

Efficacy of etidronic acid, BioPure MTAD and SmearClear in removing calcium ions from the root canal: An *in vitro* study

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ABSTRACT

Objective: The purpose of this study was to quantify the amount of calcium ions removed from the root canal by etidronic acid (HEBP), BioPure MTAD, and SmearClear using atomic absorption spectrophotometer. **Materials and Methods:** Fifty ($n = 50$) freshly extracted human mandibular premolar teeth were collected and decoronated at the cemento-enamel junction. The canals were prepared in a crown down fashion using the rotary system and copiously irrigated with 1.0% sodium hypochlorite. All specimens were rinsed with the deionized water. Based on the type of chelating agent used, the samples ($n = 10$) were randomly divided into five (four test and one negative control) groups. Accordingly, Group I - 9% HEBP, Group II - 18% HEBP, Group III - SmearClear, Group IV - BioPure MTAD, and Group V - normal Saline. Subsequent to irrigation, the solution was collected in a test tube and subjected to atomic absorption spectrophotometer for the quantification of calcium ions removed from the root canal. **Results:** The mean concentration of calcium ions removed from the root canal (mean \pm standard deviation) in all groups (I–V) were 13.32 ± 0.54 $\mu\text{g/ml}$, 16.36 ± 0.27 $\mu\text{g/ml}$, 20.04 ± 0.24 $\mu\text{g/ml}$, 18.15 ± 0.39 $\mu\text{g/ml}$, and 8.74 ± 0.49 $\mu\text{g/ml}$, respectively. **Conclusions:** SmearClear was the most effective agent for the removal of calcium ions from the root canal. Hence, its combined use with an organic solvent can be recommended for efficient smear layer removal.

Key words: Atomic absorption spectrophotometry, BioPure MTAD, etidronic acid, SmearClear

INTRODUCTION

Although, the mechanical instrumentation is an essential step in the success of root canal therapy, the generation of the smear layer is an inevitable consequence of instrumentation regardless of the type of instruments and techniques used. McComb and Smith^[1] were first to describe the smear layer on the surface of the instrumented root canal wall. Lester and Boyde^[2] described the smear layer as “organic matter trapped within translocated inorganic dentin.”

Controversies still persist with regard to the role of smear layer in root canal treatment outcome. However,

the smear layer may be considered as deleterious because it adheres to the root canal walls and might partially or completely occlude the dentinal tubules and

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complex root canal morphology, which (a) prevents the penetration of disinfecting agents, (b) acts as a barrier between canal wall and the filling materials, (c) is a potential avenue for microleakage, and (d) provides shelter and nutrition to microbes. It is believed that the smear layer removal eliminates the attached microbiota and their toxins from the root canal walls, thus reducing the probability of bacterial survival with the reproduction and improve the seal of root fillings.^[1,3]

Sodium hypochlorite (NaOCl) is the most widely used irrigant in the disinfection of the root canal system. However, it is unable to dissolve inorganic components of the smear layer in spite of its excellent antimicrobial action and capacity of dissolving organic materials. Its association with chelating agents that act on the inorganic matter, is therefore necessary for complete smear layer removal.^[1,4]

Chelators are stable complexes formed as a result of the bond between metal ions and chelator itself (ligand) having more than one pair of free electrons. They induce changes in calcium and phosphorus ion concentration in the root canal dentin.^[5] The demineralizing effect of chelators acts simultaneously on the smear layer and the root canal dentin, resulting in collagen exposure and reduction of dentin microhardness. Reduction in microhardness of the most superficial layer of root canal dentin is more advantageous (50 μm per canal wall). It can help in negotiation and facilitation of endodontic instrumentation in fine calcified canals and smear layer removal increases the penetration of the irrigants into the dentinal tubules to permit adequate disinfection.^[6]

