

Original Article**A Scanning Electron Microscopic Evaluation of the Effectiveness of Etidronic Acid, SmearClear and MTAD in Removing the Intracanal Smear Layer**Hemant Kumar Yadav¹, Rakesh Kumar Yadav², Anil Chandra³, Aseem Prakash Tikku³¹ Senior Resident, Division of Conservative Dentistry and Endodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, India.² Additional Professor, Dept. of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India.³ Professor, Dept. of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India.**KEY WORDS**Etidronic Acid;
MTAD;
SmearClear;
Smear Layer;
Scanning Electron
Microscope;**ABSTRACT****Statement of the Problem:** Root canal therapy should not simply be the extirpation of the pulp and widening of the canal. But one should also focus on how to completely remove the loosely-attached smear layer because it has adverse effects on the final outcome of the treatment.**Purpose:** This study compared the efficacy of Etidronic acid, SmearClear and MTAD to remove the smear layer created during instrumentation in different regions of the root canal.**Materials and Method:** Fifty single-rooted mandibular premolars were decoronated from the cemento-enamel junction and instrumented using the ProTaper universal rotary file system along with copious irrigation by 1.0% sodium hypochlorite and distilled water. On the basis of the type of chelating agent used for irrigation, samples (n=10) were then randomized into five groups as: Group I- 9% etidronic acid, Group II- 18% etidronic acid, Group III- SmearClear, Group IV- MTAD and Group V- normal saline. Subsequent to irrigation, all samples were rinsed, dried and sectioned longitudinally for evaluation of the smear layer removal under scanning electron microscope (2000X). Data were statistically analyzed by two-way analysis of variance and Tukey's post hoc test with statistical significance set at $p < 0.5$.**Results:** The result showed that SmearClear was the most efficient in removing the smear layer. However, etidronic acid was found inferior than both SmearClear and MTAD.**Conclusion:** Chelators are essential for complete smear layer removal in association with organic solvent.**Corresponding Author:** Hemant Kumar Yadav, Division of Conservative Dentistry and Endodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, India.
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An inevitable consequence of any hand or rotary instrumentation is the generation of substantial amount of debris shattered from the mineralized tissues. This forms a nonhomogenous structure, called the smear layer, on the walls of the cavity and root canal. The

smear layer consists of inorganic and organic components such as dentin, remnants of odontoblastic processes, pulpal tissue and bacteria. [1] Despite controversies regarding smear layer removal, the general accord is that the smear layer has adverse effect on the final outcome because it is a potential avenue for microleakage,

harbors microorganisms, reduces dentin permeability, compromises adequate disinfection by limiting the diffusion of endodontic disinfectants inside dentinal tubules and a fluid tight seal by acting as a barrier between the obturating materials and canal walls. [2] Therefore, root canal therapy should not simply be the extirpation of the pulp and widening of the canal. But one should also focus on how to completely remove the loosely-attached smear layer.

Various methods such as laser, ultrasonic, numerous chemicals and their combinations have been tested for complete smear layer removal (both organic and inorganic phases), while none of which are completely efficient along the whole canal length or used unani- mously. [2-3] However, the use of chemical irrigation is frequently considered as the method of choice to re- move the smear layer. [4] Although sodium hypo- chlorite (NaOCl) has excellent antimicrobial action and the capacity to dissolve organic materials, it alone is not completely effective to remove the smear layer from the instrumented canal walls and to prevent the accumula- tion of hard tissue debris in uninstrumented areas, espe- cially the ramifications. [4] Therefore, chelating agents such as ethylenediaminetetraacetic acid (EDTA) are recommended as an adjunct for removal as well as pre- vention of generation of the smear layer on the canal walls. [1, 4-5]

Although EDTA has been used since 1957 as an effective agent for softening of the root dentin, remov- ing the inorganic smear layer and increasing dentin permeability in different concentrations and formula- tions at different time periods, [2, 6] it causes dentinal erosion and has limited antibacterial activity. [3] There- fore, several alternatives to EDTA, such as EDTAC (EDTA + cetavlon); EDTA-T (EDTA + anionic deter- gent), CDTA, EGTA, citric acid, and so on have been investigated to assess their calcium complexing and disinfecting efficiency. [7-8]

