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ORIGINAL RESEARCH

Comparative Scanning Electron Microscopic Study of Removal of Intracanal Smear Layer using Different Concentrations of Three Root Canal Irrigants: An *in vitro* Study

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ABSTRACT

Aim: To evaluate the effect of root canal irrigation using 2.25 and 5% sodium hypochlorite (NaOCl), 17 and 3% ethylenediaminetetraacetic acid (EDTA), and 50 and 100 mg/ml doxycycline hydrochloride (DH) on intracanal smear layer and to assess which of these is more efficient in removal of smear layer.

Methods: A total of 65 extracted maxillary anterior teeth were used for the study. After access opening and establishment of working length, cleansing and shaping were done by step back preparation using K-flex files, then enlarged to size 30 file and irrigated with 2.25% NaOCl. Specimens were then divided into six treatment groups of 10 specimens each and a control group of five specimens. During cleansing and shaping to size 50 file, the treatment groups group 1 and group 2 were irrigated with 2.25 and 5% NaOCl, respectively, group 3 and group 4 were irrigated with 17 and 3% EDTA respectively, and group 6 and group 7 were irrigated with 50 and 100 mg/ml DH respectively. The control group group 7 was irrigated with sterile water. The roots were then split and observed under scanning electron microscope. Data were statistically analyzed.

Results: Scanning electron microscopic examination revealed that smear layer was effectively removed in groups treated with 17 and 3% EDTA and 50 and 100 mg/ml DH. The dentinal tubules were better expressed in groups treated with DH, and 2.25 and 5% NaOCl did not remove the smear layer.

Conclusion: These findings suggest that smear layer is best removed when DH is used as an irrigant.

Keywords: Doxycycline hydrochloride, EDTA, Irrigation, Root canal irrigants, Scanning electron microscope, Smear layer.

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INTRODUCTION

Cleaning and shaping root canals produce a smear layer containing inorganic and organic substances that include fragments of odontoblastic processes, microorganisms, and necrotic materials.[1-3]

Though the influence of this layer on the success rate of endodontic treatment has not yet been definitely determined, it is currently considered important to eliminate this layer.

It has been shown that the complete removal of the smear layer requires both organic and inorganic solvents used alternatively. Various organic acids, chelating agents, ultrasonic instruments, and lasers have been used to remove the smear layer, the most commonly used irrigants being sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA). Recently, doxycycline which has been used during periodontal treatment because of its antibacterial and chelating ability as well as its substantivity has been used as an endodontic irrigant.

But there is little information available concerning the efficacy of smear layer removal using doxycycline hydrochloride (DH) compared with NaOCl and EDTA at different concentrations.

Hence the aim of this study was to evaluate the effect of root canal irrigation using 2.25 and 5% NaOCl, 17 and 3% EDTA, and 50 and 100 mg/ml DH on intracanal smear layer and to assess which of these is more efficient in removal of smear layer.

MATERIALS AND METHODS

This study was conducted in the Department of Conservative Dentistry and Endodontics, MR Ambedkar Dental College, Bengaluru, and Indian Institute of Science, Bangalore. The study comprised of 65 maxillary anterior teeth extracted because of periodontal problems. Caries-free teeth with straight canals and completely formed

apices were used. The teeth were rinsed under tap water to remove blood and tissue debris. Soft tissue tags, bone, or calculus was removed, and then teeth were stored in 10% formalin solution for not more than 2 months until their use in the study. After access opening, a no 10 K-flex file was placed in the canal until it was just visible at the apical foramen, and 1 mm was subtracted from the length to establish the working length. The apical portion of the root tip was covered with sticky wax. Cleansing and shaping were done according to the conventional step back preparation using K-flex files, then enlarged to size 30 file and irrigated with 2.25% NaOCl. Following this, the teeth were randomly divided into seven groups according to the irrigant to be tested. Solutions and method to irrigate were group dependent.

Distribution of Samples

Group 1 – Specimens were irrigated with 2.25% NaOCl
Group 2 – Specimens were irrigated with 5% NaOCl
Group 3 – Specimens were irrigated with 17% EDTA
Group 4 – Specimens were irrigated with 3% EDTA
Group 5 – Specimens were irrigated with 50 mg/ml DH
Group 6 – Specimens were irrigated with 100 mg/ml DH
Group 7 – Specimens were irrigated with sterile water (control)

During instrumentation, the pulp chamber was flooded with the test irrigant, and 1.5 ml of the tested solution was left in the chamber during cleansing and shaping to size 50 file.

