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# Comparative evaluation of root canal dentin erosion after using different smear layer removing solutions for root canal irrigation by scanning electron microscope (SEM) and energy dispersive x-ray spectroscopy (EDS): An *in-vitro* study

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**Abstract**
**Objective:** To evaluate and compare the effect of different final irrigating solutions (17% EDTA, Smear Clear, QMix and BioPure MTAD) on root canal dentin erosion in the coronal, middle and apical third of root by Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS).
**Materials and Methods:** A total of 105 recently extracted mandibular premolars were taken and decoronated to a standardized root length of 12mm. They were prepared using ProTaper system up to size F3. Initial rinse was done with sodium hypochlorite. After completion of root canal preparation, all root canals were subjected to final irrigation protocol. Group 1 was irrigated with 17% EDTA for 1 min. Group 2 was irrigated with Smear Clear for 1 min. Group 3 was irrigated with QMix for 1 min. Group 4 was irrigated with MTAD for 5 min. Group 5 was irrigated with distilled water. Out of 21 samples in each group, 10 were analysed by SEM and 11 were analysed by EDS.
**Results:** The results of the SEM analysis revealed that the use of 17% EDTA and Smear Clear resulted in significantly more erosion than the use of QMix and MTAD. No statistically significant difference was observed at the coronal, middle and apical thirds in the amount of dentin erosion. The results of EDS analysis revealed that all treatment groups significantly decreased calcium content in comparison to control group. However, no significant difference was observed among EDTA, Smear Clear, Qmix and MTAD in decreasing calcium levels in dentin.
**Conclusion:** BioPure MTAD and QMiX cause negligible dentin erosion and thus can be used as alternative final irrigating solutions after sodium hypochlorite instead of EDTA and Smear Clear.

**Keywords:** Smear layer, dentin erosion, EDTA, smear clear, BioPure MTAD, QMiX

## Introduction
Bacteria have long been recognized as the primary etiologic factors in the development of pulp and periapical lesions [1, 2]. The success of root canal treatment depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. Instrumentation of the root canal system produces an amorphous, irregular surface layer called the "smear layer" that covers the canal walls [3]. This layer contains inorganic and organic materials such as vital pulp tissue, odontoblastic processes, necrotic debris, and microorganisms and their metabolic products [4]. The total removal of the smear layer is preferred in order to improve the adaptation of the obturation materials in the root canal dentin, decrease apical and coronal microleakage and facilitate the diffusion of the irrigant solutions and intracanal medications into the root canal system [5].
Various chemical irrigants have been used to remove the smear layer. Sodium hypochlorite (NaOCl) in concentrations from 0.5% to 6% is the most commonly recommended irrigating solution. It has strong antibacterial and tissue dissolving effects [6]. However, it has no effect on the inorganic part of the smear layer [5]. The inorganic portion of the smear layer is removed by the use of decalcifying agents [7].

The most commonly used chelating solution is EDTA (ethylenediaminetetraacetic acid). However, it has no bactericidal activity [8]. Syringe needle irrigation with NaOCl (0.5–6.15%) followed by a final rinse with EDTA (15-17%) is the recommended protocol for endodontic irrigation [9].

Studies have shown that alternate use of NaOCl solution and EDTA can lead to intermittent erosion of the canal walls [10]. Dentinal erosion is the extensive loss of intertubular and peritubular dentin that is characterized by the widened and interconnected tubular orifices [10]. The decrease in mineral content of dentin also results in weakening of structural and physical properties of dentin such as elastic modulus, flexural strength and fatigue strength which can lead to late complications such as vertical root fracture [11, 12]. Use of chelating agents also results in alteration of the chemical composition of dentin by removal of major inorganic elements such as calcium ions ($Ca^{2+}$) present in the hydroxyapatite crystals. Changes in the $Ca^{2+}$ ratio changes the permeability, microhardness and solubility of root canal dentin and may also adversely affect the sealing ability of resin-based cements and sealers to root canal dentin [13, 14, 15].

