

Antimicrobial Efficacy and Irrigating Potential of Irrigation Solutions using Different Activation Methods

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SUMMARY

The objective of this *in vitro* study was to establish the antimicrobial efficacy and the effect of different activation methods on the smear layer at the coronal level of straight root canals of four different root canal irrigation solutions. The four irrigation solutions were 3.5% sodium hypochlorite liquid (NaOCl), 2% sodium hypochlorite gel, chlorhexidine gluconate liquid and a mixture of 100mg doxycycline capsules with 2ml sterile water.

Antimicrobial Effects: The surfaces of four agar plates were inoculated with *Enterococcus faecalis* and divided into four equal quadrants. Ten microlitres of each test solution was dispensed onto the four filter paper disks on each agar plate. The antibacterial activity of materials was apparent from circular clear inhibition zones forming around the filtration paper. The diameters of these inhibition zones were measured using a micrometer gauge.

Effect on Smear Layer: Access cavities were prepared on fifty, extracted, single rooted, human teeth and the root canals prepared with rotary files. The teeth were randomly divided into five groups (n=10) and each group irrigated with a different irrigation solution. Different activation methods were used in the coronal portion of each root canal. The solutions were activated in the canals using one of the following methods: a 30 gauge needle (Control), a sonic scaler tip, and a rotary brush. After sampling, the roots of the treated teeth were fractured and prepared for Scanning Electron Microscopy (SEM) according to standard methods. The one-way ANOVA test was used to determine whether there were any statistical significant differences between the different groups. The average zones of inhibition for 3.5% NaOCl, 2% NaOCl, 2.5% chlorhexidine and doxycycline were 2.7mm, 2.0mm, 11.2mm and 12.4 mm respectively. Sterile water, 3.5% NaOCl and 2% NaOCl had no significant effect on the smear layer. However, when chlorhexidine and doxycycline solutions were activated with a rotary brush, 90 and 80 per cent of the observed surfaces were

free of smear layer respectively. Doxycycline and 2.5% chlorhexidine demonstrated the highest antimicrobial activity against *Enterococcus faecalis* and removed most of the smear layer when the solutions were activated with a rotary brush.

Keywords: Irrigation solutions, Activation, Ultrasonics, Rotary Brush.

INTRODUCTION

Antony van Leeuwenhoek was the first person to describe bacteria in a root canal¹. Almost three centuries later Kakehashi and co-workers reported that pulpal necrosis and periradicular lesions (of teeth with exposed pulps) developed only in rats with a normal microbiota and not in germ-free rats². Later the important role of bacteria in periradicular lesions was confirmed in a study using human teeth wherein bacteria were only found in root canals of pulpless teeth with periradicular bone destruction³. Light and electron microscopy demonstrated that the root canals of all periapically affected teeth contain a variety of bacteria⁴. All current evidence implicate bacteria in the pathogenesis of periradicular tissues.

The bacteria isolated from infected root canals include a specific group of species compared to the total flora of the human oral cavity. A correlation seems to exist between the size of the periapical lesion, the number of bacterial strains and the amount of bacterial cells present in the root canal. The larger the periapical lesion, the higher the density of bacteria in the root canal¹. This suggests that specific changes of the bacterial flora take place in the root canal with time. Limited information on how the microorganisms persist and survive after the completion of root canal therapy exists.

The microbial flora from canals with persistent apical lesions differs markedly from that of untreated necrotic canals. Necrotic canals are typically polymicrobial, with approximately equal proportions of gram-positive and gram-negative bacteria and domi-

nated by anaerobic bacteria, whereas the microbial flora of failed retreatments are mono infections of predominantly gram-positive microorganisms with approximately equal proportions of facultative and obligate anaerobes⁵.

Enterococcus faecalis has been found to be one of the predominant bacteria in teeth in which root canal therapy had failed. *E. faecalis* strains are extremely resistant to several medicaments, including calcium hydroxide⁶ and its eradication by conventional means may be extremely difficult.

