



Original article

Evaluation of the different irrigation regimens with sodium hypochlorite and EDTA in removing the smear layer during root canal preparation

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ARTICLE INFO

Article history:

Received 24 April 2013

Received in revised form 25 June 2013

Accepted 27 June 2013

Keywords:

Dentine

Root canal preparation

Microscopy

Smear layer

ABSTRACT

Objectives: The aim of this study was to compare, by scanning electron microscopy analysis, the cleaning efficacy of a 2.5% sodium hypochlorite (NaOCl) and a 17% ethylenediaminetetra-acetic acid (EDTA) solution with the two solutions either applied alternately or mixed together for smear layer removal after the use of each endodontic file in different root thirds.

Materials and methods: Fifty-four single-rooted human maxillary premolars were used and divided into three groups. Manual instrumentation was performed with K-Flexofiles with the crown-down technique; and divided in Group 1: canal preparation was performed with 2.5% NaOCl mixed with 17% EDTA in the root canal. Group 2: irrigation was performed alternately with 2.5% NaOCl and 17% EDTA. Group 3: only 2.5% NaOCl was used during all instrumentation and EDTA for 3 min at the final. The mean scores for the smear layer by SEM after the use of each file were calculated and analysed.

Results: A statistically significant difference ($P < 0.05$) was found among the instrumentation groups between the apical third and the middle and coronal thirds. In the apical third, the canal walls were often contaminated by inorganic debris and smear layer.

Conclusions: The alternate or mixed use of EDTA during instrumentation with 2.5% sodium hypochlorite was the most effective form of irrigation for the removal of smear layer on the cervical and middle thirds. No form of irrigation was sufficiently effective to remove the smear layer in the apical third.

Clinical relevance: The importance of the alternating use of 17% EDTA and 2.5% sodium hypochlorite during root canal instrumentation.

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Peer review under responsibility of Saudi Society of Microscopes.



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1. Introduction

The elimination of microorganisms from the root canal is an important step in the success of endodontic therapy. The colonisation of dentinal walls with biofilm, along with the anatomical complexity of the root canal and the possibility of invasion of dentinal tubules, can compromise the success of endodontic therapy [1–3]. The root canal wall, when submitted to the action of each instrument (manual or rotary), becomes coated with a layer predominantly composed of grinding debris, reported as the smear layer [4]. Because it is of dentinal origin, it is composed

of organic and inorganic matter. The organic matter consists of collagen decomposition products, odontoblastic processes, pulp tissue, coagulated proteins, blood cells, and – in cases of pulp necrosis – bacteria and their products [5,6]. The inorganic portion is composed mainly of calcium hydroxyapatite and tricalcium phosphate [5–8].

The morphology of the smear layer is composed of two layers. The superficial layer is firmly adhered to the dentine surface, and the deep layer is formed by smaller particles that are compacted into the dentinal tubules, making the deep layer difficult to remove [7–10]. This compaction causes the reduction of dentine permeability by 25–49%, which would protect the bacteria previously installed inside the dentinal tubules [11]. Furthermore, technological advances in instrumentation techniques allow more efficiency but on the other side reduce the contact time of the endodontic irrigants [12].

The clinical implications of the deleterious effects of the smear layer produced during root canal instrumentation indicate the need for its effective removal. As there is no single solution that has the ability to dissolve organic tissues and to demineralize the smear layer, the sequential use of organic and inorganic solvents has been recommended [13]. Sodium hypochlorite, considered one of the most efficient endodontic irrigants, merely removes the organic portion of the smear layer; therefore, a decalcifying substance such as EDTA is needed to remove the inorganic matter. The combination of these two irrigants complements the cleaning of the root canal, especially in areas of difficult access, such as dentinal tubules and lateral canals [1,2,14,15]. However, there is a variation in irrigation regimens that employ these two substances, giving predominance to the use of sodium hypochlorite during all of the shaping phases of the root canal with a final irrigation using a demineralizing agent, and others authors state the order of use has not yet been defined [8,16].