1-hydroxyethylidene-1,1 bisphosphonat (HEBP or etidronic acid) is a highly biocompatible bisphosphonate and has been tried as a potential alternative to EDTA or citric acid, and shows no short-term reactivity with so- dium hypochlorite. [9-10] SmearClear (SybronEndo; Orange, CA) is a 17% EDTA solution containing a cati- onic (cetrimide) and an anionic surfactant. It has also been investigated as an effective smear layer removing

and root canal cleansing agent in some previous studies. [11-13] BioPure MTAD (Dentspl;, Tulsa Dental, Tulsa, OK, USA), a biocompatible material [14] has been found as effective as EDTA in solubilizing the pulp and dentin. [15] A previous study [16] has tested the afore- mentioned agents for removing calcium ions from the root canal which is advantageous for the inorganic smear layer removal as well as negotiation and instru- mentation of fine calcified canals. However, the effect of these agents in a particular region of the root canal (coronal, middle , and apical), especially the apical one from which calcium ions preferably elute, could not be inferred. Therefore, this *ex vivo* scanning electron mi- croscope (SEM) study was conducted to assess the ef- fectiveness of 9% etidronic acid, 18% etidronic acid, SmearClear and MTAD in removing the smear layer from the coronal, middle and apical third regions of the instrumented root canal.

Materials and Method

Sample selection and preparation

Fifty recently extracted human mandibular premolars were collected from oral and maxillofacial surgery de- partment. They were cleaned free of debris and calculus and then stored in 0.5ml of thymol solution until used. Teeth with complete root formation, patent single canals and without anatomic variations and resorption were included in the study. Teeth having curved root and calcified canal were not included in the study. All teeth were decoronated from the cementoenamel junction (CEJ) with a low-speed rotary diamond disk (90 µm; Microdont, Brazil) under coolant water to standardize the root length and to simulate similar conditions. The working length was established by subtracting 1 mm from the length recorded until a #10 or #15 K-file (Dentsply Maillefer; Ballaigues, Switzerland) intro- duced inside the canal became just visible at the apex. Root canal preparation was accomplished with the rota- ry system (ProTaper Universal; Dentsply Maillefer, Ballaigues, Switzerland) and torque control electric motor (X-Smart endodontic motor; Dentsply Interna- tional, Inc) according to manufacturer's instructions till master apical file # F5 for efficient debridement and disinfection. Each instrument was used for preparing only the four canals. During instrumentation, each sam- ple was copiously irrigated with 1.0% sodium hypo-

chlorite at each instrument change. Subsequently, all samples were rinsed with 20 ml of triple distilled water (Milli-Q water purification system; Merck Millipore India Private Limited, Bengaluru, India) for removal of possible dentin chips.

Test chelating solutions

Depending upon the type of chelating solution used, all samples (n=50) were then randomly distributed into five (four experimental and one negative control) groups (N=10) as follows: Group I- 9% etidronic acid, Group II- 18% etidronic acid, Group III- SmearClear, Group IV- MTAD and Group V- normal saline. 9% and 18% etidronic acid solutions were prepared from 60% aqueous solution of etidronate (Sigma–Aldrich; Bengaluru, India) by adding triple distilled water.

Scanning electron microscopy

All samples in each group were irrigated with 5 ml of the concerned chelating solutions for 5 min (1ml/min) using 30 gauge needles, attached to the Luer Lock syringe. Irrigation was done by inserting the needle inside the canal apically 1 to 2 mm short of the working length. After this, all specimens were rinsed with 5 ml of triple distilled water to further neutralize the action of each chelating solution and to remove any possible precipitate formed. All samples were then dried with sterile absorbent paper points, and grooved longitudinally in a buccolingual plane with a slow speed diamond disc

without penetrating the canal to facilitate their vertical splitting. Subsequent to grooving, all samples were split into two halves using a chisel and mallet. Further, each half of all samples were coded, secured on metallic (aluminum) stubs, dried in the critical point dryer, sputter coated with gold and observed under scanning electron microscope (DSM 950; Carl Zeiss, Oberkochen, Germany). After general evaluation of the canal wall, SEM photomicrographs at the center of coronal, middle and apical regions of each sample were taken in a backscatter mode at magnification of 2000X (Figure 1-5).

