
The effect of application time of EDTA and NaOCl on intracanal smear layer removal: an SEM analysis

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Abstract

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Aim To verify, under the scanning electron microscope (SEM), the influence of irrigation time with ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) on intracanal smear layer removal.

Methodology Twenty-one extracted human permanent teeth with single straight root canals were included. The root canals of the teeth were instrumented and, at the end of preparation, were irrigated with 3 mL of 15% EDTA, followed by 3 mL of 1% NaOCl for 1 min (group 1), for 3 min (group 2), and for 5 min (group 3). The canals of teeth in group 4 (control) did not receive the final irrigation. The teeth were sectioned longitudinally and prepared for an SEM. The dentinal wall of cervical, middle and apical thirds was graded

according to the amount of debris and smear layer remaining on the walls. The results were analysed using the Kruskal–Wallis and Conover–Inman tests.

Results In all the canals of experimental groups irrigation with EDTA and NaOCl completely removed the smear layer from the cervical and middle thirds. In the apical third, the dentine surface were partially covered, particularly in the teeth of group 1, where there was significantly more smear layer when compared with the other thirds in the same group ($P < 0.007$). However, the Kruskal–Wallis test showed overall that there were no significant differences between groups 1, 2 and 3 ($P > 0.05$).

Conclusion In this limited laboratory study, canal irrigation with EDTA and NaOCl for 1, 3 and 5 min were equally effective in removing the smear layer from the canal walls of straight roots.

Keywords: chelating agent, EDTA, irrigating solutions, smear layer, sodium hypochlorite.

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Introduction

During canal preparation, dentine chips created by the action of endodontic instruments add to the remnants of organic material and irrigating solutions, forming a smear layer that adheres to the canal walls. This layer can form two zones: the first, 1–2 μm -thick, made up of organic matter and dentine particles; the second, extending into dentinal tubules to a depth of 40 μm (smear plugs) is formed largely of dentine chips (Mader *et al.* 1984).

It is known that the smear layer may harbour bacteria, preventing the canal from being disinfected (Berutti *et al.* 1997). In addition, it has been demonstrated that the removal of this layer promotes dentine permeability (Pashley *et al.* 1981), enhancing diffusion and the action of intracanal medication (Ørstavik & Haapasalo 1990), allowing and producing greater penetration of filling material into lateral canals and dentinal tubules (Gutiérrez *et al.* 1990, Lloyd *et al.* 1995).

Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer. In an effort to remove this layer completely, many authors suggest the use of several solutions (Baumgartner & Mader 1987, Abbott *et al.* 1991, Barkhordar *et al.* 1997). Neutral ethylenediaminetetraacetic acid (EDTA) solutions, in a 15–17%

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concentration, are effective in demineralizing the dentine (Nygaard-Östby 1957, O'Connell *et al.* 2000, Çalt & Serper 2002), and can be used to remove the smear layer (McComb & Smith 1975, Goldman *et al.* 1981, Baumgartner & Mader 1987, O'Connell *et al.* 2000, Çalt & Serper 2002). However, as it does not dissolve organic matter (Goldman *et al.* 1981, Baumgartner & Mader 1987), EDTA has been used with sodium hypochlorite (NaOCl) solution which, in addition to acting on pulp tissue remnants (Goldman *et al.* 1981, Baumgartner & Mader 1987, Abbott *et al.* 1991), has antimicrobial properties (Baumgartner & Cuenin 1992).

Although many authors indicate canals should be irrigated at the end of instrumentation with the sequential use of EDTA and NaOCl (Goldman *et al.* 1982, Baumgartner & Mader 1987, Abbott *et al.* 1991), the literature demonstrates variation in the volume of solution and, above all, the duration of irrigation. For example, the time these solutions stay in contact with the canal walls has been reported to be from 30 s to 10 min (Goldman *et al.* 1981, Abbott *et al.* 1991, Garberoglio & Becce 1994, Lloyd *et al.* 1995). There are few reports simulating a clinical situation, comparing the results obtained from the removal of the smear layer as a function of the duration of the final irrigation.

The present investigation sought to verify, through an SEM study, the influence of irrigation time with EDTA and NaOCl on the removal of intracanal smear layer.