If it is only evaluated the final cleaning of the root canal it is not observed if the requirements of the auxiliary chemical substances really occurred along the shaping phase. The root canal is considered shaped after the use of at least 3 sequence instruments of crescent diameter. In this way, the formation of smear layer occurs when the first endodontic file is used. Thus, the use of an alternating or combined regimen of irrigation to remove it has been examined in the literature [8,17–19], and the study of interactions between chemical substances has been revisited [14,20–22]. The aim of the present study was to compare the cleaning efficacy of 2.5% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetra-acetic acid (EDTA) solutions, used either alternately or simultaneously, in the removal of the smear layer, compared with the isolated use of NaOCl during shaping and the use of EDTA only during final irrigation.

2. Material and methods

2.1. Sample preparation

A total of fifty-four freshly extracted permanent human premolars, with a single root canal and complete root formation, extracted for orthodontic or periodontal reasons,

were used. The samples were divided into three groups of 18 teeth and were randomly separated.

2.2. Root canal instrumentation

The procedure for the root canal instrumentation was performed with samples stabilised in a clamp, which was fixed on a table bench. After coronal flaring, the working length was established with the introduction of a #10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) in the root canal, which was visualised in the foramen; this measurement was reduced by 1 mm to obtain the working length of each sample. Thus, a cervical preparation of the samples with Gates-Glidden (GG) #3 and #2 (Dentsply/Maillefer, Ballaigues, Switzerland) was performed. The apical preparation was extended up to a #45 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) following the crown-down technique, and irrigation using an up-and-down motion was performed at every change of file. The irrigation was performed with the regimen established for each group. After the first file was adjusted to the working length, the root canal was considered prepared after the use of three consecutively larger diameter files in the apical zone.

2.3. Irrigation procedures

The auxiliary chemicals used in this study were a 2.5% sodium hypochlorite (Asfer Chemical Industry Ltd., São Caetano do Sul, SP, Brazil) and a 17% ethylenediaminetetra-acetic acid trisodium salt solution (Asfer Chemical Industry Ltd., São Caetano do Sul, SP, Brazil). The irrigation was performed with a plastic syringe and needles of #30 gauge NaviTip (Ultradent Dental Products, South Jordan, UT, USA) inserted to the proximities of the working length and suction cannulas #20 gauge (Becton-Dickinson, NJ, USA) in the pulp chamber. The solutions were combined for the following proposed irrigation schemes.

Group 1: NaOCl with EDTA simultaneously (at same time) in the canal. The irrigation cycles were inundated with equal parts of solutions used in accordance with the permitted volume to each canal, instrumentation for 2 min, after an irrigation and aspiration with 2 mL of NaOCl to completion. This process was repeated for each instrument.

Group 2: NaOCl alternated with EDTA. The irrigation cycles consisted of irrigation with 1 mL NaOCl, instrumentation for 2 min, another irrigation and aspiration with 1 mL of NaOCl, and irrigation with approximately 1 mL of 17% EDTA, instrumentation for 2 min, and final irrigation with 1 mL of 2.5% NaOCl. This procedure was repeated for each file until the use of the third, and largest, file.

Group 3: Instrumentation with NaOCl solution and final irrigation with EDTA. The irrigation cycles consisted of irrigation with 1 mL NaOCl, instrumentation for 2 min, another irrigation and aspiration with 1 mL of NaOCl, instrumentation for 2 min, and final irrigation with 1 mL of EDTA. This procedure was repeated for each file until the use of the third, and largest, file.

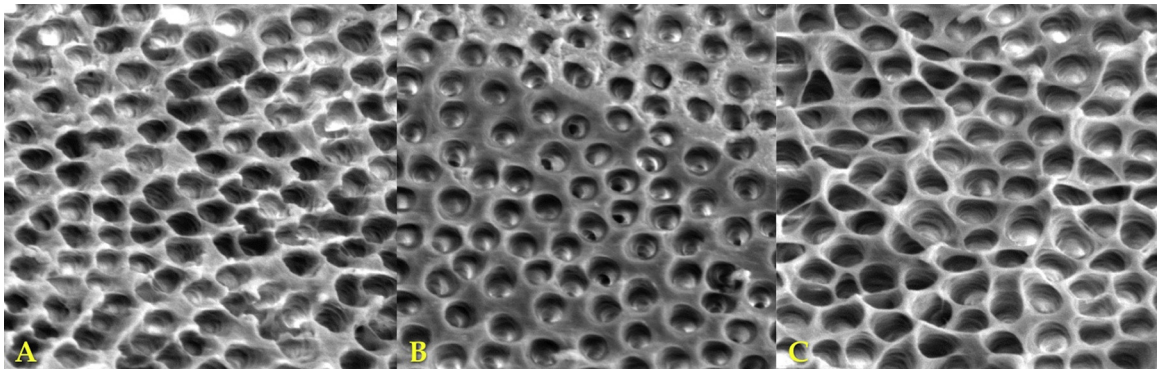


Fig. 1. SEM micrographs of Group 1 after the use of two instrument/file, in the apical (A), middle (B), and cervical (C) thirds. The dentine surface was free of the smear layer and showed open dentinal tubules (2000 $\times$).