Each photomicrograph was evaluated qualitatively for the amount of smear layer remaining on the canal wall according to the scoring system codified by Spano *et al.* [17] as 1 for no smear layer (0%), 2 when a few areas (nearly 33.3%) are covered by smear layer and many dentinal tubules are visible, 3 when most of the areas (nearly 66.6%) are covered by smear layer, and only a few dentinal tubules are visible; and 4 when all areas (100%) are covered by smear layer, and dentinal tubules are not visible.

All photomicrographs were scored independently by an examiner who was not aware of the coding system in order to avoid observer bias. Scoring was repeated twice to make sure intraexaminer uniformity.

Statistical analysis

The smear layer score of all groups was compared by

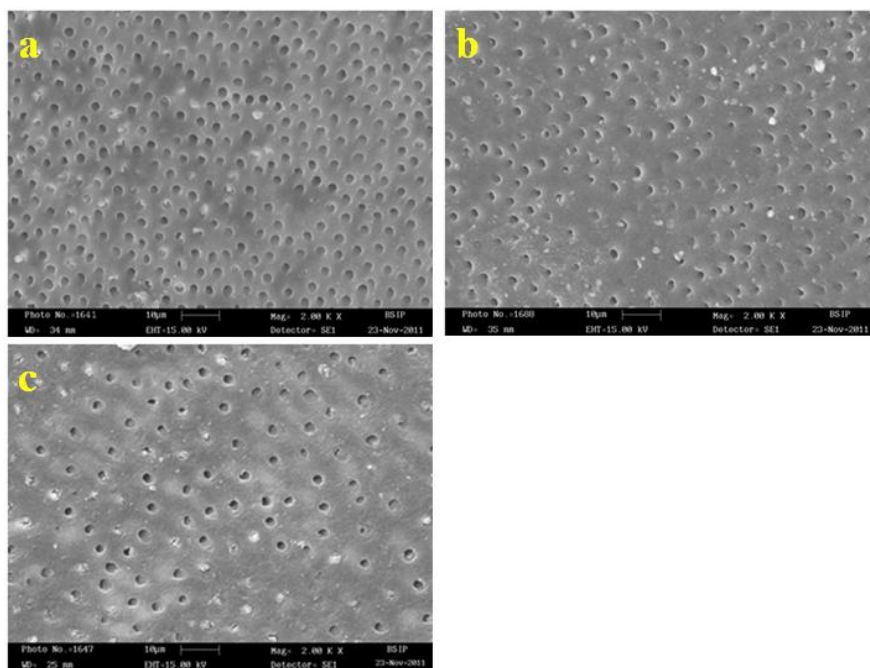


Figure 1: SEM Microphotographs (2000×) of the dentinal surfaces of the samples treated with 9% etidronic acid at **a:** coronal **b:** middle and **c:** apical third regions.