Materials and methods

Using radiographs taken in a mesio-distal direction, 21 human canine teeth with single straight root canals were identified and selected. After extraction, the teeth were cleaned by removing the remaining soft tissue and then stored in 0.1% thymol solution at room temperature. After washing under tap water for 24 h, each tooth was numbered on the buccal and palatal surfaces of the root. Endodontic access was obtained, and the lengths of the teeth were determined by the introduction of a size 15 K-file into the root canal until the tip reached the apical foramen. The working length (WL) for preparation of the canal was set 1 mm shorter than these measurements.

Having covered the root apexes with sticky wax, the canals were prepared using a standardized stepback, or telescopic, technique (Mader *et al.* 1984) with sequentially sized Flexfiles and K-files (Dentsply Maillefer,

Ballaigues, Switzerland). The apical stop was created through the use of three files larger than the initial one, and because of their similar anatomy, the apical size of the canals was enlarged to 40 or 45. In the sequence, three more instruments were employed, stepping back 1 mm at each change. Preparation was completed using Gates–Glidden burs (Dentsply Maillefer) of numbers 2, 3 and 4, with a stepback of 2, 4 and 6 mm, respectively, in relation to the length of the last instrument used. Patency of the apical foramen was maintained throughout preparation, with the help of a size 15 file. Between the use of each file or bur, canals were irrigated with 2 mL of 1% NaOCl.

Eighteen canals were divided into three groups, and irrigated with 3 mL of 15% EDTA and 3 mL of 1% NaOCl, using different durations for each solution in the canal: 1 min (group 1), 3 min (group 2) and 5 min (group 3). The irrigant was delivered with an endodontic syringe and a 22-gauge blunt-end needle (Ibras, São Paulo, Brazil) 2 mm short of the WL. No final irrigation was conducted in the three canals of group 4 (control).

The canals were dried with absorbent paper points, and the entrance to each of the canals was protected with a cotton pellet. Using carborundum discs, the crowns were removed at the cementum–enamel junction, and deep grooves were cut on the buccal and palatal surfaces of the roots, without perforating the root canal. The roots were then split with a chisel and a hammer. One half of each tooth was selected and prepared for SEM examination. After assembly on coded stubs, the specimens were placed in a vacuum chamber and sputter-coated with a 300 Å gold layer (Bal-Tec SCD 005; Bal-Tec Co., Balzers, Liechtenstein). The specimens were then analysed using a Philips SEM XL 30 (Philips, Eindhoven, the Netherlands). The dentinal wall of the cervical, middle and apical thirds was observed at magnifications of up to $\times 1000$ for the presence/absence of smear layer and visualization of the entrance to dentinal tubules. Photomicrographs ($\times 1000$) of those areas representative of the pre-predominant dominant condition on each of the thirds were taken.

The cleaning of root canal walls was evaluated individually by two previously calibrated examiners who, blind to the irrigation regimens employed for each group, attributed scores according to the rating system developed by Rome *et al.* (1985): 0 = no smear layer, dentinal tubules open, free of debris; 1 = moderate smear layer, outlines of dentinal tubules visible or partially filled with debris; 2 = heavy smear layer,

outlines of dentinal tubules obliterated. Attributed scores were tabulated and submitted to statistical analysis using the Kruskal–Wallis test to determine if there were significant differences between groups. Where significant differences were identified, a Conover–Inman multiple comparison test was used. Statistical significance was set at $P = 0.05$.

Results

Scores attributed to each specimen on the three-thirds of the canals are presented in Table 1. Data were examined with the nonparametric tests described above. The means of such scores are shown in Table 2 (comparison of thirds in each group), and Table 3 (comparison of thirds between the experimental groups).

Group 1 (1 min)

The smear layer on the dentine wall of the cervical and middle thirds was removed completely (Fig. 1a,b). The entrances to the dentinal tubules were visible and slightly enlarged. On the apical third, the dentine smear layer was partly removed on five of the six analysed specimens (Fig. 1c). When compared with the other

Table 1 Scores^a attributed to the dentinal surface of teeth in the various groups

Groups	Tooth	Cervical third	Middle third	Apical third
1 (1 min)	1	0	0	1
	2	0	0	1
	3	0	1	1
	4	0	0	1
	5	0	0	1
	6	0	0	0
2 (3 min)	7	0	0	0
	8	0	0	1
	9	0	0	0
	10	0	0	1
	11	0	0	0
3 (5 min)	12	0	0	0
	13	0	0	0
	14	0	0	0
	15	0	0	1
	16	0	0	1
	17	0	0	0
	18	0	0	0
4 (control)	19	2	2	2
	20	2	2	2
	21	1	2	2

^a0, No smear layer; 1, moderate smear layer; 2, heavy smear layer.