Irrigation used after each hand instrument consisted of 1.5 ml of the selected solution for 1to 2 minutes. Root canals were finally irrigated with 3 ml of sterile water to remove any precipitate that may have formed from the test irrigant, and the canals were dried with paper points. Then a cotton pellet was placed in the access chamber and the teeth stored in a plastic container and placed in a humidor. After storage, the crowns of the specimen teeth were sectioned at the cementoenamel junction using a double-sided diamond disk. A longitudinal groove was cut on the facial and lingual surface without penetrating the canal and split into two halves and observed under scanning electron microscope (SEM), and photomicrographs were taken at 1500 × magnifications.

Presence of smear layer and debris was determined.

Grading of the Specimens

An arbitrary ranking system was devised to evaluate the condition of dentin surface as follows:

- Smear layer absent, dentinal tubules open and free of debris.
- Smear layer present only in the apertures of dentinal tubules.
- Smear layer covers the surface outline of dentinal tubules, indiscernible tubular apertures covered by debris, the location of tubule indicated by a crack.
- Heavy smear layer, indiscernible tubule apertures.

The data were analyzed using chi-square test and Mann–Whitney test.

RESULTS

This study evaluated the effect of root canal irrigation using 2.25 and 5% NaOCl, 17 and 3% EDTA, and 50 and 100 mg/ml DH on intracanal smear layer and assessed which of these is more efficient in removal of smear layer (Tables 1 to 3).

The results can be summarized as follows:

- Scanning electron microscopic examination of Group 7, which was irrigated with sterile water, showed the presence of an intact smear layer.
- Scanning electron microscopic examination of Groups 1 and 2, which were irrigated with 2.25 and 5% NaOCl, showed the presence of a typical amorphous smear layer with some exposed dentinal tubules.
- Scanning electron microscopic examination of Groups 3 and 4, which were irrigated with 17 and 3% EDTA, revealed that the smear layer was effectively removed, and the tubular apertures were almost always clean and open.
- Scanning electron microscopic examination of Groups 5 and 6, which were irrigated with 50 and 100 mg/ml DH, revealed that the smear layer was eliminated and the dentinal tubules were better expressed than when the teeth were irrigated with EDTA.

Table 1: Irrigant score - cross tabulation

	Score				
	1	*2*	*3*	*4*	*Total*
Irrigant 2.5% NaOCl	0	0	2	8	10
5% NaOCl	0	0	3	7	10
17% EDTA	3	7	0	0	10
3% EDTA	2	8	0	0	10
500 mg/ml DH	8	2	0	0	10
100 mg/ml DH	8	2	0	0	10
Sterile water	0	0	0	5	5
Total	21	19	5	20	65

DH: Doxycycline hydrochloride; EDTA: Ethylenediaminetetraacetic acid

Table 2: Chi-square test ($p < 0.001$)

	Value	*df*	*Asymp. sig. (2 sided)*
Pearson chi-square	89.913[a]	18	0.000
Likelihood ratio	102.521	18	0.000
Linear-by-linear association	15.356	1	0.000
No. of valid cases	65		

Table 3: Mann–Whitney test in order to find out among which pair of irrigants there exists a significant difference

Irrigant 1	*Irrigant 2*	*p-value*
2.25% NaOCl	5% NaOCl	0.739
2.25% NaOCl	17% EDTA	<0.001*
2.25% NaOCl	3% EDTA	<0.001*
2.25% NaOCl	50 mg/ml DH	<0.001*
2.25% NaOCl	100 mg/ml DH	<0.001*
2.25% NaOCl	Sterile water	0.31
5% NaOCl	17% EDTA	<0.001*
5% NaOCl	3% EDTA	<0.001*
5% NaOCl	50 mg/ml DH	<0.001*
5% NaOCl	100 mg/ml DH	<0.001*
5% NaOCl	Sterile water	0.206
17% EDTA	3% EDTA	0.739
17% EDTA	50 mg/ml DH	0.028*
17% EDTA	100 mg/ml DH	0.028*
17% EDTA	Sterile water	0.001*
3% EDTA	50 mg/ml DH	0.009*
3% EDTA	100 mg/ml DH	0.009*
3% EDTA	Sterile water	0.001*
50 mg/ml DH	100 mg/ml DH	1.000
50 mg/ml DH	Sterile water	0.001*
100 mg/ml DH	Sterile water	0.001*

