The removal of the smear layer using EGTA: a scanning electron microscopic study

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The smear layer associated with endodontic instrumentation is currently thought to be a thin layer that occludes the orifices of the dentinal tubules and covers the intertubular dentin of the prepared canal wall. The purpose of this study was to compare the effect of ethylene glycol bis (β – amino ethyl ether) – N, N, N', N' – tetra acetic acid (EGTA) and ethylene diamine tetra acetic acid (EDTA) on removal of the smear layer through the scanning electron microscopy. Twenty four single rooted teeth were selected, instrumented and irrigated with various solutions and the specimens were processed for scanning electron microscopy. It was found that though both EGTA and EDTA completely removed the smear layer, EDTA caused erosion and conjugation of the tubules, whereas, EGTA effectively removed the smear layer without inducing any erosion. It was thus concluded that EGTA can be effectively used as an alternative chelator for the removal of the smear layer.
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INTRODUCTION

The smear layer associated with endodontic instrumentation is currently thought to be a thin layer that occludes the orifices of the dentinal tubules and covers the intertubular dentin of the prepared canal wall.[1] Whether it is beneficial or detrimental to a successful root canal therapy is still controversial. Though the smear layer was considered beneficial since it reduces the permeability of dentin[2,3] it was considered deleterious, since it prevents the penetration of irrigants, medications and filling materials into the dentinal tubules or it may even impede contact with the canal wall.[4-7] Thus, it is imperative that the smear layer be removed in order to eliminate bacteria, facilitate antibacterial effect of intracanal disinfectants and improve the ultimate seal of the root canals.

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To date, no single irrigant has been demonstrated to be capable of dissolving the organic pulpal material and predentin as well as demineralizing the organic calcified portion of the pulp canal wall. Thus combination of various irrigants have been recommended to accomplish these goals.[8] It is widely accepted that the most effective method to remove the smear layer is to irrigate the root canals with 10ml of 17% EDTA followed by 10ml of 5% NaOCI.[9,10] EDTA chelates with Ca^{2+} and other divalent cations, demineralizes dentin, and removes the inorganic components of the smear layer[11,12] and also causes erosion. Yet another chelator, ethylene glycol-bis (β-amino ethyl ether) – N,N,N^1,N^1-tetra acetic acid (EGTA) is reported to bind Ca^{2+} more specifically[13] without inducing any erosion; and there by removes the inorganic component of the smear layer effectively.

The purpose of this scanning electron microscope study was to evaluate the effects of EGTA on the removal of the smear layer on the pulp canal wall as an alternative to EDTA.

MATERIAL AND METHODS

Twenty four (24) extracted maxillary permanent central incisors were used for the study. The teeth were stored in saline solution until further use. The teeth were divided into 4 groups of 6 teeth each depending upon the type of irrigant used (Table 1).

Conventional access was prepared through the crowns, and the pulpal tissue was removed with a barbed broach. Teeth were instrumented to the apex using K-files up to size 60. After instrumentation, root canals of the first group were irrigated with 10ml of

Table 1. Distribution of the 4 groups

Group	Table No. of teeth	Irrigants used	Volume
Group 1	6	Normal saline	10ml
Group 2	6	5% NaOCl	10ml
Group 3	6	17%EDTA and	5% NaOCl
	10ml	10ml	
Group 4	6	17% EGTA and	5% NaOCl
		10ml	10ml

Table 2.

Score	Criteria
0	• Clean open tubules, no smear layer, no debris • No conjugation of dentinal tubules • No signs of erosion
1	• Clean open tubules, no smear layer, no debris • Presence of conjugation of dentinal tubules. • Signs of erosion
2	• Partial smear layer, some tubules open and some tubules partially occluded, scattered debris
3	• Total smear layer, debris present very few or no open dentinal tubules