Recent years have seen various modifications in the composition of the irrigating solutions in an attempt to improve the cleaning efficiency, to supplement the antimicrobial action and to decrease dentin erosion to permissible level. One of such agents is BioPure MTAD (Dentsply Tulsa Dental Specialties, Johnson City, TN) which is a mixture of 3% tetracycline isomer (doxycycline), 4.25% citric acid, and 0.5% detergent [16]. It has been recommended as a final rinse after sodium hypochlorite for effective smear layer removal [17].

Smear Clear (SybronEndo, Orange, CA) is another final irrigation solution that contains 17% EDTA, cetrimide, and surfactant. The rationale of adding a surfactant is its ability to lower surface tension of solutions and increase their wettability [18].

QMix 2 in 1 (Dentsply Tulsa Dental, Tulsa, OK, USA) introduced in 2011, is a novel irrigant solution with antimicrobial agents for the smear layer removal, proving to be as effective as 17% EDTA [19]. Its chemical composition contains EDTA, chlorhexidine, and a specific detergent [20]. It is recommended after the use of sodium hypochlorite (NaOCl) during root canal instrumentation.

MTAD, Smear Clear and QMix are relatively new irrigating solutions and their effect on dentin erosion has not been established. Extensive review of literature has shown paucity of studies that compare the effect of final irrigation with EDTA, Smear clear, QMix and Biopure MTAD on amount of dentin erosion and calcium content of root canal dentin. The aim of the present study was to evaluate the root canal dentin erosion after using different smear layer removing solutions for root canal irrigation by Scanning Electron Microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS).

## Materials and Methods

The present study was carried out in the Postgraduate Department of Conservative Dentistry And Endodontics, Government Dental College and Hospital Srinagar. A total of 105, single-rooted human mandibular premolar teeth having a single canal and fully developed apices extracted for orthodontic reasons were selected for the study. The teeth were disinfected in 5% sodium hypochlorite solution for 30 minutes. They were cleaned of soft tissue tags and debris with ultrasonic scaler and kept in normal saline until used. The teeth were decoronated to a standardized root length of 12 mm with a diamond disc. The working length of each specimen was measured by deducting 1 mm from length recorded when the tip of #15 K-file (DENTSPLY Maillefer) was just visible at the apical foramina. All apices of the root were sealed with wax to simulate clinical conditions.

Before root canal preparation, all the roots (n=105) were randomly divided into five groups (n=21) according to the solution to be used in the final rinse protocol: Group 1 (EDTA), Group 2 (Smear Clear), Group 3 (QMix 2 in 1), Group 4 (BioPure MTAD) and Group 5 (Control). The root canals were then instrumented with the ProTaper (DENTSPLY Maillefer) rotary file system up to F3 file. Between each file, canals were irrigated with 2 ml of 5% NaOCl, except for the roots in the MTAD group, where 1.3% NaOCl was used (manufacturer' recommendation) [21].

**All groups were then subjected to final irrigation protocol as follows**

**Group 1 (EDTA):** irrigation with 5 ml of 17% EDTA for 1 min.

**Group 2 (Smear Clear):** irrigation with 5 ml of Smear Clear for 1 min.

**Group 3 (QMix 2 in 1):** irrigation with 5ml of QMix for 1 min.

**Group 4 (BioPure MTAD):** irrigation with 5 ml of MTAD for 5 min.

**Group 5 (Control):** irrigation with distilled water for 1 min.

Out of 21 samples in each group, 10 were analysed by SEM for evaluation of dentin erosion and 11 were analysed by EDS for evaluation of calcium content.

