Scientific

Comparison of smear layer removal using four final-rinse protocols

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Abstract

Objectives: This study aimed to compare the efficacy of Tetraclean and 17% EDTA as final irrigants in the removal of the smear layer in the coronal, middle and apical thirds of the instrumented root canal. **Methods and Materials:** Forty extracted human permanent teeth (n=10) were randomly assigned to 4 groups: no smear layer removal (group 1); EDTA rinse (group 2); liquid component of Tetraclean only (group 3); Tetraclean (group 4). The specimens were analyzed using scanning electron microscopy analysis at 500X and 1000X magnification and cleaning was evaluated at the apical, middle, and cervical levels using a three-point scoring system. Data were statistically analyzed using Kruskal-Wallis analysis of variance test (5% significance level). **Results:** When the entire canal was considered, groups were ranked in the following order: 1>2≥3=4 ($p<0.05$). For different sections of the canal space, distance from the apex (2, 6 and 10 mm) influenced smear layer removal within each group ($p<0.05$). **Discussion:** Differences between EDTA and Tetraclean were only evident at 6 mm from the apex, whereas at 2 mm both protocols had similar performances in smear layer removal from the root canal system of single-rooted permanent teeth. **Conclusions:** the use of a chelating agent leads to a higher removal of smear layer from the root canal walls.

Key words

EDTA, Endodontic treatment, irrigation, smear layer, sodium hypochlorite.

Introduction

The main purpose of root canal therapy in infected teeth is the elimination of debris, toxins and microorganisms by chemomechanical preparation. However, even after cleaning and shaping, total sterilization of the root canal system remains difficult to achieve.[1]

Studies have shown that mechanical instrumentation of root canals implies the formation of a smear layer covering the dentinal walls[2] and containing both inorganic and organic materials.[2] The presence of the smear layer may considerably delay or prevent the penetration of antimicrobial agents, such as endodontic irrigants and

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intracanal medications, into the dentinal tubules,[3] as well as interfere with the adhesion of root canal sealers to the root canal walls, thus compromising the quality of the root canal filling.[4]

Keeping or removing the smear layer is a highly controversial subject. Nevertheless, it seems that the smear layer itself may be infected and may harbor bacteria within the dentinal tubules.[5] This is significant in teeth with infected root canal system where the outcome of the endodontic treatment depends on the elimination of bacteria and their byproducts from the root canal system. In these cases at least, removing the smear layer appears to be of importance.[6]

For effective removal of both organic and inorganic components of the smear layer, combined application of sodium hypochlorite (NaOCl) and a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), is recommended.[7] The combination of these substances is capable of removing the smear layer, mainly from the middle and cervical thirds.[8] However, the application of EDTA for more than 1 minute[9,10] and in volume more than 1 ml[9,10,11] has been reported to be associated with dentinal erosion. It is also noteworthy that chemical interactions between NaOCl and EDTA should be taken into account. Mixing them caused a complete loss of free available chlorine from NaOCl in less than one minute.[7] This suggests that in an alternating irrigating regimen, copious amounts of hypochlorite should be administered to rinse out chelator remnants and allow the NaOCl to develop its antimicrobial and tissue dissolving potential. However, the interaction between NaOCl and EDTA makes usage of this two component difficult.[12]

In 2003, Torabinejad[9] proposed the use of an irrigant to be used in association with 1.3% NaOCl to remove smear layer from canal walls and facilitate the elimination microorganism from infected dentin.[13] This irrigant (MTAD, Dentsply Tulsa Dental, Johnson City, TN USA) is a solution containing a mixture of an antibiotic (doxycycline), an acid (citric acid), and a detergent (Tween-80). Citric acid works as a chelating agent in association with the lower chelating action of the antibiotic, while surfactant is able to facilitate the penetration of the solution into the root canal system. While Shabahang and Torabinejad[13] demonstrated the efficacy of this solution, other studies have shown several important limits. Tay et al.[14] demonstrated that the solution was more aggressive against intertubular dentin, leading to a reduction of collagenic matrix exposed. A new irrigant, Tetraclean (Ogna Laboratori Farmaceutici, Milano, Italy), has been developed containing a mixture of a tetracycline isomer, an acid and 2 detergents. It is recommended to be used as a final rinse after root canal preparation.[15] It is similar to MTAD but with a reduced amount of doxicycline (50mg/5ml instead of 150mg/5ml for MTAD), with polypropylene glycol (a surfactant), citric acid, and