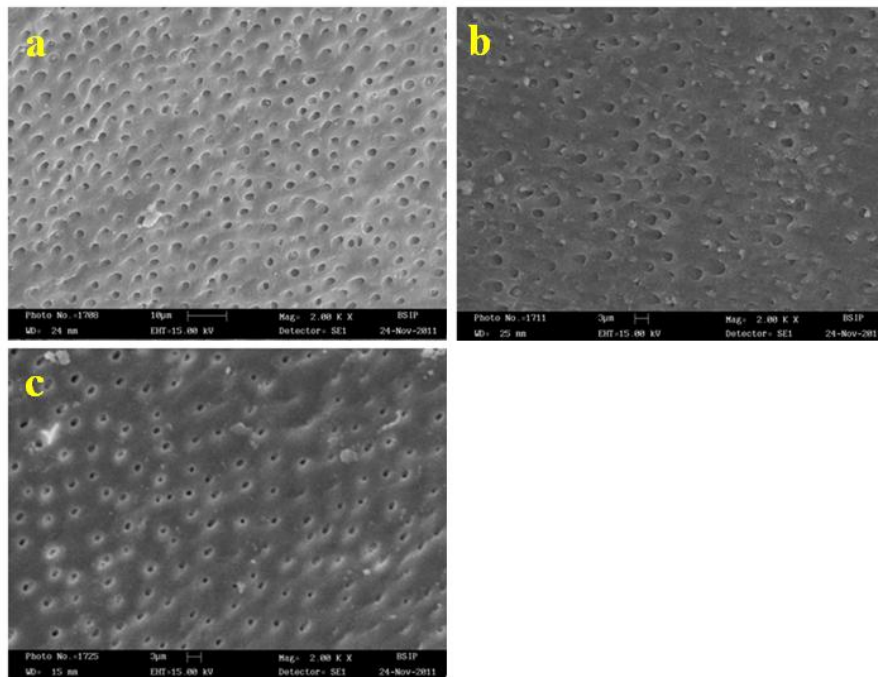


Figure 2: SEM Microphotographs (2000 \times) of the dentinal surfaces of the samples treated with 18% etidronic acid at **a:** coronal **b:** middle and **c:** apical third regions.

two-way (root canal regions and groups) analysis of variance (ANOVA) using general linear models (GLM) and the significance of mean difference within and among the groups was done by Tukey's post hoc test after transforming the score by square root transformation [$\sqrt{\text{score} + 0.5}$]. A two-tailed ($\alpha=2$) $p < 0.05$ was considered statistically significant. All analyses were carried out on GraphPad Prism version 5.0 for Windows (GraphPad software, LaJolla, CA, USA).

Results

The smear layer score of five groups at three different root canal regions is summarized as Mean \pm SD in Table 1. Among groups, SmearClear showed least smear layer score followed by MTAD, 18% etidronic acid, 9% etidronic acid and normal saline. The smear scores of all experimental groups were found least in the coronal region followed by middle and apical region. The two-way ANOVA test revealed significant ($p < 0.001$) effect

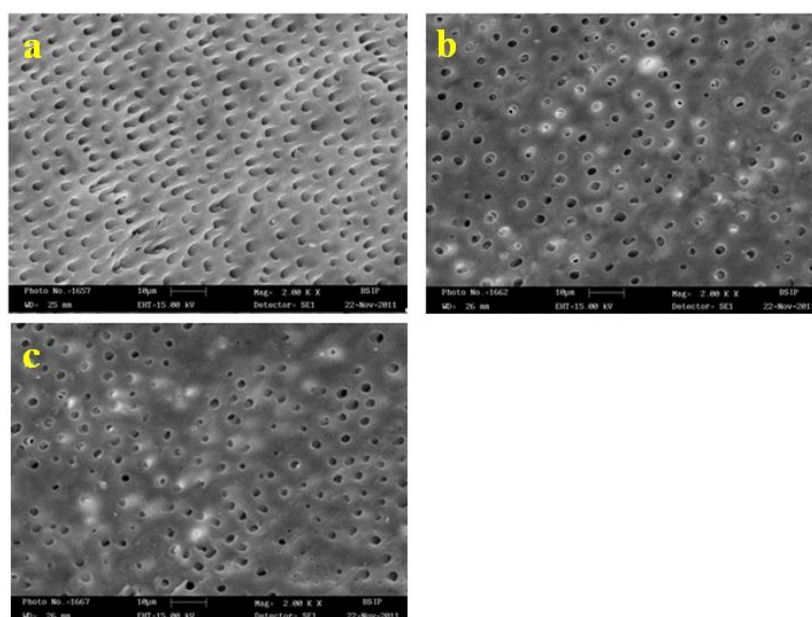


Figure 3: SEM Microphotographs (2000 \times) of the dentinal surfaces of the samples treated with SmearClear at **a:** coronal, **b:** middle and **c:** apical third regions.

Table 1: Smear layer score (Mean \pm SD, n=10) of all groups in coronal, middle and apical regions of the root canal.