The aim of root canal therapy is to eliminate the microorganisms from the root canal system and to prevent reinfection. The main cause of root canal failure is the persistence of microorganisms and relevant antigens after therapy or the recontamination of the canal system because of an inadequate coronal seal⁶.

Studies show that currently used methods of root canal preparation produce a smear layer that covers root canal walls and the openings to the dentinal tubules⁷. The smear layer consists of organic and inorganic material, including fragments of odontoblastic processes, microorganisms and necrotic materials. The smear layer prevents penetration of intracanal irrigants and medication into the irregularities of the root canal system and the dentinal tubules and also prevents complete adaptation of obturation materials to the prepared root canal surfaces⁸. Irrigation during instrumentation is imperative⁹ and necessary to remove debris from root canals, eliminate microorganisms and serve as a lubricant.

No single root canal irrigant meets all the requirements and a great range of different solutions have been tested¹⁰.

Sodium hypochlorite (NaOCl) has been used as an endodontic irrigant for more than 75 years. Sodium hypochlorite is an alkaline solution with a pH of approximately 11 to 12. Introducing it beyond the tooth apex can cause a violent tissue reaction and unbearable pain. It causes injury primarily by oxidation of proteins. A safer and biocompatible irrigant is desirable¹¹.

A combination of 2% chlorhexidine gluconate with 2.5% sodium hypochlorite produced a significant reduction of post-irrigant positive cultures compared to the use of sodium hypochlorite alone but not significant compared to the use of chlorhexidine gluconate alone^{12,13}. Chlorhexidine gluconate is the most effective antibacterial substance against selected gram-positive and gram-negative bacteria in necrotic canals¹⁴. Although chlorhexidine gluconate is a safer and more effective antimicrobial irrigant, it has been proven to be ineffective in dissolving pulpal tissue¹⁵.

Various antibiotics such as penicillin, bacitracin and streptomycin have been used without success to disinfect the root canal system without success¹⁶. Tetracycline is a broad-spectrum antibiotic effective against a wide range of microorganisms. Tetracycline has a low pH and thus can act as a calcium chelator stimulating enamel and root surface demineralisation. Doxycycline-HCl in a concentration of 100mg per ml, was found to be more effective in removing the smear layer compared to EDTA and 2.5% sodium hypochlorite¹⁷. In another study, both tetracycline HCl 100mg/ml and 50% citric acid removed the smear layer, but tetracycline did not widen the tubule apertures and destroy as much peritubular dentine as did citric acid¹⁸.

The aims of this *in vitro* study were:

- to establish the antimicrobial efficacy of four different root canal irrigation solutions, and

- to determine the effect of different irrigation solutions and activation methods on the smear layer at the coronal level of straight root canals.

MATERIALS AND METHODS

The four different irrigation solutions that were used in this study were as follows:

- **3.5% Sodium hypochlorite (NaOCl)** (Reckitt Benckiser South Africa (Pty) Ltd., Elandsfontein, Gauteng, South Africa) (Batch no: 0055368),
- **2% Sodium hypochlorite gel (Thick Jik)**, (Reckitt Benckiser South Africa (Pty) Ltd., Elandsfontein, Gauteng, South Africa) (Batch no: 055341),
- Water soluble **chlorhexidine gluconate liquid** (Dental Warehouse, Wendywood, SA) (Batch no: 07 2007), and
- Mixture of **100mg doxycycline capsules** (Pharmacare SA) (Batch no: 1468) with 2ml sterile water.

A. ANTIMICROBIAL EFFECTS

A MacFarland Standard 1 suspension¹⁹ was prepared from an overnight culture of *E. faecalis* (ATCC 49474) and spread onto ten casein-peptone-soymeal-peptone agar plates (CASO-Agar) (Merck SA (Pty) Ltd., Halfway House, South Africa) with a sterile glass rod.

The prepared surface of each agar plate was divided into four equal quadrants. In each quadrant a sterilized, five millimetre diameter filter paper disk was placed. Ten microlitres of each test solution was dispensed onto the four filter paper disks on each agar plate.