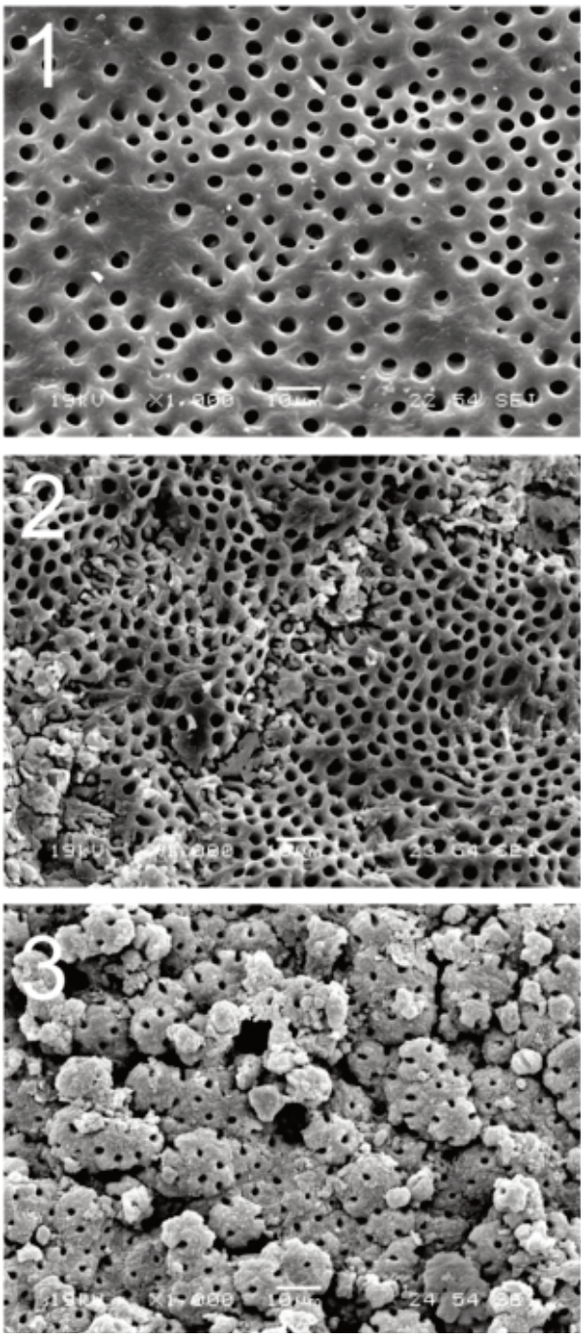

Figure 1: SEM MICROGRAPHS SCORE 1 = No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open; 2 = Moderate smear layer. No smear layer was observed on the surface of root canal, but tubules contained debris; and 3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules.

Table 1

Group	Coronal third scores			Median	Middle third scores			Median	Apical third scores			Median	P value
	1	2	3		1	2	3		1	2	3		
Control (n=9)	0	1	8	3.000	0	0	9	3.000	0	0	9	3.000	<0.05
EDTA (n=10)	3	6	1	2.000	0	6	4	2.000	0	7	3	2.000	<0.05
Tetraclean Liquid (n=9)	7	2	0	1.000	3	6	0	2.000	0	7	2	2.000	<0.05
Tetraclean Liquid+Powder (n=10)	9	1	0	1.000	7	3	0	1.000	0	8	2	2.000	<0.05

Group	Whole root Canal scores			Median	P value
	1	2	3	3.000	
Control (n=29)	0	2	27	2.000	<0.05
EDTA (n=30)	3	19	8	2.000	<0.05
Tetraclean Liquid (n=29)	11	15	3	1.000	<0.05
Tetraclean Liquid+Powder (n=30)	16	12	2		<0.05

cetrimide. This substance is supposedly capable of eliminating all bacteria and smear layer from the root canal system when used as a final irrigation.