Nowadays, ethylenediaminetetraacetic acid (EDTA) and citric acid are the most commonly used chemicals for the instrumentation of root canals and smear layer removal. However, alternative chemicals to remove the smear layer have also been suggested such as EDTAC (EDTA + cetavlon), EGTA (ethylene glycolbis (β -aminoethylether)-N, N, N', N'-tetraacetic acid, malic acid, peracetic acid, etc. Studies have reported that EDTA or citric acid strongly reacts with NaOCl, thus making the latter ineffective.^[7-9] Consequently, etidronic acid (1-hydroxyethylidene-1,1 bisphosphonate or HEBP) has been investigated as a potential alternative. HEBP is nontoxic and has been systematically used to treat bone diseases.^[10] Like EDTA, it has chelating property and is commonly used as an adjunct in personal care and household products such as soaps.

SmearClear (SybronEndo, Orange, CA) is a 17% EDTA solution containing cetrimide (a quaternary ammonium compound) and an additional proprietary surfactant (polyoxyethylene (10) iso-octylcyclohexyl ether). It has also been investigated for smear layer removal and root canal cleansing.^[11-13]

BioPure MTAD (Dentsply, Tulsa Dental, Tulsa OK), a mixture of antibiotic (doxycycline hyclate: 150 mg/5 ml (3%), citric acid (4.25%), and a detergent (0.5% polysorbate 80 detergent or Tween 80), is a biocompatible material. Doxycycline has also been used in periodontal therapy due to its antimicrobial and chelating ability as well as its substantivity. BioPure MTAD has been investigated as an effective solution for both removing the smear layer and disinfecting the root canal system.^[14]

Most of the studies have analyzed only the ability of chelators to remove the smear layer.^[12,15] Limited studies have determined the concentration of calcium ions eluted from root dentin^[16,17] and there are as yet no studies that show the comparative calcium ion removal by HEBP, BioPure MTAD and SmearClear from the root canal wall. The present study was therefore conducted to evaluate the efficacy of 9%, 18% HEBP, SmearClear and BioPure MTAD for removal of Ca^{2+} ions from the root canal using flame atomic absorption spectrophotometry (AAS).

MATERIALS AND METHODS

Sample selection and preparation

Fifty freshly extracted human mandibular premolars were collected from the Oral and Maxillofacial Surgery Department. They were cleaned, free of debris and calculus and then stored in 0.1% thymol solution until used. Teeth were selected based on criteria: Teeth with complete root formation, patent canals and without anatomic variations. Teeth having curved root, root resorption, and calcified canal were not included in the study. Each tooth was decorated from the cemento-enamel junction (CEJ) by using a slow speed, water-cooled diamond saw (90 μm ; Microdont, Brazil). The working length was checked with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) introduced into the root canal of each tooth up to the point until it became visible at the apex and then pulled back 1 mm. Root canal preparation was done in a crown down fashion using the rotary system (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland). Master apical preparation

was done till finishing file F5. All procedures were performed with a torque control electric motor (X-Smart endodontic motor Dentsply International, Inc). During biomechanical preparation, the root canals were irrigated with 5 ml of 1.0% NaOCl at each instrument change. The 1% NaOCl solution was obtained by dilution of a 5.25% solution with triple distilled water. The 100 ml 1% NaOCl solution was prepared by adding 19.05 ml of 5.25% NaOCl solution in triple distilled water. All the samples were rinsed with 20 ml of deionized water obtained by using the Milli-Q water purification system (Merck Millipore India Private Limited, Bengaluru, India) to remove the possible dentinal chips. Subsequently, the apex was sealed with composite resin to keep the test irrigating solutions inside the root canal.

Test chelating solutions

Based on the type of chelating agents used, the samples ($n = 10$) were randomly divided into five (four test and one negative control) groups. Accordingly, Group I - 9% HEBP, Group II - 18% HEBP, Group III - SmearClear, Group IV - BioPure MTAD, and Group V - normal saline. 9% and 18% HEBP solutions were prepared from 60% aqueous solution of etidronate (Sigma-Aldrich, Bengaluru, India) by adding triple distilled water.