Plates were incubated anaerobically using Anaerocult A (Merck SA (Pty) Ltd., Halfway House, South Africa) at 37° C for 24 hours, and antibacterial activity evaluated using the conventional agar plate diffusion method. The antibacterial activity of materials was apparent from circular clear inhibition zones forming around the filtration paper. The diameters of these inhibition zones were measured using a micrometer gauge. Measurements were done after incubation at three different positions, for each paper disk. An average was calculated for the nine measurements per paper disc on each plate.

The one-way ANOVA test was used to determine the differences between the inhibition zones of the different irrigation solutions.

B. EFFECT ON SMEAR LAYER

Fifty, single rooted human teeth were collected from the extraction clinic at the University of Pretoria. Immediately after extraction the teeth were rinsed under running water and stored in containers filled with sodium azide (Merck SA (Pty). Ltd. Halfway House, South Africa), at 4°C until needed. Pre operative radiographs were taken of each extracted tooth to eliminate teeth with aberrant canal anatomy, caries, resorption, calcifications, multiple canals, or any other condition, which may negatively influence the irrigating procedure. Only teeth with straight, patent single canals were used.

Preparation of specimens

Standardized access cavities were prepared using diamond burs and long shanked round burs. Canals were explored using size 10 K-flexfiles (Dentsply Maillefer, Baillaigues, Switzerland)

and the canals were irrigated with distilled water, confirming apical patency and establishing the working length, for each individual tooth. Working lengths were noted for each tooth, at the time of preparation. Root canals were prepared using nickel-titanium rotary files (ProTaper, Dentsply Maillefer, Baillaigues, Switzerland) with a crown-down technique. After each size file and as often as needed, during the preparation procedure, the canals were irrigated using a sterile saline solution.

The teeth were randomly divided into five groups (n=10) and irrigated as follows:

Group 1: 3.5% Sodium Hypochlorite liquid (3.5% NaOCl)

Group 2: 2% Sodium Hypochlorite gel (2% NaOCl)

Group 3: 2.5% Chlorhexidine Gluconate (CHX)

Group 4: Doxycycline-HCl 100mg/ml (D-HCl)

Group 5: Sterile distilled water (control).

The solutions for Groups 1 and 2 were standard solutions, obtained commercially.

The solution for Group 3 was 2.5% commercially available water soluble chlorhexidine gluconate liquid (Dental Warehouse, Wendywood, SA). The solution for Group 4 was produced by mixing 100mg doxycycline powder (Pharmacare SA) with 5ml sterile water.

Different activation methods were used in the coronal portion of each root canal to see if this would have an effect on the removal of the smear layer. Each canal was irrigated for 5 minutes. The solutions were delivered into the canals with a 30 gauge needle and activated with one of the following methods:

- a 30 gauge needle (Ultradent)(Control) moved up and down in the root canal,
- a sonic scaler tip (Satelec) activated by a Satelec P5 scaler operating at 30% power, and
- a rotary brush (Ultradent) (Figure 1) operating at 250rpm driven by a X-Smart electric motor (Dentsply).

After sampling, the roots of the treated teeth were fractured and prepared for Scanning Electron Microscopy (SEM) according to standard methods^{20, 21}.

Two different SEM photomicrographs were taken for each sample. They were coded and examined blind. Two investigators scored the presence or absence of the smear layer on the surface of the root canal or in the dentinal tubules at the coronal level. For semi-quantative evaluation, the photographs were divided into 10 sub areas by overlaying a grid, which permitted a more precise determination of the ratio of smear free to smeared surfaces. For each of the 10 sub areas, the absence or presence of the smear layer was rated and scored according to the following three appearances:

1. Regularly distributed open dentinal tubule orifices and free of smear layer, scored 10.
2. Scattered open tubule orifices and partially free of smear layer, scored 5.
3. No visible tubule orifices, surfaces with complete smear layer coverage, scored 0.