This study aimed to compare the efficacy of Tetraclean and 17% EDTA in the removal of smear layer from the coronal, middle and apical thirds of instrumented root canals. The null-hypothesis tested was that there are no statistically significant differences between different protocols for smear layer removal.

Materials and Methods

Sample preparation

Forty human single-rooted teeth with a straight single canal recently extracted for periodontal reasons were selected for the study under a protocol approved by the local ethical committee. Exclusion criteria were: teeth shorter than 20 mm, apex larger than #25 before instrumentation, presence of caries, root fissures or fractures. All teeth were stored in saline at 4°C and used within one month after extraction.

To standardize canal instrumentation, crowns were removed by cutting the teeth 12 mm above the apex, using a water-cooled slow-speed Isomet saw (Buehler, Lake Bluff, IL). Size 10 K-file was inserted into each canal until it was seen through the apical foramen. The working length was established by reducing this length by 0.5 mm. The canals were shaped with nickel-titanium rotary instruments (FlexMaster, VDW, Munich, Germany). Size 30/.06 taper was the last file used at the working length. Irrigation with 5% NaOCl (Niclor 5 Dentale, Ogna, Muggio', MI) was performed during instrumentation using a syringe with a 30-gauge needle (Perio/Endo Irrigation Needle, Biaggio, Switzerland), and the teeth were then randomly divided into four groups (N=10). The exterior part of the apical third of each root was covered with sticky wax to prevent irrigants from dripping through the apical foramen. This was done after placing a calibrated Fine-Medium gutta-percha cone (Mynol Curaden Healthcare SRL, Saronno, VA) at the working length in order to avoid wax intrusion into the apex and the cone was removed after the wax had set.

After instrumentation, each group of teeth underwent a specific final irrigation protocol. For group 1 (control), 5% NaOCl was used (3ml); for group 2 (EDTA), 17% \EDTA (3ml, Ogna, Muggio', Milano, Italy) was used for 1 minute

followed by 5% NaOCl (3ml); for group 3 (Tetraclean liquid, polypropylene glycol and citric acid), the liquid component of Tetraclean was used for 1 minute (3ml), followed by 5% NaOCl (3ml); and for group 4 (Tetraclean), Tetraclean (powder+liquid, 3ml, polypropylene glycol, citric acid and Doxycycline 50 mg/5 ml) was used for 1 minute followed by 5% NaOCl (3ml). The solutions were introduced into the root canals using a 30-gauge needle (Miraject, Hager Werken, Duisburg, Germany), which penetrated to 1-2 mm of the working length. The root canals were then irrigated with 5ml of distilled water and dried with paper points.

SEM observations

Two longitudinal grooves confined to dentin were prepared on the buccal and lingual surfaces of each root using a diamond disc. The roots were then immersed for 30 seconds in a bowl containing liquid nitrogen, which was sufficient for most of them to generate a separation of the two root halves, otherwise a chisel was introduced into the grooves to separate the two root halves. For each root, the half containing the most visible part of the apex was conserved and coded. The coded specimens were then mounted on metallic stubs, gold sputtered, and examined using a scanning electron microscope (SEM JSM-6060LV, JEOL, Tokyo, Japan). Pictures taken at 500X and 1000X were used to evaluate the coronal (10 mm from apex), middle (6 mm from apex), and apical (2 mm from apex) levels of each specimen. The amount of smear layer remaining on the surface of the root canal or in the dentinal tubules was scored according to the following criteria:[7] no smear layer on the surface of the root canals, all tubules were clean and open (score 1); no smear layer was observed on the surface of root canal, but tubules contained debris (score 2); and smear layer covering the entrances of the tubules (score 3) (figure 1). Approximately 250 scanning electron microscopy photomicrographs were scored by two expert endodontists who were unaware of the coding system in order to exclude observer bias. In the case of disagreement between the operators, the higher score was assigned.