Table 2 Mean of scores attributed to teeth in groups 1, 2, 3, 4, according to the thirds

Group third	G1 (1 min)	G2 (3 min)	G3 (5 min)	G4 (control)
Cervical	0.000	0	0	1.667
Middle	0.167	0	0	2.0
Apical	0.833	0.333	0.333	2.0
<i>T</i>	9.92	4.25	4.25	2
<i>P</i>	0.007	0.1194	0.1194	0.3679

Vertical bars indicate statistical equivalence (Kruskal–Wallis, and Conover–Inman tests, $P > 0.05$).

Table 3 Mean of scores attributed to the thirds of teeth in groups 1, 2 and 3

Group	Cervical	Middle	Apical
1	0	0.167	0.833
2	0	0	0.333
3	0	0	0.333
<i>T</i>	0	2	3.78
<i>P</i>	>0.999	0.3679	0.1512

Vertical bars indicate statistical equivalence (Kruskal–Wallis test, $P > 0.05$).

thirds in this group, a significant difference was seen ($P = 0.007$).

Groups 2 (3 min) and 3 (5 min)

Results for these two groups were identical. The dentine wall of the cervical and middle thirds on all the samples were free from smear layer, the entrances to the tubules were visible and enlarged. Despite no significant difference being seen between the thirds, smear layer was completely removed from the apical third on four specimens (Fig. 2), and in two of them the dentine surface was partly covered as in group 1 (Fig. 1c).

Overall, comparing the thirds between experimental groups 1, 2 and 3 (Table 3), no significant difference was found ($P > 0.05$).

Group 4 (control)

With the exception of the cervical third in one specimen, the dentinal surface of all the other samples was completely covered with the smear layer (Fig. 3). No significant difference exists between the thirds in this group ($P > 0.05$).

Discussion

The association of EDTA and NaOCl solutions has proved effective in removing smear layer formed during