Each SEM photomicrograph was finally scored by adding the scores of the 10 sub- areas, thus expressing the result as a percentage of smear layer free surface. The final result for each

segment of the root canal was obtained by calculating the mean of all the photomicrographs.

RESULTS

Antimicrobial Results – Inhibition Zones

The means and standard deviations of the zones of inhibition for all the test solutions are presented in Table 1. Figure 2 shows an example of the inhibition zones obtained for each irrigation solutions.

The average zones of inhibition for 3.5% NaOCl, 2% NaOCl, 2.5% chlorhexidine and doxycycline were 2.7mm, 2.0mm, 11.2mm and 12.4mm respectively. Figure 3 shows the comparison of the average areas of inhibition for the undiluted irrigation solutions.

Table 2 shows the statistical comparisons between the different inhibition zones for the irrigation solutions. Statistical analysis using the one-way ANOVA test showed a statistical significant difference between the inhibition zones obtained for 2% and 3.5% sodium hypochlorite compared to 2.5% chlorhexidine ($p < 0.05$) and doxycycline ($p < 0.05$).

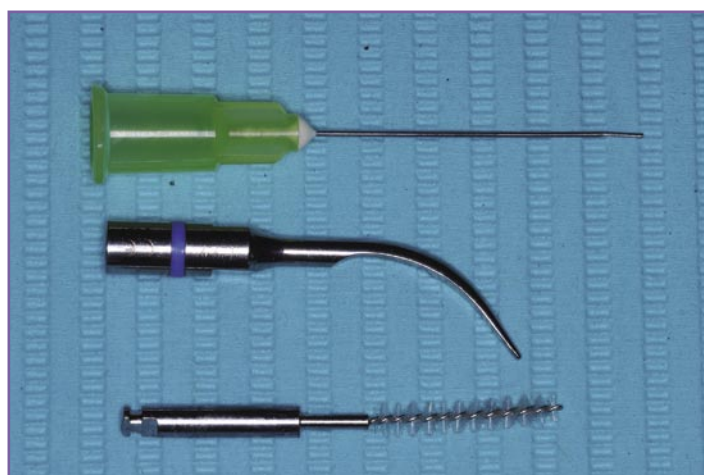


Figure 1: 30 gauge needle (Ultradent), sonic scaler tip (Satelec) and rotary brush (Ultradent) used to deliver and activate irrigation solution.

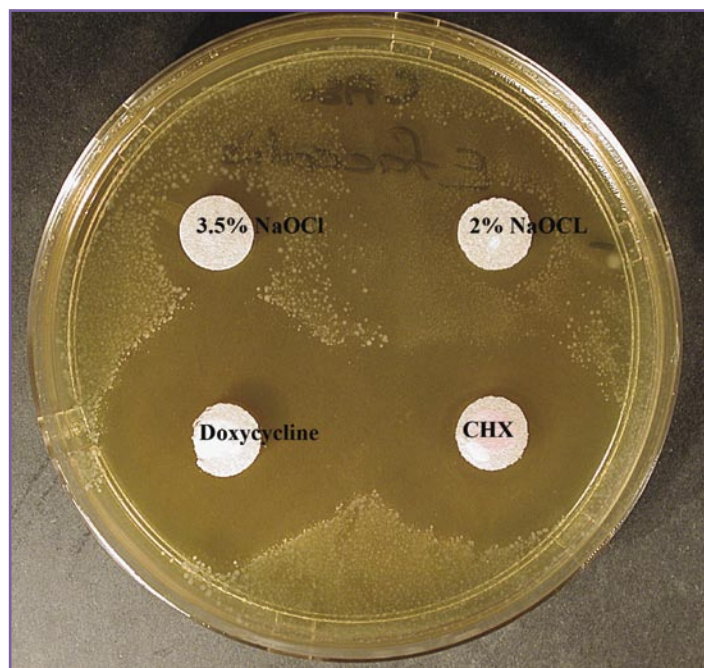


Figure 2: A representative photograph of an *E. faecalis*- seeded CASO-Agar plate incubated for 24 hours after placement of 3.5% sodium hypochlorite, 2% sodium hypochlorite, chlorhexidine and doxycycline.