In all groups, the samples conducted to SEM to analysis were irrigated with an additional final irrigation of 2 mL of saline solution to eliminate the waste from the irrigating substances. The instrumentation was stopped at three different times for sample analysis. The assessment was conducted after the instrumentation with the first instrument, after the second instrument, and after the last for each group.

2.4. SEM observation

To examine the removal of the smear layer during chemomechanical instrumentation at different thirds of the root canal, we used tooth samples from each group and compared the results. The evaluation of the samples was performed at three distinct moments of instrumentation, i.e., after preparation with the first instrument, after the use of two instruments, and after the use of three instruments. Each instrument was one size larger than the instrument previously used.

Crowns were sectioned at the cemento-enamel junction with a diamond bur, and longitudinal grooves were made using a 0.30-mm-thick diamond disc (KG Sorensen, São Paulo, SP, Brazil) on the root surface. The root was then split with a stainless chisel into two corresponding halves. The most suitable hemi-section of each sample tooth was

selected for SEM examination. The specimens were dried and mounted on a single stub, sputter-coated with gold in a high-vacuum evaporator, and analysed under a scanning electron microscope (Zeiss DSM 940A, Oberkochen, Germany) with 2000 $\times$ magnification at the cervical, middle, and apical levels of each root half (Figs. 1–3).

2.5. Evaluation and statistical analyses

An examiner evaluated the root canal walls, and the scoring criteria were based on the rating system developed by Rome et al. [23] as follows: 0, no smear layer, dentinal tubules open and free of debris; 1, moderate smear layer, outline of dentinal tubules observable or partially filled with debris; and 2, heavy smear layer, cannot distinguish outlines of tubules.

The data were analysed using the nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests at the significance level of 5% ($P < 0.05$). Multiple comparisons with Dunnett's test were applied to determine the significance of detected differences among the groups.

3. Results

Table 1 shows the mean smear layer scores for the three levels of each group. After the use of one instrument, the

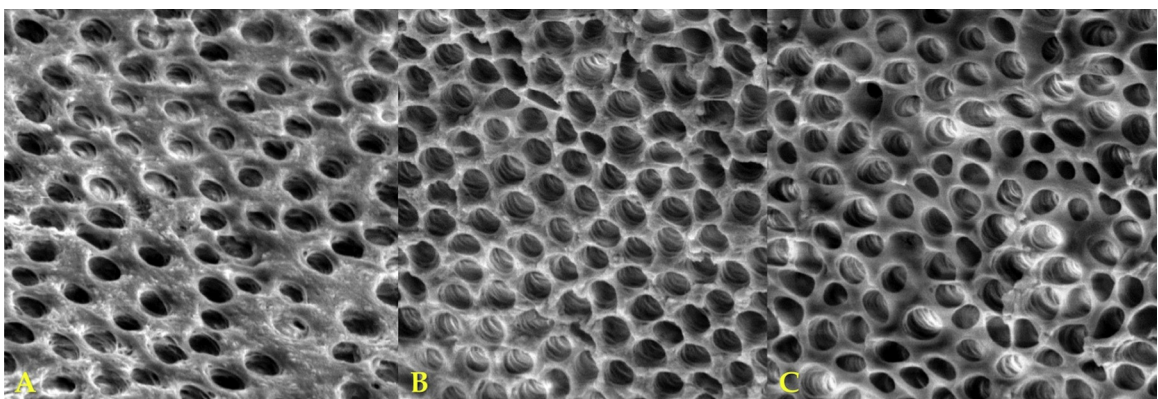


Fig. 2. SEM micrographs of Group 2 after the use of two instruments/files, in the apical (A), middle (B), and cervical (C) thirds. The dentine surface was free of the smear layer and showed open dentinal tubules (2000 $\times$).