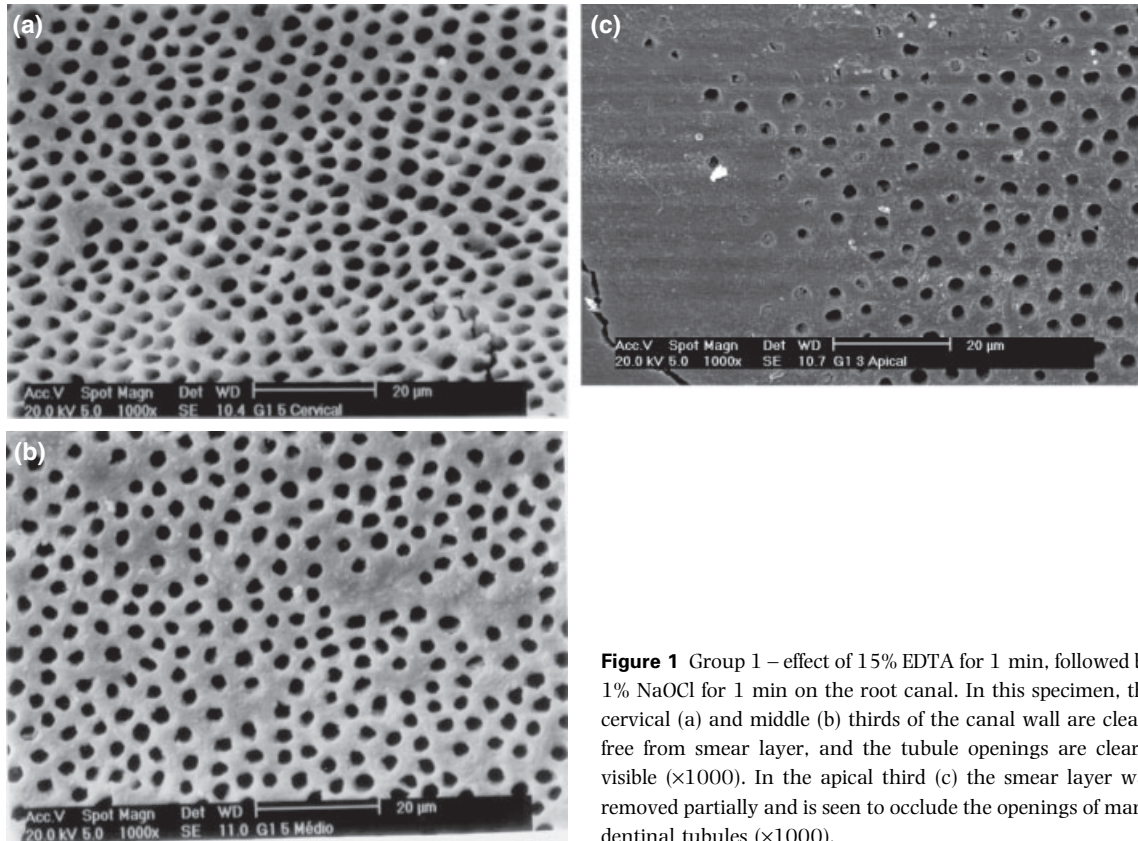


Figure 1 Group 1 – effect of 15% EDTA for 1 min, followed by 1% NaOCl for 1 min on the root canal. In this specimen, the cervical (a) and middle (b) thirds of the canal wall are clean, free from smear layer, and the tubule openings are clearly visible ($\times 1000$). In the apical third (c) the smear layer was removed partially and is seen to occlude the openings of many dentinal tubules ($\times 1000$).

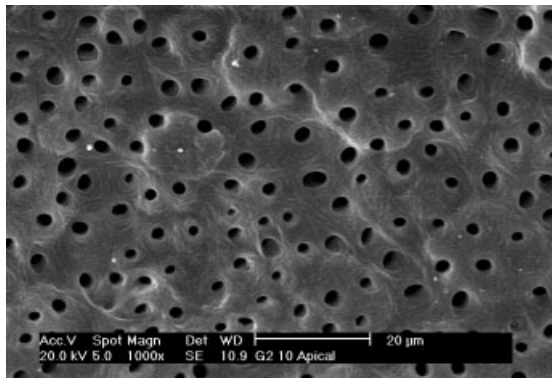


Figure 2 Group 2 – effect of 15% EDTA for 3 min, followed by 1% NaOCl for 3 min on the apical third of the root canal. The smear layer was completely removed, and all of the tubule openings were clearly visible on four of the six specimens ($\times 1000$). The group 3 has the same results.

endodontic instrumentation (Goldman *et al.* 1982, Baumgartner & Mader 1987, Abbott *et al.* 1991). EDTA acts upon the inorganic components of the smear layer, causes the decalcification of peri- and

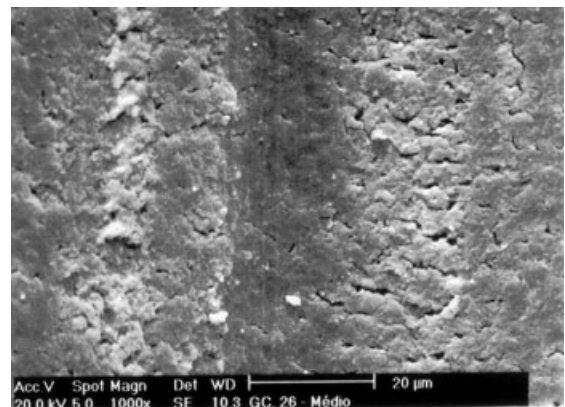


Figure 3 Group 4 – control – teeth without final irrigation. Dentine surface along the whole length of the canal was covered with a dense smear layer ($\times 1000$).

intertubular dentine, and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen, leaving the entrances to the dentinal tubules more open and exposed (Goldman *et al.* 1982, Baumgartner & Mader 1987).

Considering the time these solutions remain in contact with the canal walls can be varied (Goldman *et al.* 1981, 1982, Abbott *et al.* 1991, Lloyd *et al.* 1995), the present study was designed to verify the effect of time in removing the dentinal smear layer.