Groups	Root Canal Regions		
	Coronal	Middle	Apical
9% Etidronic Acid	1.80 \pm 0.63 ^{A, a}	2.60 \pm 0.52 ^{A, B, a}	3.20 \pm 0.92 ^{B, C, a}
18% Etidronic Acid	1.50 \pm 0.53 ^{A, a}	2.00 \pm 0.67 ^{A, a, b}	3.00 \pm 0.82 ^{B, a, c}
SmearClear	1.20 \pm 0.42 ^{A, a}	1.40 \pm 0.52 ^{A, b}	1.50 \pm 0.53 ^{A, b}
MTAD	1.30 \pm 0.48 ^{A, a}	1.60 \pm 0.52 ^{A, B, c, b}	2.20 \pm 0.42 ^{B, C, c, b}
Normal Saline	3.80 \pm 0.42 ^{A, b}	3.90 \pm 0.32 ^{A, d}	4.00 \pm 0.00 ^{A, a}

Means followed by the similar superscript lowercase letters in columns indicate no statistically significant differences ($p > 0.05$) among groups for each root canal region. However, dissimilar superscript lowercase letters in columns indicate statistically significant differences among groups for each root canal region ($p < 0.05$)

Means followed by the similar superscript uppercase letters in rows indicate no statistically significant differences ($p > 0.05$) among different root canal regions for each group. However, dissimilar superscript uppercase letters in rows indicate statistically significant differences among different root canal regions for each group ($p < 0.05$)

of groups and different root canal regions on smear layer. Further, the interaction effect of root canal regions and groups on smear layer was also found to be significant ($p = 0.006$).

The intergroup comparison of mean smear layer scores revealed no significant ($p > 0.05$) difference in smear scores among all experimental groups in the coronal region of the canal. In both coronal and middle regions, the smear scores were significantly ($p < 0.05$) different and lower for all experimental groups (I-IV) than normal saline (Table 1). SmearClear and MTAD groups showed significantly ($p < 0.001$) lower smear scores than control in the apical region. The smear layer scores for SmearClear and MTAD were also significantly ($p < 0.05$) lower than 9% etidronic acid in both middle and apical regions. Moreover, at apical site, SmearClear also showed significantly ($p < 0.001$) lower smear score as compared to 18% etidronic acid.

Intragroup comparison of smear layer scores between coronal and middle region revealed no statistically significant difference ($p > 0.05$) for all groups. 9% etidronic acid, 18% etidronic acid and MTAD showed significantly ($p < 0.05$) higher smear scores in the apical region as compared to the coronal. Further, 18% etidronic acid also showed significantly ($p < 0.05$) higher score in the apical region than the middle. However, SmearClear showed comparatively lower, but statistically non-significant ($p > 0.05$) scores in both coronal and middle regions than apical.

Discussion

During root canal instrumentation, the final apical enlargement or preparation size is still a matter of debate. [18] Therefore, all potential mechanisms should be explored for the best possible chemomechanical debridement of the canal to reduce microbial load. The strategy

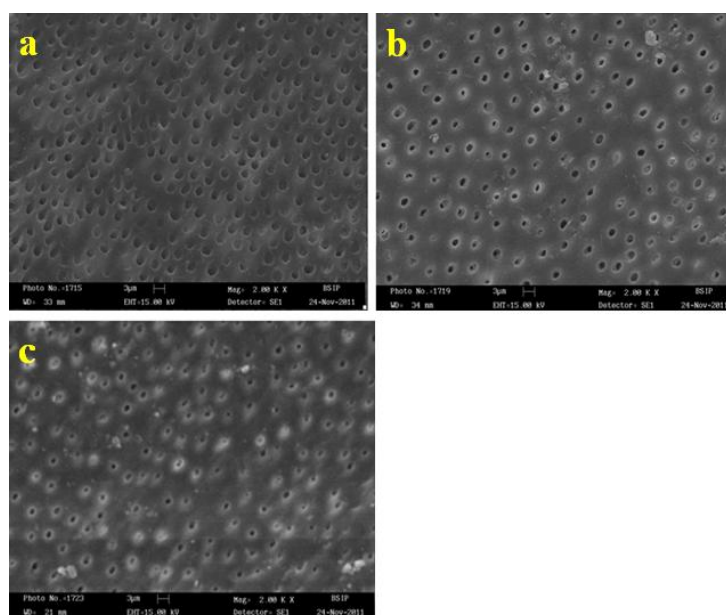


Figure 4: SEM Microphotographs (2000 \times) of the dentinal surfaces of the samples treated with MTAD, **a:** coronal, **b:** middle and **c:** apical third regions.