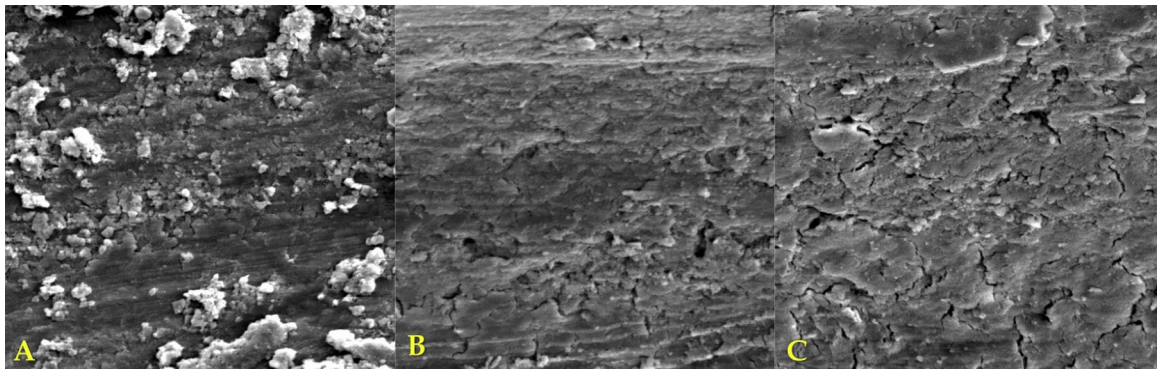


Fig. 3. SEM micrographs of Group 3 after the use of two instruments/files, in the apical (A), middle (B), and cervical (C) thirds. At this stage, the EDTA had not been used (2000 $\times$).

smear layer was more evident at the cervical, middle, and apical thirds of Group 3, although statistically significant differences were not evident for any root sections of the three that were studied ($P > 0.05$).

After the use of the two instruments, it became evident that the outlines of the dentinal tubules were imperceptible with a higher presence of smear layer in all samples of Group 3 (score 2). Statistically significant differences were shown in the middle ($P = .007$) and cervical ($P = .000$) thirds of the three tested groups after using the two instruments, with a massive presence of smear layer in Group 3 (score 2). In Group 2, a smaller amount of debris and smear layer was evident (score 0), followed by Group 1 (Table 2).

The Kruskal–Wallis test showed that there was a statistically significant difference among the root sections (apical, middle, and cervical) when the instrument groups were compared ($P < 0.05$), especially with the use of two instruments. A nonparametric Dunnett's multiple range tests showed that there was no significant difference between Group 1 and Group 2, but when both were compared with Group 3 in the middle and cervical thirds after the use of two instruments, there was a significant difference ($P < 0.05$).

In the cervical third, the mean amounts of smear layer scored in the second and third instruments of Group 1 were the lowest ($P < 0.05$), followed by the cervical and middle

scores in the second instrument of Group 2 ($P < 0.05$). In the apical third, there was extensive smear layer in all groups, and no significant difference was observed ($P > 0.05$). The coefficient of agreement showed a Kappa value of 0.82.

4. Discussion

Baumgartner and Ibay [14], via a stoichiometric study of reactions between sodium hypochlorite and EDTA or citric acid, showed that sodium hypochlorite in aqueous solution presents a chemical equilibrium that is dependent on the pH of the medium. When the pH becomes low, the equilibrium shifts to a predomination of hypochlorous acid non-dissociated (HOCl/instable and more active), and the release of available chlorine becomes pronounced. In a high pH, the equilibrium shifts to a predomination of dissociated form (OCl⁻/stable and less active). The antibacterial efficacy of hypochlorite preparations is a function of their free available chlorine (OCl⁻ and HOCl) in solution [15], as is their tissue-dissolution potential [19]. Therefore, in a pH acid, the antimicrobial activity of sodium hypochlorite is accentuated by the increased release of available free chlorine and oxygen [14].

The stability of chlorine products depends on the maintenance of the pH high, in which there is a chemical equilibrium [14]. The root canal presents organic residues,

Table 1
Results of smear layer scores at root canal sections [n (%)].