*Denotes a significant difference; DH: Doxycycline hydrochloride; EDTA: Ethylenediaminetetraacetic acid

- Chi-square test was used to evaluate the association between the irrigants and the scores, and it was concluded that there is a significant association between the irrigants and the scores ($p < 0.001$).
- In order to find out among which pair of irrigants there exists a significant difference, Mann–Whitney test was done.
- From the results of Mann–Whitney test, it can be concluded that there is a significant difference between 2.25% NaOCl and 17% EDTA, 3% EDTA, 50 and 100 mg/ml DH ($p < 0.001$).
- There is also a significant difference between 5% NaOCl and 17% EDTA, 3% EDTA, 50 and 100 mg/ml DH ($p < 0.001$).
- It can be concluded that there is no significant difference between 17 and 3% EDTA ($p > 0.05$). But there is a significant difference between 17% EDTA and 50 and 100 mg/ml DH ($p < 0.05$). The difference between 17% EDTA and sterile water is also significant ($p < 0.001$).
- There is a significant difference between 3% EDTA and 50 and 100 mg/ml DH ($p < 0.01$). There is also a significant difference between 3% EDTA and sterile water ($p < 0.001$).
- There is no significant difference between 50 and 100 mg/ml DH ($p > 0.05$), but there is a significant difference between 50 mg/ml DH and sterile water ($p < 0.001$).
- There exists a significant difference between 100 mg/ml DH and sterile water ($p < 0.001$).

DISCUSSION

One of the greatest challenges of root canal treatment is the effective chemomechanical preparation of the root canal system and achieve an adequate obturation.

However, studies have shown that current methods of cleaning and shaping root canals, especially rotary instrumentation techniques, produce a smear layer that covers root canal walls and the openings to the dentinal tubules.

Though the influence of this layer on the success rate of endodontic treatment has not yet been definitely determined, it is currently considered important to promote techniques and products that can prevent the formation of, or eliminate, this layer.[4,5]

A large number of agents have been used as root canal irrigants, none of which have been totally effective or have received total acceptance in removing smear layer.

Scanning electron microscopy, which allows an examination of morphologic details of the surfaces of prepared root canal, has been used to determine the effectiveness of various irrigants to remove smear layer.

This study evaluated the effect of root canal irrigation using 2.25 and 5% NaOCl, 17 and 3% EDTA, and 50 and 100 mg/ml DH on intracanal smear layer and also assessed which of these is more efficient in removal of smear layer.

- Under the conditions of this *in vitro* study, the examination with SEM showed that smear layer was not removed in Groups 1 and 2, which were irrigated with 2.25 and 5% NaOCl, and in Group 7, which was irrigated with sterile water.
- In Groups 1 and 2, the instrumented portion of root canals was covered with a typical amorphous smear layer with some exposed dentinal tubules.

The results of this study confirm previous reports that NaOCl irrigation during instrumentation leaves the prepared canal wall entirely covered with a smear layer.

Sodium hypochlorite has a pH of approximately 11 to 12, and when hypochlorite contacts tissue proteins, nitrogen, formaldehyde, and acetaldehyde are formed within a short time and peptide links are broken, resulting in dissolution of the proteins. During the process, hydrogen in the amino groups is replaced by chlorine, thereby forming chloramines that play an important role in antimicrobial effectiveness. As a consequence, NaOCl is highly toxic to vital tissues at undiluted high concentrations.

The active principle of NaOCl solutions is the amount of undissociated HOCl molecules, which are consumed in the interaction with organic matter. However, its action does not affect inorganic material.

Studies have shown that a smear layer with some exposed dentinal tubules was seen on all instrumented surfaces regardless of concentration of NaOCl.[6]

Studies evaluating the effect of NaOCl on dentin microhardness have shown that 6% NaOCl caused a more significant decrease in microhardness compared with 2.5% NaOCl concentration.[7] Bearing in mind that there is only little antibacterial advantage to the use of higher concentrations of NaOCl[4] and that higher concentration of NaOCl is cytotoxic,[8] it may be prudent to not select higher concentrations of NaOCl so that the physical properties of dentin are not hampered.

- In the present study, examination of specimens in Groups 3 and 4, which were irrigated with 17% EDTA and 3% EDTA, revealed that the smear layer was effectively removed, and the tubular apertures were almost always clean and open. The differences between the solutions, however, were not statistically significant.