Statistical analysis was performed using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison tests to reveal differences among the groups at $p<0.05$.

Results

One specimen in the control group and one in group 3 were excluded from the study because the canals had been perforated by the disc during the preparation for SEM evaluation. The results obtained in terms of smear layer scores are shown in Table 1. Statistically significant differences were found among the groups in relation to the irrigant used. When the levels were compounded, groups were ranked in the following order: $1>2\geq3=4$ ($p<0.05$). For different sections of the canal space, the distance from the apex (2, 6 and 10 mm) influenced the smear layer removal within each group ($p<0.05$).

Analysis of the smear layer removal at different locations revealed that at 10 mm from the apex, the control group showed the highest score without significant differences with group 2. Groups 3 and 4 revealed the lowest scores ($p<0.05$). At 6 mm the result obtained were similar to those at 10 mm but group 4 performed significantly better than group 2 (fig.2) ($p<0.05$). At 2 mm from the apex the control group showed the highest score with a statistical significant difference with all the other groups ($p<0.05$).

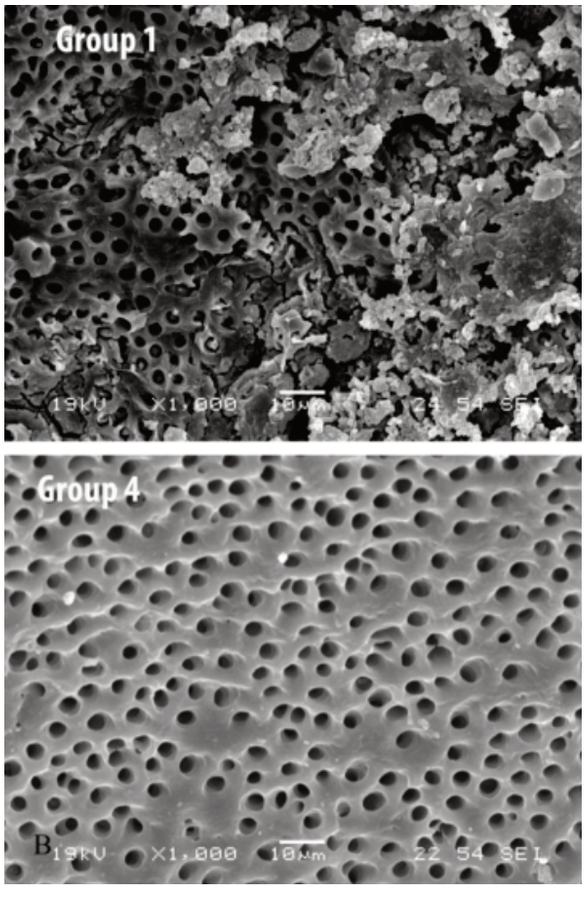

Figure 2: SEM micrographs representing different smear layer removal ability at 6 mm from the apex between group 1 and 4.

Discussion

The null-hypothesis tested in the study had to be rejected

since there were statistical differences between the smear layer removal ability of the different irrigation protocols.

In the present study, 3ml of chelating solutions were used. There is no agreement in the literature concerning the volume of chelating agent or the contact time required in final rinse protocols.[7,9,11] EDTA and Tetraclean were not used according to usually recommended durations but according to experimental ones. As it has been shown that EDTA is effective in removing smear layer without affecting intra and peritubular dentin,[11] 1min application of EDTA was chosen as protocol, and tetraclean application time was mirrored to that of EDTA. It is noteworthy that different application times might yield different results.