Score	After one instrument			After two instruments			After three instruments		
	Apical	Middle	Cervical	Apical	Middle	Cervical	Apical	Middle	Cervical
Group 1									
0	1 (16.6%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	3 (50.0%)	6 (100%)	2 (33.3%)	2 (33.3%)	5 (83.3%)
1	2 (33.3%)	4 (66.6%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	–	2 (33.3%)	1 (16.6%)	–
2	3 (50.0%)	–	1 (16.6%)	2 (33.3%)	–	–	2 (33.3%)	3 (50.0%)	1 (16.6%)
Group 2									
0	–	4 (66.6%)	5 (83.3%)	3 (50.0%)	6 (100%)	6 (100%)	–	2 (33.3%)	3 (50.0%)
1	3 (50.0%)	–	–	1 (16.6%)	–	–	1 (16.6%)	3 (50.0%)	3 (50.0%)
2	3 (50.0%)	2 (33.3%)	1 (16.6%)	2 (33.3%)	–	–	5 (83.3%)	1 (16.6%)	–
Group 3									
0	–	1 (16.6%)	1 (16.6%)	–	–	–	1 (16.6%)	1 (16.6%)	1 (16.6%)
1	–	1 (16.6%)	–	1 (16.6%)	–	–	3 (50.0%)	5 (83.3%)	5 (83.3%)
2	6 (100.0%)	4 (66.6%)	5 (83.3%)	5 (83.3%)	6 (100%)	6 (100%)	2 (33.3%)	–	–

0: Canal wall with absence of dentinal smear layer, dentinal tubules opened, and free debris.

1: Moderate presence of smear layer, contour of dentinal tubules visible or partially obliterated with debris.

2: Canal wall completely covered by smear layer, contour of dentinal tubules imperceptible.

Table 2
Statistical results of canal cleanliness at root canal sections with combinations between groups*.

Apical level			Middle level			Cervical level		
1st. Instrument	2nd. Instrument	3rd. Instrument	1st. Instrument	2nd. Instrument	3rd. Instrument	1st. Instrument	2nd. Instrument	3rd. Instrument
$\chi^2 = 3.616$ Sig. 0.164 G1 × G2	$\chi^2 = 3.542$ Sig. 0.170 G1 × G2	$\chi^2 = 0.708$ Sig. 0.702 G1 × G2	$\chi^2 = 6.530$ Sig. 0.038 G1 × G2	$\chi^2 = 9.917$ Sig. 0.007 G1 × G2	$\chi^2 = 5.989$ Sig. 0.050 G1 × G2	$\chi^2 = 6.701$ Sig. 0.035 G1 × G2	$\chi^2 = 12.750$ Sig. 0.000 G1 × G2	$\chi^2 = 8.362$ Sig. 0.015 G1 × G2
$\chi^2 = 0.789$ Sig. 0.789 G1 × G3	$\chi^2 = 0.733$ Sig. 0.733 G1 × G3	$\chi^2 = 0.071$ Sig. 0.071 G1 × G3	$\chi^2 = 0.727$ Sig. 0.727 G1 × G3	$\chi^2 = 0.056$ Sig. 0.056 G1 × G3	$\chi^2 = 0.498$ Sig. 0.498 G1 × G3	$\chi^2 = 0.338$ Sig. 0.338 G1 × G3	$\chi^2 = 1.000$ Sig. 1.000 G1 × G3	$\chi^2 = 0.386$ Sig. 0.386 G1 × G3
$\chi^2 = 0.058$ Sig. 0.058 G2 × G3	$\chi^2 = 0.149$ Sig. 0.149 G2 × G3	$\chi^2 = 0.733$ Sig. 0.733 G2 × G3	$\chi^2 = 0.061$ Sig. 0.061 G2 × G3	$\chi^2 = 0.005$ Sig. 0.005 G2 × G3	$\chi^2 = 0.434$ Sig. 0.434 G2 × G3	$\chi^2 = 0.055$ Sig. 0.055 G2 × G3	$\chi^2 = 0.001$ Sig. 0.001 G2 × G3	$\chi^2 = 0.091$ Sig. 0.091 G2 × G3
$\chi^2 = 0.056$ Sig. 0.056	$\chi^2 = 0.121$ Sig. 0.121	$\chi^2 = 0.083$ Sig. 0.083	$\chi^2 = 0.154$ Sig. 0.154	$\chi^2 = 0.001$ Sig. 0.001	$\chi^2 = 0.923$ Sig. 0.923	$\chi^2 = 0.027$ Sig. 0.027	$\chi^2 = 0.001$ Sig. 0.001	$\chi^2 = 0.241$ Sig. 0.241

Kruskal–Wallis and Mann–Whitney Test.