To prevent the diameter of canals and the extension of preparation from having an effect on the amount of smear layer, the teeth were carefully selected and the mechanical preparation was conducted under a standard modality. During final irrigation it was possible to introduce the needle up to 2 mm short of the WL in all canals. Thus, considering the standard conditions of these factors, and also the quantity of the solutions employed, it is expected the difference in results is explained by the different periods of time the solutions acted upon the dentinal walls. The dentinal surface of canals, which did not receive the final irrigation, were covered with the smear layer (Fig. 3).

In some of the specimens in the experimental groups it was possible to see globular dentine, or calcospherites (Fig. 4). The action of NaOCl on the uninstrumented walls of canals dissolves the predentine and exposes the globular dentine, as reported in other studies (Baumgartner & Mader 1987, Baumgartner & Cuenin 1992, O'Connell *et al.* 2000).

In general, analysis of the dentinal wall of all the specimens in the experimental groups demonstrated that cleaning had been effective. Most notably on the cervical and middle thirds the surfaces were clean, permitting the visualization of the entrances to the dentinal tubules. It is possible that the size of the canals in these thirds, when compared with the apical third, allowed better circulation and action of the irrigating

solution, making the complete removal of the smear layer possible. Such results are in agreement with those of various authors (Baumgartner & Mader 1987, Abbott *et al.* 1991), who have also observed an effective cleaning action on these thirds even when different quantities of solutions and times of irrigation were employed.

In the apical third, the smear layer was partially removed in five specimens of group 1 and in two of groups 2 and 3. Here, in spite of the irrigating needle going as deep as 2 mm short of the WL, removal of the smear layer was not as effective as that seen on the cervical and middle thirds, particularly for teeth of group 1 where the solutions remained in the canal for 1 min. It is possible a deeper introduction of the needle would permit better cleaning. Such introduction, however, in clinical practice would bring greater risk of injuring the periapical tissues on account of the possible extrusion of the irrigants.

Results for the apical third agree with those of other studies showing how difficult it is to remove the smear layer in that region (Goldman *et al.* 1982, Barkhordar *et al.* 1997, O'Connell *et al.* 2000, Çalt & Serper 2000). On the other hand, Garberoglio & Becce (1994), using EDTA for 30 s, reported good cleaning of the apical third, although they did notice the presence of smear plugs in some of the specimens. Upon irrigating the canals for 5 min, Lopes *et al.* (1996) reported that the mechanical stirring of EDTA for 2 min using a Lentulo spiral allowed for the near complete removal of the dentinal smear layer from the apical third. The authors explained that, on account of the reduced dimension of the root canal, air bubbles frequently remain trapped and prevent total filling with the irrigant. Mechanical stirring with a Lentulo spiral removes the air bubbles, favours improved contact of EDTA with the canal walls, and takes the solution to areas that are not reached by the irrigating needle.

Although no significant differences existed when compared with the other groups, the time of 1 min (group 1) proved insufficient in cleaning the apical third, as the dentinal wall in five of the six specimens remained partly covered with smear layer. The longer application time of EDTA and NaOCl, produced the best results in the apical third.

Conclusion

The association of EDTA and NaOCl solutions proved effective in removing the smear layer from the cervical and middle thirds for all times of application (1, 3 and

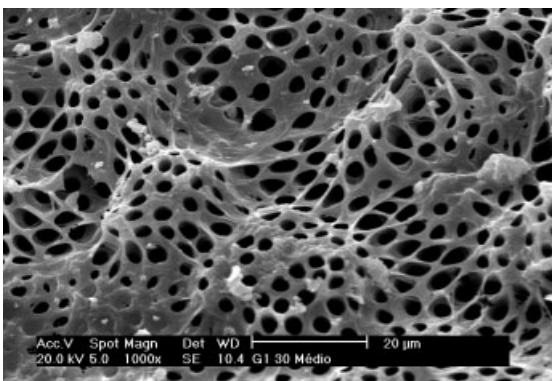


Figure 4 In casually uninstrumented dentine walls, the NaOCl action exposed the calcospherites due to dissolution of the predentine ($\times 1000$).

5 min). In the apical third the efficacy of the smear layer removal was decreased, particularly in group 1.

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