Table 3. Showing the Kruskal Wallis Test Scores
Test statistics a, b

Group	Test	Apical	Middle	Coronal
Group 1	H	4.250	0.000	0.000
	P	0.199+	1.000+	1.000+
Group 2	H	0.000	0.442	0.000
	P	1.000+	0.802+	1.000
Group 3	H	2.000	0.000	0.000
	P	0.368+	1.000+	1.000+
Group 4	H	0.000	2.000	0.000
	P	1.000+	0.368+	1.000+

+: Not significant
a. Kruskal – Wallis test
b. Grouping variable : Examiner

normal saline; second group irrigated with 10ml of 5% NaOCl. The third group was irrigated with 10ml of 17% EDTA (SD Fine Chemicals, Mumbai, India) followed by 10 ml of 5% NaOCl. The fourth group was irrigated with 10ml of 17% EGTA (Sigma, St. Louis, MO) followed by 10ml of 5% NaOCl. The pH of the EDTA and EGTA solutions was adjusted to 7.5 by the addition of NaOH. Irrigation procedure was continued for 2min and then the crowns of the teeth were removed at the cervical line by a high-speed handpiece and the roots were split longitudinally and processed for SEM (JEOL, Japan, Model 5309).

The photomicrographs were coded by 3 examiners who allocated the grades. The photomicrographs were graded according to amount of debris and smear layer as depicted in Table 2.

Inter examiner variability was tested using the Kruskal Wallis test and was found to be not significant (Table 3). Hence the readings of the first examiner were taken into account for the further study purpose.

RESULTS

The following observations were also noted based on the photomicrographs from the scanning electron microscope.

Group 1. (Normal saline)
The specimens which were irrigated with normal saline predominantly had a score of 3 in all the sections barring few sections where a score of 2 was obtained. These specimens showed extensive amount of debris and smear layer. It was full of hard and soft tissue debris. Higher magnification (X 4000) showed the organic debris embedded into the background of the smear layer. Though the saline solution was effective in cleaning the pulpal remnants, yet the smear layer was present and was quite conspicuous (Figures 1 to 3).

Group 2. (5% NaOCl)
The specimens which were irrigated with 5% NaOCl had a score of 3 in majority of the sections and in 1 or 2 sections, a score of 2 was obtained. These specimens showed less amount of soft tissue debris. At higher magnification (X 4000) they revealed a typical amorphous smear layer, which completely obscured the dentinal tubules and much less soft tissue remnants were present throughout the canal (Figures 4 to 6).

Group 3. (17% EDTA and 5% NaOCl)
All the specimens which were irrigated with 17% EDTA and 5% NaOCl had a score of 1. This irrigation regimen produced a clear surface with occasional strands of isolated superficial debris, but the patent dentinal tubules were clearly seen. Though this irrigating solution efficiently removed the smear layer, the following effects were observed. There was conjugation of the dentinal tubules and erosion, was seen both at peritubular as well as Intertubular dentin. (Figures 7 to 9)

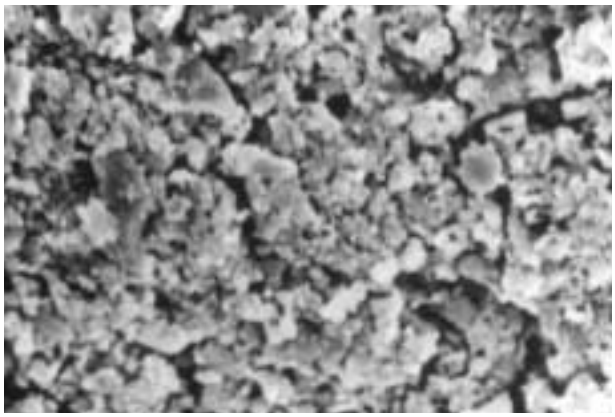

Figure 1.

Figure 2.