* Statistically homogeneous subsets ($P < 0.05$).

in addition to changes of dentine in the chemical balance of sodium hypochlorite [22]. This stoichiometric study has been demonstrated in investigations such as that by Zehnder et al. [15], who examined the chemical interactions of sodium hypochlorite and substances with demineralising abilities. They found that the mixture of sodium hypochlorite with EDTA maintained the antibacterial properties of hypochlorite in dilutions up to 1:10 after 15 min of incubation. They also observed that this mixture led to an almost total loss of free available chlorine. In turn, sodium hypochlorite did not affect the ability of the demineralising EDTA to remove the smear layer, but is necessary a copious amounts of NaOCl to rinse out chelators remnants. In a review study, Rossi-Fedele et al. [19] investigated the influence of changes in pH of the medium in sodium hypochlorite, observing that a reduction in pH improves its antimicrobial properties, but reduces its tissue solvent capacity. Soares et al. [18] evaluated the antimicrobial activity of two endodontic irrigation schemes: sodium hypochlorite during shaping with final irrigation using EDTA, and a regimen alternating between sodium hypochlorite and EDTA to eliminate the intracanal biofilms of *Enterococcus faecalis*. The authors observed that the irrigation regime influenced the elimination of biofilm and that the alternating irrigation method demonstrated greater long-term effectiveness. Ozdemir et al. [17] evaluated the effect of root canal irrigation with sodium hypochlorite and EDTA alone or in combined regimens on both young and elderly human dentine colonised by biofilms of *E. faecalis*. The authors found that the mixture of the two solutions also increased the antimicrobial action.

Photomicrographs 1 and 2 showed that after the use of two instruments during biomechanical preparation and cleaning with the irrigation schemes, there was less accumulation of smear layer on the walls of the canal. In the case of the irrigation system of Group 3, the tubules were obliterated, which reduced dentine permeability to sodium hypochlorite [8]. This smear layer removal only occurs after the final irrigation with EDTA; this fact should reduce the effectiveness of sodium hypochlorite in the early stages of preparation by reducing dentine permeability [5,7].

Tunga et al. [24], in evaluating the effectiveness of the F-file with NaOCl on the removal of the smear layer, observed that in the apical third, there was extensive smear layer in all specimens. The authors attributed these results to the comparatively smaller apical canal diameter hindering the penetration of the root canal irrigants and chelating agents, resulting in limited contact with the root canal. It is necessary to use an irrigation regimen and preparation technique that is effective in removing the smear layer because of the anatomical complexity of the root canal system [2]. An important step in biomechanical cleaning is the removal of anatomic interferences along the root canal, so that an estimate of where the first file binds at the apical region is more precise and enables the endodontic irrigation cannulas to work better [25].

According to other studies, the cleaning of the apical third is critical regardless of the irrigation regimen [7,24]. Some investigations support the theory that larger preparations and frequent and abundant irrigation with

antimicrobial substances play an important role in maximising the effectiveness of chemomechanical preparation [24]. However, irrespective of the irrigation regimen or the instrumentation technique, most specimens still contained living bacteria. This finding confirmed that instruments and irrigants failed to penetrate confined areas of the root canal system. The instruments used in this study, along with fine needles and syringes, did not allow the efficacy necessary for the apical region. Thus, other physical methods must be used to improve the effectiveness of the combined irrigation with sodium hypochlorite and EDTA, especially in the apical third.

The alternating or simultaneously use of 17% EDTA during instrumentation with 2.5% sodium hypochlorite after the use of each instrument during the shaping stage of root canal preparation has been shown to be the most effective form of irrigation in the removal of the smear layer, after use of each instrument during the shaping stage of the root canal from the cervical and middle thirds. The use of 2.5% sodium hypochlorite during root canal preparation and a final flush with 17% EDTA showed results similar to Groups 1 and 2 only at the end of the instrumentation. No form of irrigation was sufficiently effective to remove the smear layer in the apical third.

Conflict of interest

The authors do not have any conflict of interest in connection with this manuscript.

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