Atomic absorption spectrometry analysis

Each sample was irrigated with 5 ml of test irrigants for 5 min (1 ml/min) using 30 gauge needle attached to a syringe. Irrigation was performed by introducing the needle inside the canal as far apically as possible. Irrigating solution from each sample was collected in

test tube placed beneath the Eppendorf tube holding the sample [Figure 1] and prepared for AAS by using an air-acetylene flame to determine the concentration of calcium ions removed from the root canal of each sample. Instrument-specific condition for the analysis of calcium metal on flame AAS is as follows: (Wave length (nm) - 422.7, sensitivity check (mg/L) - 0.5, linear range (mg/l - 3.0-0.05, expected absorbance units - 0.25-0.50, optimum working range ($\mu\text{g/ml}$) - 0.01-3).

The samples were then analyzed on Atomic Absorption Spectrophotometer (GBC Avanta Σ , Australia) with background correction. Standard stock solutions (1000 $\mu\text{g/ml}$) were purchased from E. Merck Mumbai with National Institute of Standards and Technology traceability and diluted to working standard solution within the linear range of calcium element. Calibration curve was drawn for 1, 3, 5, and 10 $\mu\text{g/ml}$ standard solution ($R^2 = 0.992$).

Statistical analysis

All the results of the concentration of calcium ions were analyzed statistically. Data were summarized as a mean \pm standard deviation. The concentrations of calcium ions of five independent groups were compared by one-way analysis of variance (ANOVA) and significance of the mean difference between the groups were compared with Tukey's *post-hoc* test after ascertaining the homogeneity of variance among the groups by Bartlett's test. All the analysis was performed on GraphPad Prism version 5.0 for windows (GraphPad software, La Jolla, CA, USA).

RESULTS

The mean concentration of calcium ions removed from the root canals by HEBP, SmearClear, and BioPure MTAD is shown in Table 1 and Figure 2. ANOVA revealed the significant ($P < 0.001$) effect of each group (chelating agents) on mean concentration of calcium ions removed from the root canal ($F = 1223.74$, $P < 0.001$). Tukey's *post-hoc* multiple comparison tests revealed the significantly ($P < 0.001$) different removal of Ca^{2+} ions from root canal between the groups and was found the highest in SmearClear followed by BioPure MTAD, 18% HEBP, 9% HEBP, and normal saline the least.

DISCUSSION

The main objectives of endodontic therapy are to remove the diseased tissue, eliminate the bacteria present in the canals and dentinal tubules, and prevent recontamination after treatment. These objectives

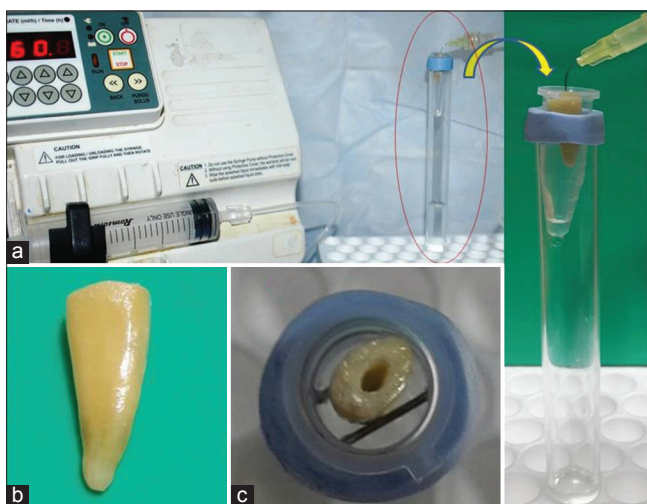


Figure 1: (a) The apparatus used to collect irrigating solution. The arrow indicates the magnified view of encircled region, (b) the outer surface of the tooth sample coated with bonding agent and thin cellophane strip, (c) the tooth sample held in an Eppendorf tube

are achieved by thorough cleaning, shaping, and disinfecting the root canal system.^[18] In the present study, human mandibular premolars decoronated from CEJ were used to ensure similarity in size, shape, and canal anatomy. This makes the homogeneous effect of chelators on calcium ion removal from the root canal.