### Scanning Electron Microscope Analysis

Ten specimens from each group were prepared for SEM analysis (n= 50). Two longitudinal grooves were prepared on the buccal and lingual surfaces of each root using a diamond disc, avoiding penetration into the canal. The roots were then split longitudinally with a bi-bevelled chisel and a mallet in corono-apical axis, exposing the entire root canal. One half of each root was selected depicting the entire root canal length and prepared for scanning electron microscope examination. SEM photomicrographs were then taken at magnification of 4000× at 15kV in the coronal third (9mm from apex), middle third (6mm from apex), and apical third (3mm from apex). Three calibrated examiners viewed the SEM photomicrographs, analyzed independently and in a blind manner, scored the degree of erosion of the dentinal tubules according to the criteria used by Torabinejad *et al*. [22].

Score 1 = No erosion, All tubules look normal in appearance and size.

Score 2 = Moderate erosion, Peritubular dentin is eroded.

Score 3 = Severe erosion, Intertubular dentin is destroyed, and tubules connected to each other.

### Energy Dispersive X-Ray Spectroscopy (EDS) Analysis

After the final rinse protocol, 11 specimens from each group were prepared for EDS analysis. Three root dentin blocks with a thickness of approximately 1.5 mm were horizontally sectioned from each tooth at the coronal (9mm from apex), middle (6mm from apex) and apical third (3mm from apex) and prepared for EDS analysis. A total of 4 areas, toward the mesial, buccal, distal, and lingual surfaces, were selected and

area scanning function was used to determine the weight percentage of calcium in dentin. Levels of elemental calcium (Ca) were measured in weight percentage by EDS at a voltage of 15 kV and the mean value of calcium was calculated.

**Fig 1a:** Mounting of specimens for SEM

**Fig 1b:** EDS analysis on metallic stubs

## Results

Statistical analysis was performed using SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as Mean±SD. Analysis of variance (ANOVA) was employed for comparison of various parameters and for multiple comparisons, least significant difference (LSD) test was applied. A P-value of less than 0.05 was considered statistically significant.

**SEM Analysis:** The mean values of scores of degree of dentin erosion for 4 experimental groups at coronal, middle and apical levels are given in Table 1 and Table 2. SEM photomicrographs showed the presence of heavy smear layer in control group so the degree of erosion could not be evaluated in control group (Group 5). 17% EDTA (Group 1) and Smear Clear (Group 2) showed significantly higher peritubular dentin erosion as compared to QMix and MTAD ($P$<0.05) (Table 2). Dentinal tubule orifices were irregularly enlarged and rough in appearance (Fig. A and B). No significant difference was observed between 17% EDTA and Smear Clear in the amount of dentinal erosion ($P$>0.05). In QMix (Group 3) and BioPure MTAD (Group 4) the tubule openings were clearly visible and peritubular and intertubular dentin appeared smooth and flat with no erosive changes (Fig. C and D). Also there was no significant difference between QMix and MTAD in the amount of dentinal erosion. No significant difference was observed in the amount of erosion at coronal, middle and apical levels in all groups ($P$>0.05).

**EDS Analysis**: The mean values of calcium content of all groups at coronal, middle and apical levels are shown in Table 3 and Table 4. Calcium levels were significantly decreased after treatment with all irrigating solutions when compared with control group ($P$<0.05). No significant difference was observed between Group 1 (17% EDTA), Group 2 (Smear Clear), Group 3 (QMix) and Group 4 (MTAD) at coronal, middle and apical levels ($P$>0.05).