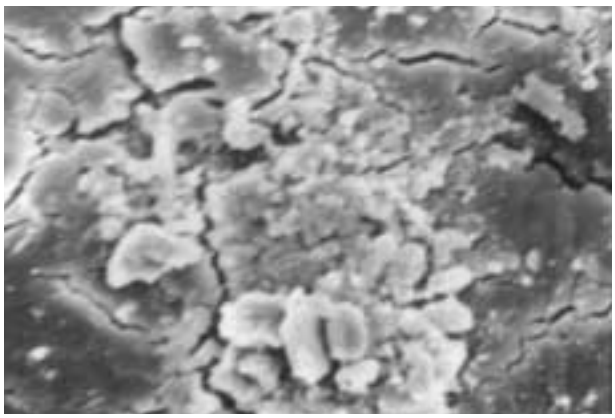

Figure 3.

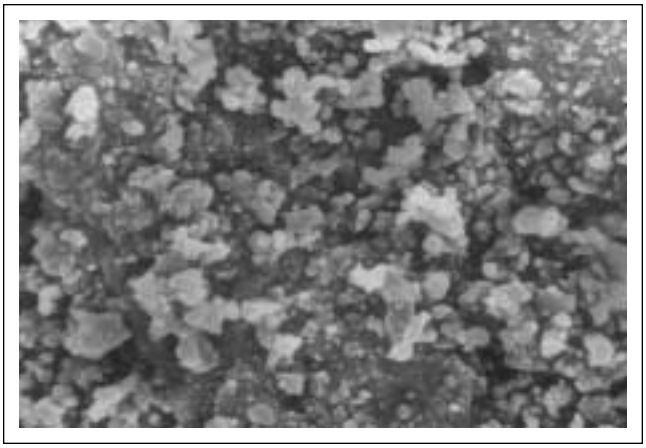

Figure 4.

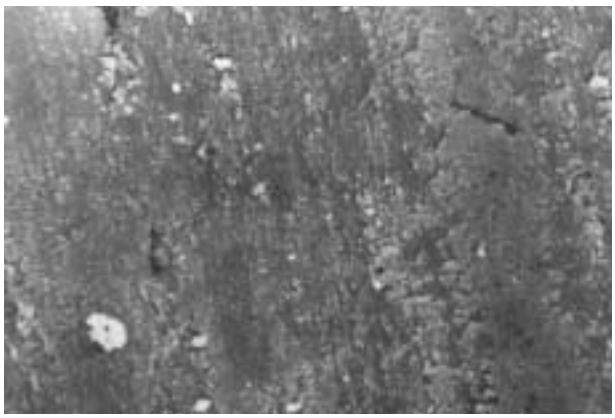

Figure 5.

Figure 6.

Figure 7.

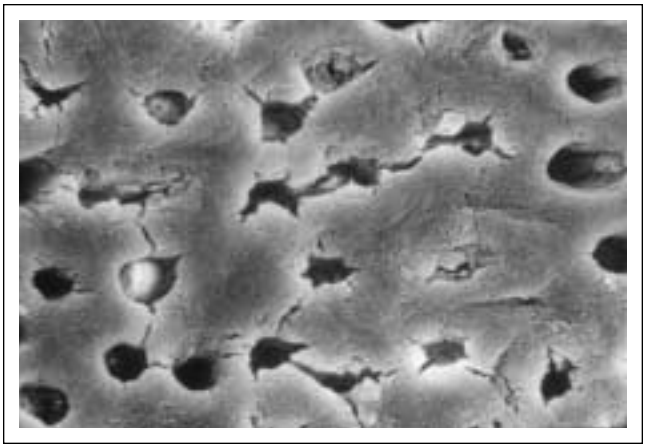

Figure 8.

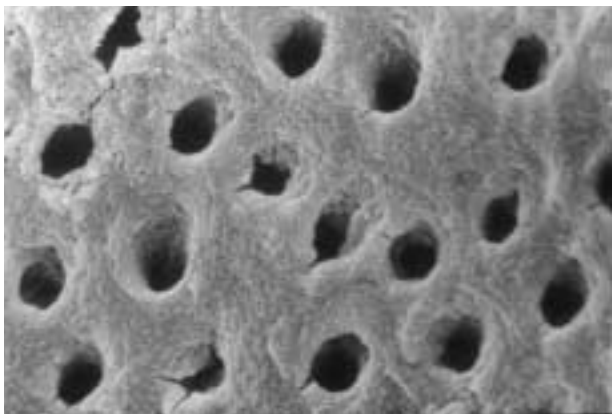

Figure 9.