The choice of concentration of NaOCl (0.5–5.25%) is still a matter of debate, despite its antibacterial, tissue-dissolving, and lubricating properties. Serious incidents have been reported after inadvertent penetration of NaOCl to periradicular tissues or its leakage through the rubber dam onto the patient’s skin.^[19,20] Therefore, simply increasing NaOCl concentrations over 1% to render them more effective may not be advisable. Hence, in the present study, 1% NaOCl was used separately for copious irrigation and prevention of all these risk factors and negative interactions with EDTA and citric acid.^[8,9]

The decalcifying effect of chelators in the removal of inorganic component of the smear layer and negotiation of the fine, tortuous, and calcified canal to ascertain patency depends on the root length, application time, diffusion in the dentin, relationship between the amount of available active substance (chelator), and the canal wall surface area and, especially, the solution pH, because the demineralization process

continues until all chelating agents have formed complexes with calcium. The recommended pH of EDTA solutions for decalcification is around 7.3 while for citric acid it is 0.8–1.9.^[9,19,21-23] In a gravimetric analysis, Seidberg and Schilder^[24] showed that the properties of chelators (EDTA) were self-limiting, because of pH changes during the demineralization of dentin. Under normal conditions, most chelators have an almost neutral pH. Because of the release of the acid by exchange of calcium from dentin with hydrogen, the efficiency of EDTA decreases with time; on the other hand, the reaction of the acid with hydroxyapatite affects the solubility of dentin.^[24] Hülsmann and Hahn^[19] in their study demonstrated that EDTA solutions demineralized dentin up to a depth of 50 µm per canal wall.

Currently, there are disagreements regarding the ideal chelator, the application time, and the association with hypochlorite. The time these solutions stay in contact with the canal walls has been reported to vary from 30 s to 10 mins.^[19,23] We have used 5 ml of test irrigants for 5 min (1 ml/min), a considerable time and volume of irrigation because during irrigation chelation is not necessarily an equilibrium reaction and is determined by a standard stability constant because the rate and ligand exchange reactions might considerably affect the chelation process.^[25] Irrigation time of 1 min was relatively short, but longer irrigation times with effective chelators such as EDTA, might affect dentin structure. A previous study has shown that a 1 min EDTA irrigation was effective in removing the smear layer; however, a 10-min application of EDTA caused excessive peritubular and intertubular dentinal erosion.^[23]

On the basis of calcium-binding capacity and stability constant of the HEBP-calcium complex, the use of 7% HEBP solution was found significantly less effective in debriding root canals than 10% citric acid.^[9,26] Further experiments showed that HEBP-calcium chelation from root canals is dependent on the concentration of the chelator in solution. With 20% HEBP solution, the amount of calcium ions eluted from the root canals was found to be similar with 17% EDTA or 10% citric

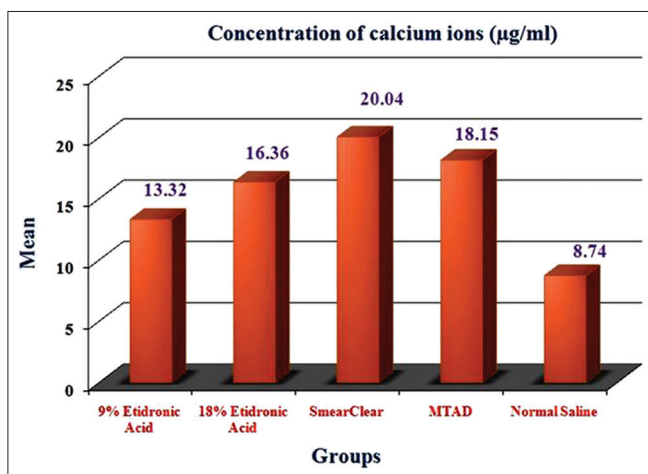


Figure 2: Mean concentration (µg/ml) of calcium ions removed from root canals by etidronic acid, SmearClear, and BioPure MTAD

