg, South Africa) in root canals was superior to that of NaOCl.[65] Marais showed that the ECA water removed the bacteria and smear layer in large areas of the root canals. However, Marais and Williams repeated this study and concluded that ECA did not demonstrate any antimicrobial effectiveness against *E. faecalis*.[66] In the present study we could not identify any antimicrobial properties for Sterilox, also an activated electrolyte solution, against *E. faecalis*.

Finally, it should be noted that discrepancies in results among the published studies and the present investigation are difficult to analyze as a result of the use of differing experimental bacterial strains, methods and materials.

CONCLUSIONS

1. No significant inhibition of *E. faecalis* was observed with sterile water (control) and the undiluted solutions of Sterilox, 10% Citric Acid and Ozonated water. However, 3.5% NaOCl, 18% EDTA, TopClear 17% EDTA, 2% Chlorhexidine, BioPure MTAD and SmearClear showed significant inhibition of *E. faecalis*.
2. For the 1/10 diluted solutions no inhibition of *E. faecalis* was observed with sterile water (control), 3.5% NaOCl, Sterilox, 10% Citric acid and Ozonated water. BioPure MTAD and SmearClear demonstrated significantly greater inhibition of *E. faecalis* compared with 18% EDTA, TopClear 17% EDTA and 2% Chlorhexidine.
3. No significant inhibition of *E. faecalis* was observed with sterile water (control) and the 1/100 diluted solutions of 3.5% NaOCl, Sterilox, TopClear 17% EDTA, 2% Chlorhexidine, 10% Citric acid, Ozonated water and SmearClear. The only solution that showed significant inhibition of *E. faecalis* at this dilution was BioPure MTAD.
4. BioPure MTAD was the only irrigation solution that inhibited growth of *E. faecalis* in the undiluted, 1/10 diluted and 1/100 diluted solutions.

Declaration: No conflict of interest declared.

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