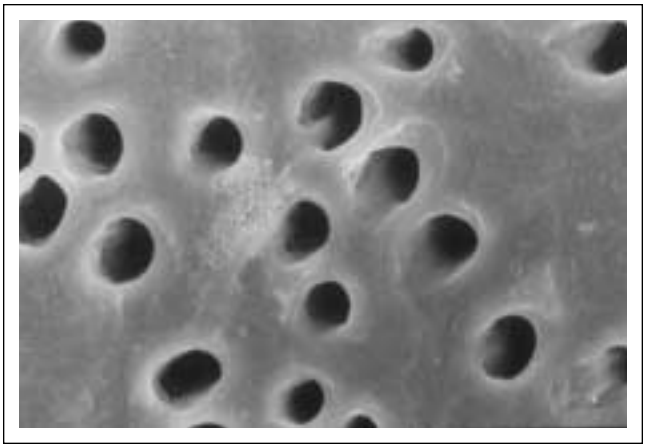

Figure 10.

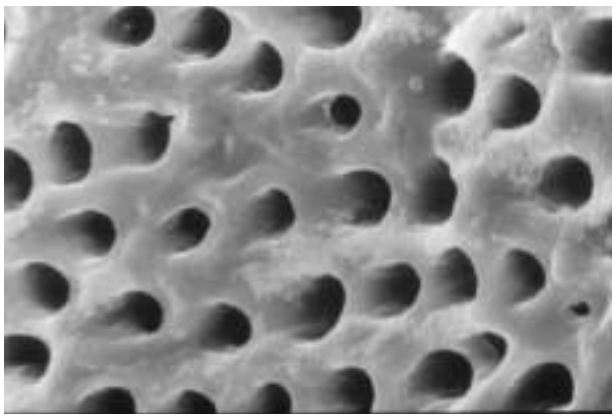

Figure 11.

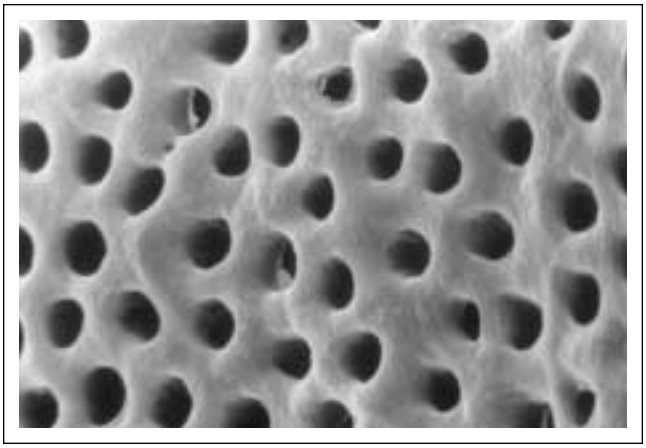

Figure 12.

Table 4.

Sl No.	Comparison of Groups	Apical		Middle		Coronal	
		Z	P	Z	P	Z	P
1.	Group 1 vs Group 2	0.000	1.000+	0.000	1.000+	2.803	0.005!
2.	Group 1 vs Group 3	3.317	0.001*	3.146	0.002!	3.317	0.001*
3.	Group 1 vs Group 4	3.317	0.001*	3.146	0.002!	3.207	0.001*
4.	Group 2 vs Group 3	3.317	0.001*	3.146	0.002!	3.207	0.001*
5.	Group 2 vs Group 4	3.317	0.001*	31.460	0.002!	2.762	0.006!
6.	Group 3 vs Group 4	3.317	0.001*	3.317	0.001*	2.138	0.033#

+ - Not significant
# - Significant
! - Highly significant
* - Very highly significant

Group 4. (17% EGTA and 5% NaOCl)
The specimens which were irrigated with 17% EGTA and 5% NaOCl had a score of 0 in all the sections, except in one coronal section, where a score of 2 was obtained. This irrigation combination produced a startlingly clean surface with all the patent dentinal tubules open. Additionally, it could be seen that there were no conjugation of the dentinal tubules when compared with that of group 3 and neither were there any signs of erosion. (Figures 10 to 12)

STATISTICAL RESULTS

Mann-Whitney 'U' Test was used for intergroup comparison and the results are depicted in Table 4.