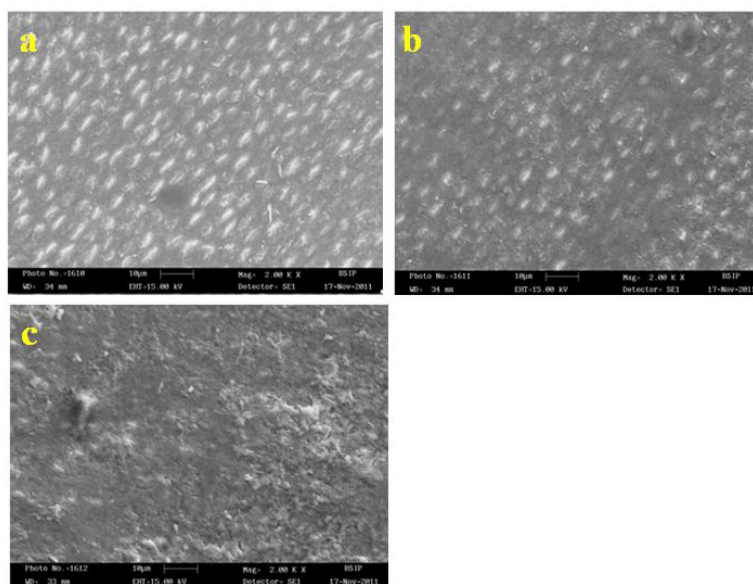


Figure 5: SEM Microphotographs (2000×) of the dentinal surfaces of the samples treated with normal saline at **a:** coronal, **b:** middle and **c:** apical third regions.

of wider apical preparation of the canal removes potentially infected dentin permitting deeper passive needle placement and subsequent irrigants penetration deeper inside the canal. [19-20] This facilitates better debridement and disinfection of canals. However, other studies found no significant difference in eradicating microorganisms during canal preparation with or without apical enlargement. [21-22] Despite controversy over the final apical preparation size, these studies suggest that the preparation should be sufficiently wide, confined to the canal space, and incorporate its original cross sections. Therefore, in the present investigation, canals were prepared till master apical file # F5 to achieve optimum debridement and disinfection.

Currently, there is no consensus regarding standardization of the scoring measurements of debris and smear layer. [23] Different areas of coronal, middle and apical regions of each sample were chosen randomly for SEM examination at 2000X magnification and scored as per Spano *et al.* [17] In this study, all the experimental groups (Group I-IV) have shown a definite decline in removing the smear layer along the entire canal length which is in concord with the results of some previous studies. [3, 24] This may be because of much more sclerosed apical root dentin. Dentin is a heterogeneous structure and get sclerosed with aging due to physiological deposition of increasing amounts of peritubular dentin which creates difficulty in identification of dentinal tubules. [25] Further, the larger canal diameter

in the coronal 2/3rd region exposes the dentin to a higher volume of irrigants, which permits better flow of the solution and, hence, the smear layer removing efficacy.

The extent of smear layer removal by any compound is directly linked to its pH, relationship between the amount of available active substance (chelator) and the canal wall surface area, diffusion in the dentin, hardness of dentin, root length and the application time because the demineralization process continues until all chelating agents have formed complexes with calcium. [26-27] Various studies have reported a wide range of time period from 30 seconds to 15 minutes required for the action of these solutions; mostly between 1 and 5 minutes. [5, 27] We have used 5 ml of each irrigating solution for 5 minutes; a substantial time and volume of irrigation. Calt and Serper [27] has been advocated that irrigation during root canal therapy with strong chelating agents such as EDTA for more than 1 minute has an erosive effect on dentin. However, the authors found no such detrimental effects on dentin even after 5 minutes of irrigation with the chelating solutions used in the present study. This may be either due to disparity in sample selection, experimental arrangement or both.