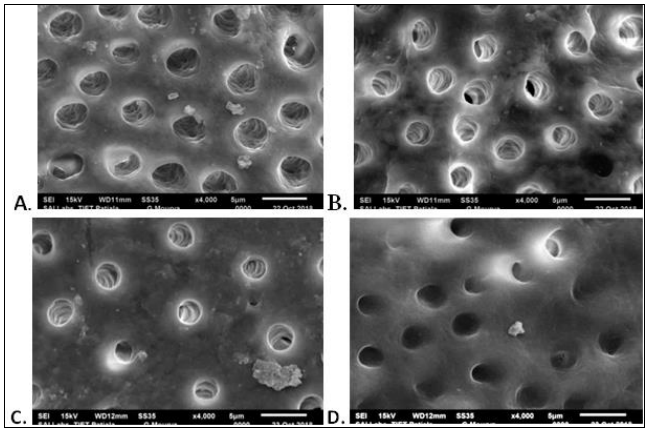


**Fig 2:** SEM images of root canal walls showing dentin erosion after final irrigation with (A) 17% EDTA (B) Smear Clear (C) QMix 2 in 1 (D) BioPure MTAD

**Table 1:** Intra-group comparison of dentin erosion

| | | Mean | SD | Min | Max | P-value |
|---|---|---|---|---|---|---|
| Group 1 | Coronal | 2.1 | 0.568 | 1 | 3 | 0.925 |
| | Middle | 2.0 | 0.667 | 1 | 3 | |
| | Apical | 2.0 | 0.707 | 1 | 3 | |
| Group 2 | Coronal | 1.8 | 0.422 | 1 | 2 | 0.761 |
| | Middle | 1.8 | 0.422 | 1 | 2 | |
| | Apical | 1.7 | 0.500 | 1 | 2 | |
| Group 3 | Coronal | 1.3 | 0.483 | 1 | 2 | 0.563 |
| | Middle | 1.2 | 0.422 | 1 | 2 | |
| | Apical | 1.1 | 0.316 | 1 | 2 | |
| Group 4 | Coronal | 1.1 | 0.316 | 1 | 2 | 0.612 |
| | Middle | 1.1 | 0.316 | 1 | 2 | |
| | Apical | 1.0 | 0.000 | 1 | 1 | |

**Table 2:** Intergroup comparison of dentin erosion among various groups

| Group Comparison | P-value | | |
|---|---|---|---|
| | Coronal | Middle | Apical |
| 1 vs 2 | 0.151 | 0.352 | 0.126 |
| 1 vs 3 | <0.001* | 0.001* | <0.001* |
| 1 vs 4 | <0.001* | <0.001* | <0.001* |
| 2 vs 3 | 0.019* | 0.008* | 0.010* |
| 2 vs 4 | 0.002* | 0.002* | 0.003* |
| 3 vs 4 | 0.334 | 0.641 | 0.623 |

*Statistically Significant Difference (P-value<0.05)

**Table 3:** Intra-group comparison of calcium content (Weight %)

| | | Mean | SD | Min | Max | P-value |
|---|---|---|---|---|---|---|
| Group 1 | Coronal | 16.54 | 1.262 | 14.92 | 19.13 | 0.220 |
| | Middle | 16.14 | 1.231 | 14.15 | 18.35 | |
| | Apical | 17.09 | 1.281 | 15.10 | 19.04 | |
| Group 2 | Coronal | 15.96 | 0.979 | 14.21 | 17.34 | 0.145 |
| | Middle | 15.77 | 0.903 | 13.98 | 17.25 | |
| | Apical | 16.60 | 1.105 | 14.81 | 17.87 | |
| Group 3 | Coronal | 16.59 | 0.686 | 15.39 | 17.35 | 0.143 |
| | Middle | 16.28 | 0.968 | 15.11 | 18.01 | |
| | Apical | 17.02 | 0.910 | 15.52 | 18.36 | |
| Group 4 | Coronal | 15.65 | 0.950 | 14.41 | 16.91 | 0.085 |
| | Middle | 15.35 | 0.968 | 13.92 | 16.91 | |
| | Apical | 16.23 | 0.758 | 15.11 | 17.42 | |
| Group 5 | Coronal | 20.11 | 1.722 | 17.63 | 22.76 | 0.081 |
| | Middle | 18.31 | 1.404 | 16.44 | 20.80 | |
| | Apical | 19.30 | 2.214 | 15.43 | 22.35 | |