Table 1: Comparison of *in vitro* antimicrobial activity of the irrigation solutions, using paper disks on agar plates, against *E. faecalis*.

100% Solution	Mean (mm)	Standard Deviation	Coefficient of variance %
H ₂ O control	0	0	0
3,5% NaOCl	2.7	± 0.51	18
2% NaOCL	2.0	± 0.22	11
2.5% CHX	11.2	0	0
Doxycycline	12.4	± 0.12	9.6

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

	H ₂ O control	3,5% NaOCl	2% NaOCL	2.5% CHX	Doxycycline
H ₂ O control				p<0.05	p<0.05
3,5% NaOCl				p<0.05	p<0.05
2% NaOCL				p<0.05	p<0.05
2.5% CHX					
Doxycycline					

Table 3: Incidence of smear layer-free surfaces at the coronal level of root canals (expressed as a percentage of observed surfaces).

Group		1	2	3	4	5
		H ₂ O control	3,5% NaOCl	2% NaOCL	2.5% CHX	Doxycycline
27 Gauge needle	Mean	0	0	0	40	50
	SD (±)	0	0	0	3.5	3.2
Sonic Scaler Tip	Mean	0	0	0	40	50
	SD (±)	0	0	0	3.3	3.1
Rotary Brush	Mean	0	30	30	80	90
	SD (±)	0	3.9	4.3	2.7	2.9

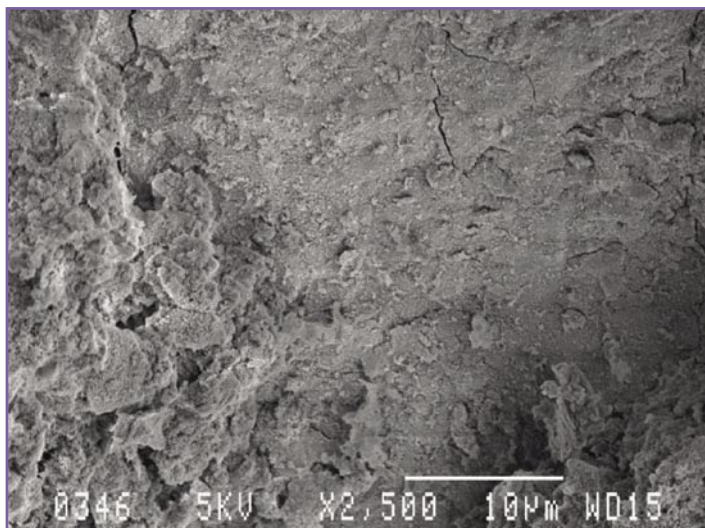


Figure 3: Coronal part of the root canal irrigated with sterile water, 3.5% NaOCl and 2% NaOCl showed consistently the presence of smear layer when the solution was delivered with the irrigation needle or activated with an ultrasonic scaling tip.

The incidence of smear-free surfaces created by the irrigation solutions are given in Table 3. Examination of the surface of the root canal walls in teeth irrigated with sterile water, 3.5% NaOCl and 2% NaOCl consistently showed the presence of heavy smear layer for all the groups tested (Figure 3). However, when these irrigation solutions were activated with the rotary brush there was some evidence of smear layer removal (30%) (Figure 4).

Most samples examined for the groups that were irrigated with 2.5% chlorhexidine and doxycycline demonstrated some modification or removal of the smear layer. When irrigation was done with a 25 gauge needle or by activating the solution with an ultrasonic scaler tip approximately 50% of the observed surfaces were free of smear layer (Figure 5).