The overall performance of etidronic acid to remove the smear layer was found inferior to SmearClear and MTAD. This may be due to its weaker chelating property. [28] For any new material to be clinically used, its potential adverse effects are the main concern. Etidronic acid is a biocompatible material and has been

used in treating osteoporosis and osteolytic diseases of jaws. [10] Etidronic acid is also used in swimming pools to prevent stains from metal ions because of its compatibility with hypochlorite. [10] In the present study, 18% etidronic acid was found to be more efficient than 9% etidronic acid. This is probably due to differences in concentration gradient and stability constant of the etidronic acid -calcium complex. [29] 9% etidronic acid was found to have smear layer removal efficacy in the coronal region as equal to that of other experimental groups. But in the apical region, it removed less smear layer when compared with SmaerClear and MTAD. This might be because of the lesser chelating action of etidronic acid on sclerosed dentin in apical regions.

To facilitate effective debris and smear layer removal, the contact of an irrigants on a solid surface (dentin walls) is essential and directly correlates to its surface tension. [30] Abou-Rass and Patonai [31] proved that reduction of surface tension of an irrigant enhanced its flow inside the main canal, accessory canals, ramifications and the dentinal tubules. These views are in support of the present investigation, where SmearClear had shown better smear layer removal than other chelators used. This may be because of its low surface tension (33mJ/m²) due to the presence of additional surfactants. However, our result is in contrast to the findings of Khedmat *et al.* [12] who used 1 ml of SmearClear for 1 minute followed by 3ml of 5.25% NaOCl as final irrigant. Moreover, some other studies have also stated reducing surface tension of an irrigant did not affect its chelating ability. [6, 11, 28] This may be due to difference in experimental design, concentration of chelators used and their application time.

The exact mechanism of action of MTAD in removing the smear layer and killing microbes is not clearly well known. It has been stated that its smear layer removing capacity, is due to its doxycycline and citric acid component. These components have been separately reported as competent agents in removing the smear layer. [32] In the present *ex vivo* study, MTAD was found as efficient to remove the smear layer as SmearClear except in the apical region where it was slightly inferior, but not statistically different. This is probably due to lesser surfactants in MTAD. Torabinejad *et al.* [3] reported that MTAD was superior to EDTA in debris removal in the apical regions with min-

imal erosive changes in the structure of dentin. However, Tay *et al.* [33] showed that MTAD was more aggressive in dentin demineralization and exposed about 2 times thicker layers of collagen matrices than EDTA. The extra added advantage of MTAD is its antimicrobial effect due to the bacteriostatic effect of the doxycycline which exerts its effect through inhibition of protein synthesis. This eliminates the risk of release of antigenic endotoxins. [34]

The present investigation was carried out in an *in vitro* environment. Therefore, the results obtained may necessarily not be extrapolated in clinical scenarios. Blood, tissue remnants, temperature, various delivery and agitation devices may affect the actions of chelating agents used during the root canal instrumentation. The highly unpredictable result could be due to dentinal sclerotic changes and root canal length, its diameter, apical topography, curvature as well as its final apical enlargement which impedes the effective flow of irrigants inside the canal. Further methodically sound *in vitro* studies and clinical trials should be carried out to confirm their comparative effectiveness in endodontic therapy.

Conclusion

Based on the outcomes and limitations of this *ex vivo* investigation, SmearClear was found most efficient in removing the smear layer in all regions of the root canal followed by MTAD, 18% etidronic acid and 9% etidronic acid. Etidronic acid was not effective in cleaning the apical region of the canal. Efficacy of cleanliness of the canal, especially in apical region was statistically similar between 9% and 18% etidronic acid, and SmearClear and MTAD.

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Conflicting Interest

Authors have declared no conflict of interest.

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