**Table 4:** Intergroup comparison of calcium content (weight %) among various groups

| Group Comparison | P-value | | |
|---|---|---|---|
| | Coronal | Middle | Apical |
| 1 vs 2 | 0.256 | 0.443 | 0.395 |
| 1 vs 3 | 0.921 | 0.776 | 0.903 |
| 1 vs 4 | 0.084 | 0.103 | 0.139 |
| 1 vs 5 | <0.001* | <0.001* | <0.001* |
| 2 vs 3 | 0.217 | 0.295 | 0.466 |
| 2 vs 4 | 0.541 | 0.379 | 0.520 |
| 2 vs 5 | <0.001* | <0.001* | <0.001* |
| 3 vs 4 | 0.068 | 0.057 | 0.173 |
| 3 vs 5 | <0.001* | <0.001* | <0.001* |
| 4 vs 5 | <0.001* | <0.001* | <0.001* |

*Statistically Significant Difference (P-value<0.05)

## Discussion

The present study was conducted to evaluate the root canal dentin erosion at coronal, middle and apical third of root after using 17% EDTA, Smear Clear, QMix and BioPure MTAD as final irrigants by utilizing Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS). Most of the previous studies investigated the extent of dentin erosion using scoring based on Scanning electron microscopic (SEM) images [23, 24]. SEM provides only qualitative analysis of the surface changes of root canal wall. In the present study, EDS was used in conjunction with SEM as it provides quantitative evaluation of dentin erosion by measuring changes in Ca (Calcium) level inside dentin after using root canal irrigants [25].

Different irrigating solutions showed different levels of erosion in the present study. EDTA and Smear Clear showed higher degree of erosion than QMix and MTAD. In EDTA group, erosion of peritubular dentin was seen in coronal, middle and apical third of root similar to previous studies [26, 27]. Peritubular dentin is highly mineralized with a lower collagen content which makes it more quickly dissolvable in acid than intertubular dentin [28]. Many studies have reported that erosion due to the use of EDTA is derived mainly from the use of NaOCl as the initial irrigant [29, 30]. This effect can be related to the loss of organic substance from the dentin by its prolonged contact with NaOCl, thereby creating diffusion channels for more rapid penetration of EDTA into the intertubular and peritubular dentin resulting in apatite dissolution [31].

In Smear Clear group, moderate erosion of peritubular dentin was seen in coronal, middle and apical third of root. These results are in disagreement to previous study that showed that Smear Clear does not cause erosion [26]. This difference could be attributed to the fact that distilled water was used as initial irrigant in the previous study whereas in our study 5% sodium hypochlorite was used which could have contributed to dentin erosion.

In QMix group, the tubule openings were clearly visible and peritubular and intertubular surface dentin appeared smooth and flat with no erosive changes. These findings are in agreement with those of previous studies which showed that QMix causes negligible dentin erosion [26, 27]. In the MTAD group, the tubule openings were clearly visible with no erosion of the peritubular and intertubular dentin. These findings are similar to a previous study which concluded that MTAD does not significantly change the structure of the dentinal tubules when used as a final irrigant [21].

All treatment groups showed significantly lower calcium level in root dentin than control group at coronal, middle and apical third. Our results are similar to previous studies which showed that the use of EDTA solution reduced the calcium level significantly from the root canal dentin [32]. Presence of EDTA in Smear Clear and QMix could have contributed to reduced calcium as evident in EDS. The decreased levels of calcium in the MTAD group could be attributed to its chemical composition i.e. citric acid, doxycycline and low pH of 2.15 [10].

## Conclusion

Based on the results of our study, it can be concluded that EDTA, Smear Clear, QMix and MTAD have a tendency to cause calcium loss from dentin. Nevertheless, Biopure MTAD and QMix can be considered better alternatives to EDTA and Smear Clear as final irrigating solutions due to their minimal erosive effects.

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