Activating the 2.5% chlorhexidine or doxycycline solutions with the rotary brush, 90 and 85 per cent of the observed sur-

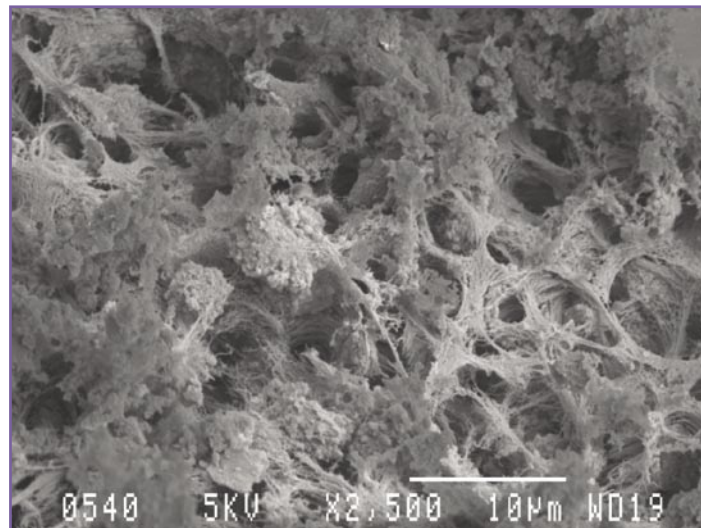


Figure 4: Coronal area of a root canal irrigated with 3.5% sodium hypochlorite liquid activated with a rotary brush. The brush disrupted the smear layer exposing the underlying dentine.

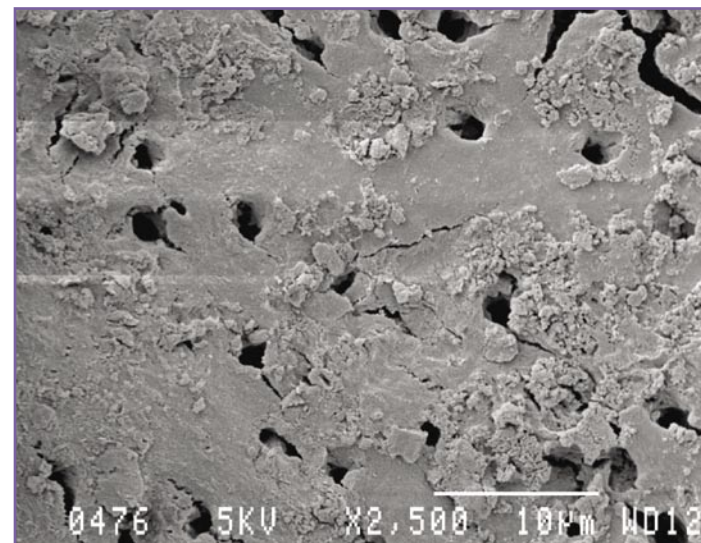


Figure 5: Coronal area of a root canal irrigated with doxycycline demonstrated some modification or removal of the smear layer when the solution was delivered with a 25-gauge needle or by activating the solution with an ultrasonic scaler tip.

faces were free of smear layer respectively (Figure 6). There was a statistically significant difference between the groups (2% and 3.5% sodium hypochlorite, 2.5% chlorhexidine and doxycycline) that were activated by the rotary brush compared to all the other activation methods (p<0.05).

DISCUSSION

One of the objectives of this *in vitro* study was to determine the effect of different irrigation solutions and activation methods on the smear layer in root canals. The main limitation of this study was that the effect of the irrigating solutions and activation methods