DISCUSSION

The main aim of this study was to compare the effects of EGTA and EDTA on the removal of the smear layer. The effectiveness of EDTA as a chelating agent in root canal therapy has been in the thick of controversy ever since it was first recommended by Nygaard and Ostby[14] who concluded from clinical and histological investigations that a chelating agent facilitated root canal enlargement.

The present study with the scanning electron microscopic pictures at all the three levels, i.e., at apical, middle and coronal areas demonstrated the presence of plenty of hard and soft tissue debris in the specimens irrigated with normal saline. This is in agreement with the study of Baumgartner *et al*.[5] where they reported a typical amorphous smear layer seen consistently on the instrumented halves and residual superficial pulpal fibers, collagen fibers of the predentin on the uninstrumented halves when the specimens were irrigated with saline.

In the group 2 where the specimens were irrigated with 5% NaOCl, there was obscurity of the dentinal tubules, although the superficial soft tissue debris was very minimal. NaOCl although being potent and an effective tissue dissolvent, it produced canal with minimal amount of superficial debris and was unable to remove the smear layer. The present study is in confirmation with that of Lester *et al*.[15] where they reported that 5% NaOCl could not remove the unmineralized predentin.

It was known that irrigation with EDTA solutions alone cannot remove the smear layer completely[16,17] and the best results can be obtained after irrigation with 10 ml of EDTA followed by 10 ml of NaOCl solution.[16,17]

From the observations of this study, the efficacy of EDTA and NaOCl regimen were equally good for all the three sections. This irrigation regimen produced clean pulp canal walls and least amount of smear layer. The results of our study are in accordance with the results obtained by Goldman *et al*.,[18] where efficacy of EDTA and NaOCl regimen was good in the coronal and middle layer.

Calt *et al*.[19] observed the conjugation of dentinal tubules and erosion of both peritubular and intertubular dentin with EDTA and NaOCl regimen. Similar observations were noted in the present study.

In the group 4, the irrigation combination of 17% EGTA and 5% NaOCl produced the cleanest pulp canal walls and all the patent dentinal tubules were visible, even at the apical regions of the canal. The present study is in accordance to the earlier study of Calt *et al*.[19] where they had reported that the irrigation regimen of EGTA and NaOCI does not cause any conjugation of the dentinal tubules, no erosions, but at the same time, efficiently removes the smear layer.

From the present observation, the action of EGTA was very effective even in the inaccessible and important apical third, contrary to the study done by Calt *et al*,.[19] It was still not clear whether the erosion and joining of the orifices from the action of EDTA is deleterious. Even though this suggests the stronger action of EDTA, EGTA can also be used as an effective root canal irrigants.

SUMMARY AND CONCLUSIONS

1. Normal saline solution did not remove the smear layer.

2. Five (5%) NaOCl, even though it had a high solvent action, it was ineffective in removing the smear layer.
3. Seventeen (17%) EDTA followed by 5% NaOCl produced a clean pulp canal wall with the opening of the patent dentinal tubuli though they caused conjugation of the tubules and erosion of both peritubular and intertubular dentin.
4. Seventeen (17%) EGTA followed by 5% NaOCl produced a remarkably clean surface of the pulp canal wall and was the most effective debridement regiment.

The results from the present study suggest that the sequential irrigation of the pulp canal walls with EGTA followed by NaOCI produced efficient and total smear free root canal walls. EGTA can be used as an alternative to EDTA in conventional root canal debridement. Further investigations regarding *in vivo* use are warranted.

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