Mean±SD of different groups (µg/ml)					ANOVA (between groups)			
Group I 9% etidronic acid	Group II 18% etidronic acid	Group III SmearClear	Group IV Biopure MTAD	Group V normal saline	df	MS	F	P
13.32±0.54	16.36±0.27	20.04±0.24	18.15±0.39	8.74±0.49	4 {45}	196.79 {0.16}	1223.74	<0.001

{ } : Error, ANOVA: Analysis of variance, df: Degrees of freedom, MS: Mean square, SD: Standard deviation

acid.^[9] In the present study, the chelating efficiency of 18% HEBP was found better than 9% HEBP because of higher concentration. Consequently, a less aggressive calcium complexing agent such as 7–10% HEBP could be administered during the whole course of root canal preparation to prevent erosive dentinal changes.

HEBP can be used in combination with NaOCl as a single irrigant during and after the instrumentation without short-term loss of the desired properties of either compound so that a smear layer is never created.^[9,27] In the current study, 18% HEBP was found more efficient than 9% HEBP in removing calcium from the root canal, but relatively weaker than SmearClear and BioPure MTAD due to the lack of surfactants which enhance their diffusion inside dentinal tubules.

Irrigants must be in contact with the dentin walls for effective debris removal and penetration more readily into the root canal system, thus making more surface area available for action.^[28,29] The closeness of this contact is directly related to its surface tension.^[30] According to Grossman and Meiman,^[31] low surface tension is one of the ideal characteristics of an irrigant. These views are in support of our study in which the SmearClear was found to be most efficient in removing Ca²⁺ ions from root canal than all others because of low surface tension (33 mJ/m²) due to the presence of additional surfactants. This leads to a better flow of chelating solution inside the canal. However, other studies have shown that the reducing surface tension of chelators did not enhance their calcium-binding ability.^[4,12,32,33] This is in contrast to our study.

BioPure MTAD is capable of removing the inorganic substances because of its components such as citric acid, doxycycline and low pH of 2.15. The chelating property of BioPure MTAD is due to the presence of citric acid and doxycycline which removes the smear layer thereby allowing the better penetration of doxycycline inside the dentinal tubules for its extended antibacterial effect.^[34] The solubilizing effect of BioPure MTAD on dentin and pulp was found similar to those of 17% EDTA and 5% citric acid except for its higher binding affinity to dentin.^[14,35] Torabinejad *et al.*^[14] reported that it has lesser erosive intraradicular changes in dentin than that of EDTA. However, De-Deus *et al.*^[36] reported that citric acid has a strong erosive effect. In the current study, BioPure MTAD was found more efficacious in removing the calcium ions from the root canal than 9 and 18%

HEBP. It may be due to the presence of a citric acid; a strong chelating agent. However, it is inferior to that of SmearClear. It may be due to a lesser number of surfactant.

The lack of studies addressing the use of 9% HEBP, 18% HEBP, SmearClear, and BioPure MTAD hinders the comparison of these findings to those published elsewhere. Further, *in vitro* studies and clinical trials should be undertaken to confirm the efficacy of these agents for root canal therapy in clinical practice.

CONCLUSION

Under these study conditions and the limitations of the present study, SmearClear was most effective in removing Ca²⁺ ions from the root canal followed by BioPure MTAD, when compared with 18% HEBP, 9% HEBP, and normal saline. Hence, their combined use with NaOCl can be recommended for efficient smear layer removal. It can also be helpful in the negotiation and instrumentation of fine and calcified canals.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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