The results of the present study are in accordance with other studies showing that NaOCl is not effective in removing the smear layer[7,9,11] when used without a chelatant. When considering the whole root canal it was evident that the use of a chelatant was imperative for removing the smear layer. Tetraclean is a helpful solution for the removal of the smear layer when used as a final rinse ex vivo: it promotes clean canal walls, with absence of smear layer and opened dentinal tubules, without changing the structure of dentine.[16] In this study, a final rinse of each canal was performed by using 3 ml of 5% NaOCl for all the experimental groups to standardize final irrigation protocols. Because this study examined only the efficacy of different protocols for smear layer removal, further studies should be conducted to examine the effect of 5% NaOCl final rinse on antimicrobial effectiveness of doxycycline component in Tetraclean and its substantivity. The liquid component of Tetraclean has been proposed for the final rinsing step, followed by 5%NaOCl (group 3), for understanding the chelating action when citric acid works with surfactants, estimating an optimal time-effect relationship for the clinical application. De Deus et al.(17) reported that demineralization kinetics promoted by 10% citric acid is faster than for 17% EDTA as demineralizing substance: real-time observation of the demineralization process in radicular dentine 17% EDTA promoted much weaker demineralization and caused less peritubular and intertubular dentine erosion when compared with 10% citric acid. The association of a powder and a liquid (group 4) is even more effective in cleaning the root canal walls. This is possibly due to the presence of an antibiotic with chelating action in the powder. Doxycycline has been used in periodontal treatments because of its antibacterial and chelating ability as well as its substantivity.[18] Barkhordar et al[19] and Haznedaeroglu and Ersev[20] recommended the use of tetracycline hydrochloride to remove the smear layer from the surface of instrumented canals and root-end cavity preparations.

At 6 mm from the apex, groups 2 and 3 gave better results than control group, and group 4 revealed statistically significant differences with all the other groups: this can be explained by the addition of a powder containing a tetracycline isomer which has a chelating action and improves the penetration ability of the solution into this narrow region of the root canal. However at 2 mm from the apex, groups 2, 3 and 4 were not statistically different, and gave lower scores when compared to the control group. At this level, the presence of the surfactant agent should have improved the penetration of the solution into dentinal tubules however, no significant differences were detected. Although images from groups 4 revealed better smear layer removal than group 2, the sample size was probably too small to allow detection of differences between these groups. The current study showed that the process of smear layer removal was more efficient in the coronal and middle thirds than in the apical third of the canals. This finding is in agreement with the results of various studies that have shown an effective cleaning action in the coronal and middle thirds of the canals even when different irrigation times and volumes of solutions were investigated.[7] A larger canal diameter in the coronal and middle thirds exposes the dentin to a higher volume of irrigants, allowing a better flow of the solution and, hence, further improving the efficiency of smear layer removal.[7] Consequently, it is important to use other methods, such as ultrasonic devices, for improving the efficiency of low-volume chelating agents used for a short application time[22] From another standpoint, Mancini et al[21] showed that the apical third is always the least cleaned as it is likely to receive less volume of irrigant when compared to the more coronal portion of the canal. In a recent study Poggio et al[16] investigating by SEM image analysis the endodontic dentinal surfaces after canal shaping with Ni-Ti instruments and irrigating with 5.25% NaOCl + different irrigating solutions as final rinse showed that NaOCl+Tetraclean group had significantly lower scores than other groups were in accordance with present study.

It is evident that increasing the instrument taper will allow a deeper penetration of the irrigation needle and improve the flushing of debris.[23] Shuping et al[24] found a better antibacterial effect using nickel-titanium (NiTi) instrumentation when NaOCl was used, but only after instrumentation exceeded ISO size #30 to #35. To overcome the potential limited irrigation in the apical area, enlargement of this area has been advocated for better cleansing.[25] For this reason it was decided to prepare the apical foramen of the samples to #30 in order to be able to

compare the outcome of the present study with other studies in literature.

It is noteworthy that when an antibiotic is included in the formulation of the irrigant, the possibility of increasing the microbial resistance to that antibiotic should be taken into account. Several mechanisms including oxygen limitation, antibiotic penetration, and the presence of a small subpopulation of 'persister' cells, could be responsible of antiobiotic susceptibilities.[26]

Therefore it can be concluded, within the limitation of this ex-vivo study, that the use of a chelating agent leads to a higher removal of smear layer from the root canal walls. Differences between EDTA and Tetraclean were only evident at 6 mm from the apex, whereas at 2 mm both protocols had similar performances in smear layer removal from the root canal system of single-rooted permanent teeth.

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The authors deny any conflict of interest.

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