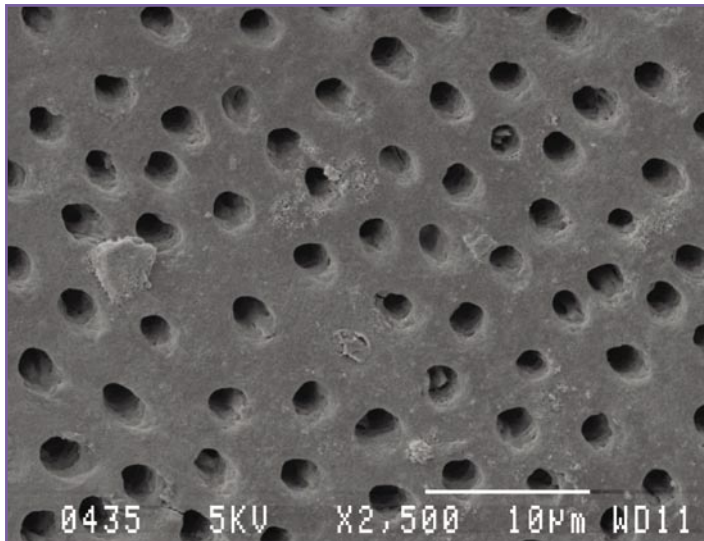


Figure 6: Coronal area of a root canal irrigated with Doxycycline demonstrated complete removal of the smear layer when the solution was activated with the rotary brush.

was only examined in the coronal portion of the root canals due to the size of the rotary brush that was used in this study. It is important to note that there are no rotary brushes commercially available for this specific application.

The most popular irrigation solution is sodium hypochlorite. It is an effective antimicrobial agent²² and an excellent organic solvent for vital, necrotic and fixed tissues²³. However, it is highly irritating to periradicular tissues, especially in high concentrations²⁴. In the present study the 2% and 3.5% NaOCl demonstrated a low level of antimicrobial properties against *E. faecalis* compared to all the other irrigation solutions tested in this study.

The results of the SEM obtained for 2% and 3.5% NaOCl confirmed previous reports that NaOCl irrigation during instrumentation leaves the prepared canal wall entirely covered with a smear layer²⁵. The only activation method in the present study that could alter the smear layer when irrigating with NaOCl was the rotary brush method.

Chlorhexidine is a potent antiseptic and its use in endodontics has been proposed both as irrigant and intracanal medication²⁶. In the present study it demonstrated excellent antimicrobial properties against *E. faecalis*. Despite this it cannot be used as the sole irrigant because it is unable to dissolve necrotic tissue remnants²⁷.

The chlorhexidine irrigation solution partially removed the smear layer when delivered with a syringe or when activated by the sonic scaler tip. When the solution was activated with the rotary brush, most of the smear layer (80%) was removed.

Tetracycline is a broad spectrum antibiotic, well researched and used in dentistry, especially in periodontology²⁸. According to Berutti and Castellucci (2005) tetracycline is absorbed and then gradually released by the mineralized tissues (dentine and cementum) of the teeth²⁸. Furthermore it carries out a chelating action contributing to the removal of the smear layer.

Doxycycline is a hydroxyl derivative of tetracycline²⁹. Barkhordar and co-workers compared solutions to remove the smear layer and found that doxycycline-HCl (100mg/ml) was the most effective in removing the smear layer compared to EDTA and NaOCl³⁰. In the present study doxycycline irrigation solution partially removed the smear layer when the solution

was delivered with a syringe or activated by the sonic scaler tip. When the solution was activated with the rotary brush, most of the smear layer (90%) was removed.

It is evident from this *in vitro* study that activating irrigating solutions with a rotary brush seems to be the most effective method to remove the dentinal smear layer. According to Berutti and Castellucci the bristles of brushes shift debris into solution for removal out of the canal in a coronal direction²⁸. More research and development is needed to design micro-brushes which can be used to activate irrigation solutions in the middle and apical part of root canal systems.

CONCLUSIONS

1. Doxycycline and 2.5% chlorhexidine demonstrated statistically significant higher antimicrobial activity against *Enterococcus faecalis* compared to all the other test groups.
2. Sterile water, 3.5% NaOCl and 2% NaOCl had no significant effect on the smear layer when the solutions were activated with a 30 gauge needle or with a sonic scaler tip.
3. 3.5% NaOCl and 2% NaOCl removed approximately 30% of the smear layer after the solutions were activated with a rotary brush.
4. Doxycycline and 2.5% chlorhexidine removed most of the smear layer when the solutions were activated with a rotary brush.

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Additional references (14-30) are available on www.sada.co.za