The results of the study are in accordance with the previous study by investigators,[9] who found that both 3 and 17% EDTA solutions effectively removed smear layer with no significant difference between the two. Investigators have also reported that EDTA solution removed superficial smear layer completely, but that some of the openings of dentinal tubules were still closed.

It appears that the chelating effect of the EDTA demineralizes and removes the inorganic component of smear layer produced during instrumentation and leaves an organic fibrous component behind on the canal wall.

In areas where the fibrous layer does not completely cover the canal wall, EDTA may demineralize the underlying dentin surface to expose some of its organic matrix.

These two hypotheses are consistent with the known ability of EDTA to demineralize inorganic calcified material.[10]

Studies have shown that increasing the contact time and concentration of EDTA from 10 to 17% has shown to increase demineralization of dentin.[11]

It was reported that 15% EDTA also reduces the hardness of dentin as a result of its excessive demineralization effect. This also suggests that the use of 15% EDTA for removal of the smear layer is not favorable in terms of the protection of dentin. Studies have also shown that concentrations of 15 and 17% EDTA are cytotoxic.

In comparing these properties, it is proposed that 3% EDTA is more useful for clinical applications.

- In the present study, specimens in Groups 5 and 6 were irrigated with 50 and 100 mg/ml DH solutions respectively. Scanning electron microscopic examination revealed that DH eliminated smear layer and the dentinal tubules were better expressed than when the teeth were irrigated with EDTA. The results indicated that DH effect was statistically significant when compared with all other experimental groups.

The results of this study are in agreement with previous study by investigators, who in their study concluded that doxycycline in a concentration of 100 and 50 mg/ml was more effective than 15% EDTA.[12]

Doxycycline has many unique properties other than its antimicrobial effect. It has a low pH and thus can act as a calcium chelator and cause enamel and root surface demineralization. It bonds directly to demineralized surface.[13] In addition, it has been shown that it is a substantive medication.

The effects of the tetracycline family of antibiotics on the removal of smear layer from the surface of instrumented root canals and root end cavity preparations have also been studied, and it has been found that tetracycline hydrochloride is effective in the removal of smear layer. Tetracycline hydrochloride at a concentration of 100 mg/ml will effectively unmask and partially demineralize dentin surfaces, providing local antimicrobial concentrations.

The wide use of doxycycline in periodontal therapy has led to its evaluation as an adjunct in endodontic therapy. Doxycycline, a hydroxyl derivative of tetracycline, is the most potent anticollagenase antibiotic among the commercially available tetracyclines. It is found to have greater antimicrobial activity against most of the microorganisms associated with primary endodontic infections when compared with NaOCl and chlorhexidine. A further benefit of doxycycline is that prolonged treatment with the drug does not facilitate bacterial mutation to generate tetracycline-resistant microorganisms.

It seems to be as effective as acidic conditioners in removing smear layer. Doxycycline is capable of disinfecting the dentin, removing the smear layer, opening the dentinal tubules, and allowing the antibacterial agents to penetrate the entire root canal system.

In another study, researchers evaluated the effect of tetracycline hydrochloride as an endodontic irrigant on smear layer removal and compared it with bidistilled water, 2.5% NaOCl, and citric acid. They concluded that in the group treated with tetracycline hydrochloride, no smear layer was present, and the surface was found to be free of debris. The apertures of the dentinal tubules were enlarged. Also, tetracycline hydrochloride was found to be significantly more effective on smear layer removal than 2.5% NaOCl.

Studies have reported that the lower concentrations of DH were significantly more effective in the presence

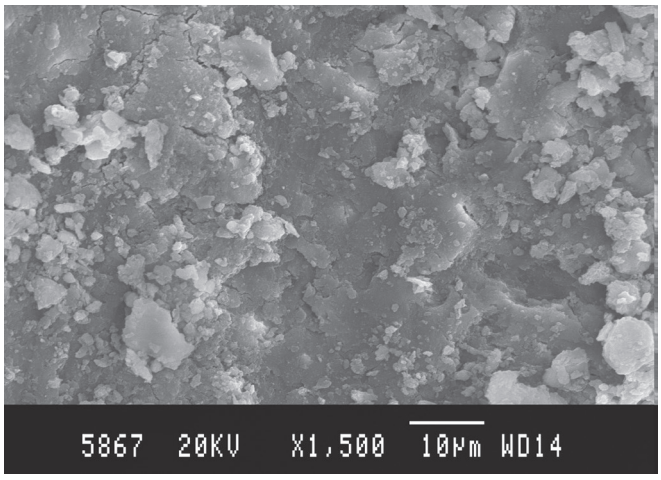

Fig. 1: Irrigated with 2.25% sodium hypochlorite

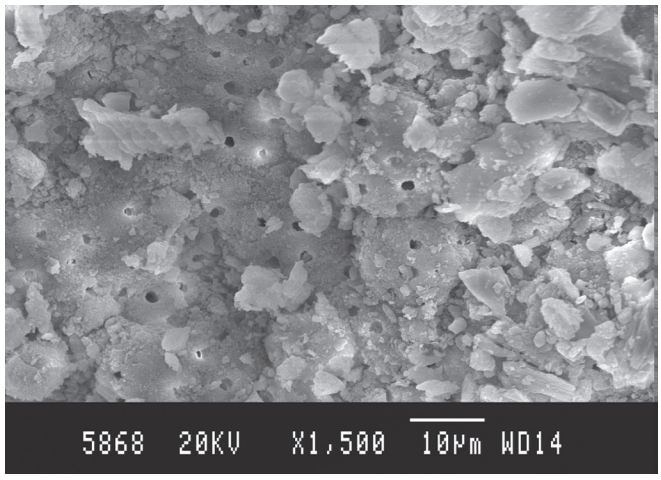

Fig. 2: Irrigated with 5% sodium hypochlorite

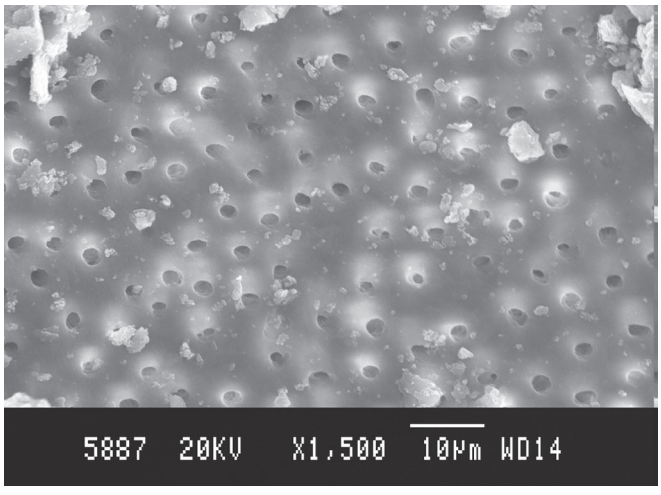

Fig. 3: Irrigated with 17% ethylenediaminetetraacetic acid

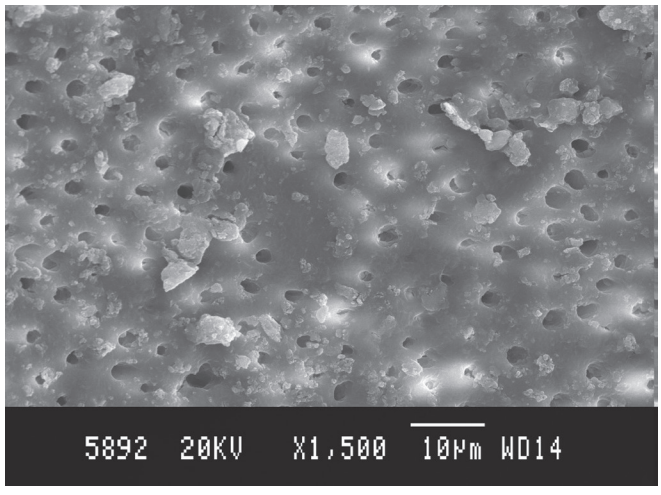

Fig. 4: Irrigated with 3% ethylenediaminetetraacetic acid

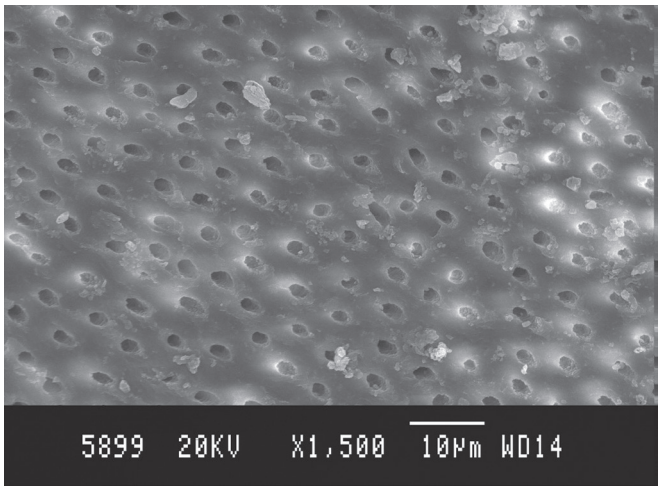

Fig. 5: Irrigated with 50 mg/ml doxycycline hydrochloride

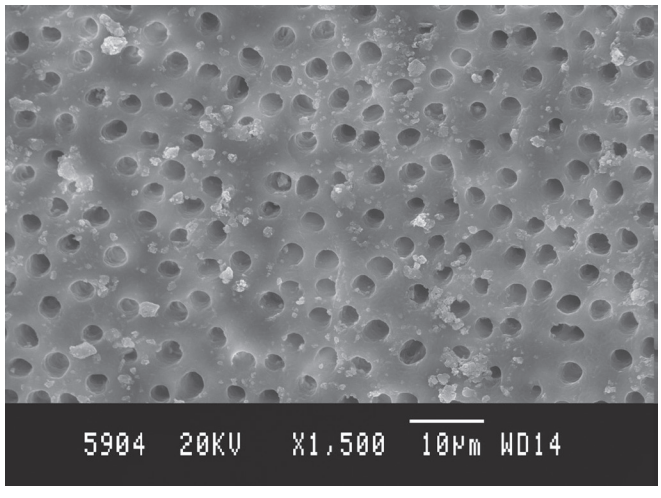

Fig. 6: Irrigated with 100 mg/ml doxycycline hydrochloride

of NaOCl than when used with saline. This raises the possibility that a combination of DH and NaOCl could be a more effective irrigant.

- From the results of this study, it seems that DH is an effective irrigant for the removal of smear layer. It not only has disinfecting property, but also does not significantly change the structure of dentinal tubules. When used in conjunction with NaOCl, it has potential in root canal treatment procedures. The solution is easily delivered to the root canal system and adheres

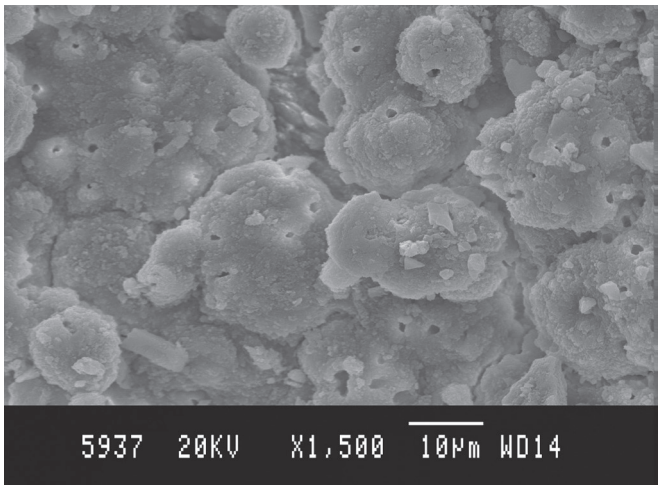

Fig. 7: Irrigated with sterile water

to the canal wall. However, further studies are needed to evaluate the *in vivo* efficacy of doxycycline in endodontic treatment.

CONCLUSION

According to the findings of this study, it can be concluded that

- 2.25 and 5% NaOCl used as a root canal irrigant did not remove the smear layer;
- 17 and 3% EDTA removed the smear layer effectively, but no significant difference was found between the two; and
- 50 and 100 mg/ml DH eliminated smear layer, and dentinal tubules were better expressed than when teeth were irrigated with EDTA.

A thorough knowledge of the irrigants used, including chemical reactions, should be mandatory for all clinicians. Further research into the physical and chemical properties of the root canal irrigants is necessary to establish the specific factors that affect their ability to remove the smear layer. Doxycycline hydrochloride is an effective irrigant for the removal of smear layer. When used in conjunction with NaOCl, it has potential in root canal treatment procedures. However, further studies are needed to evaluate the *in vivo* efficacy of DH in endodontic treatment.

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