Comparison of efficiency of ethylenediaminetetraacetic acid, citric acid, and etidronate in the removal of calcium hydroxide intracanal medicament using scanning electron microscopic analysis: An in-vitro study

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Abstract

Context: Being integral to root canal therapy, obturation can be performed adequately only after the removal of intracanal medicament. One technique involves the use of chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid. Etidronic acid, a relatively new chelator, has smear layer removal ability and lesser dentinal erosion. It is untested in calcium hydroxide (Ca(OH)$_2$) medicament removal.

Aim: The aim of this study was to compare the efficiency of irrigation protocols (EDTA, citric acid, and etidronate) in Ca(OH)$_2$ removal.

Materials and Methods: Forty-five single-rooted mandibular premolars were decoronated, instrumented, and filled with Ca(OH)$_2$. After 7 days incubation, Ca(OH)$_2$ was removed by three irrigation protocols (Group-I: 17% EDTA; Group-II: 10% citric acid; and Group-III: 18% etidronate). Roots were split and analyzed (scanning electron microscope, x1500). Chelator solution pH was tested. Data were analyzed by Kruskal–Wallis ANOVA and Mann–Whitney U-test.

Results: Group-III (coronal-third) and Groups-I and II (middle-third) had highest cleanliness scores; Groups-II and III (apical-third) had lowest scores. Comparing the thirds, all groups showed difference in scores. pH of Groups-I, II, and III were 6.8, 1.4, and 0.3, respectively.

Conclusion: The solution pH of citric acid and etidronate impacts their Ca(OH)$_2$ removal efficiency in different ways: the highly alkaline pH of Ca(OH)$_2$ increases citric acid pH toward neutrality, where it becomes an inefficient chelator; on the contrary, high acidity of etidronate compensates for its weaker chelation. Etidronate may not require 5 min duration for Ca(OH)$_2$ removal due to the likelihood of dentinal erosion.

Keywords: Calcium hydroxide; citric acid; etidronic acid; root canal irrigants; scanning electron microscopy

INTRODUCTION

Prior to obturation, the intracanal medicament should be removed. Medicament residues can reduce root canal permeability by promoting the formation of calcium carbonate particles and interfering with sealing ability of root canal sealers.[3]

Numerous studies have aimed to determine the best protocol to remove all the calcium hydroxide (Ca(OH)$_2$) medications. Irrigation, in combination with filing, has proven to be...
chelation agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid have been recommended as adjuncts in root canal therapy, since both agents show efficiency in removing the smear layer. In addition, these chelators can emulsify, neutralize, and hold Ca(OH)$_2$ debris in suspension. However, prolonged exposure to strong chelators such as EDTA and citric acid may weaken root dentin, as dentin hardness and elastic modulus are the functions of dentin mineral content.$^{[3]}$

A relatively new chelating agent utilized in in vitro studies in endodontics, and for several years, as a systemically administered agent in the treatment of malignant bone diseases is, etidronate. Etidronate (etidronic acid/1-hydroxyethylidene-1,1-biphosphonate) has shown ability in smear layer removal.$^{[4]}$ Being a relatively weak chelator compared to EDTA or citric acid, it may erode dentin to a lesser extent.$^{[3]}$ In addition, it is nontoxic$^{[5]}$ and shows no short-term interaction with sodium hypochlorite (“hypochlorite-compatible chelator”).$^{[6]}$ Hence, it has been proposed as a potential alternative to EDTA and citric acid. This chelating agent has not been tested for its efficiency in Ca(OH)$_2$ medicament removal.

The aim of this study was to compare the efficiency of three irrigation protocols involving saline and the chelators: EDTA, citric acid, and etidronate in the removal of Ca(OH)$_2$, intracanal medicament from root canals. The null hypothesis was that there will be no difference in the efficiency of irrigation by saline in combination with different chelators in the removal of Ca(OH)$_2$ intracanal medicament.

**MATERIALS AND METHODS**

Forty-five single-rooted mandibular premolar teeth with completely formed apices and single root canal were decoronated to standardize the root length to 14 mm. Teeth with resorption, developmental anomalies, root caries, fractures, and previous endodontic treatment were excluded. Working length was established by inserting #10 K-file into each root canal until just visible at the apical foramen and then subtracting 1 mm from this point. This same file was used during preparation to ensure patency at all times.

The roots were instrumented with rotary up to F4-ProTaper (Dentsply-Maillefer, Switzerland) and irrigated with 2.5% NaOCl after each instrument. This was followed by irrigation with 17% EDTA to remove smear layer. Canals were dried with paper points, then filled with freshly mixed Ca(OH)$_2$ paste obtained by mixing Ca(OH)$_2$ powder with distilled water (1:1 by volume), and placed with a lentulo-spiral until the paste extruded through the apex. Access cavities were temporarily sealed with Cavit (3M ESPE, Germany). Teeth were incubated (Tempo-Instruments and Equipments [I] Pvt. Ltd., Mumbai, Maharashtra, India) at 37ºC ± 1ºC and 100% relative humidity for 7 days. After this, canals were reopened.

Root samples were randomly divided into three experimental Groups (I, II, III) ($n = 15$). The bulk of Ca(OH)$_2$ was initially removed using saline (working solution; volume = 5 mL/canal) and re-instrumentation with master apical file (MAF) (#40) using circumferential filing. The following chelator irrigation protocol was performed:

- **Group-I (17% EDTA):** Rate = 5 mL/min; volume = 5 mL/canal
- **Group-II (10% citric acid):** Rate = 5 mL/min; volume = 5 mL/canal
- **Group-III (18% etidronate):** Rate = 1 mL/min; volume = 5 mL/canal.

Seventeen percentage EDTA (w/v) and 10% citric acid (w/v) were prepared by mixing the powder form (Sigma-Aldrich, India) with distilled water. 60% aqueous etidronate (Sigma-Aldrich, India) was diluted to 18% v/v concentration.

Finally, the canals were flushed with saline (volume = 5 mL/canal). Irrigation was performed using 5 mL disposable plastic syringes with 30-gauge needle tips placed passively into the canal up to 2 mm from apical foramen without binding.

After irrigation, a cotton pellet was inserted in canal orifice to avoid entrance of dentinal chips. Longitudinal grooves were cut on buccal and lingual root surfaces using diamond disc without penetrating the canal. Roots were then split bucco-lingually into halves using chisel and mallet. For each root, the half containing the most visible portion of apex was selected, then demarcated into three equal parts as coronal, middle, and apical-thirds (by grooving with sharp knife) at 9, 6, 3 mm, respectively, from apex. Teeth were stored in distilled water until scanning electron microscope (SEM) analysis.

Roots were dehydrated by a series of graded ethanol solutions, sputter-coated with gold layer, and subjected to SEM analysis at $\times 1500$ magnification (Inspect-FEI$^{[7]}$). A five-grade scale was used to determine the degree of Ca(OH)$_2$ medicament removal and dentinal wall cleanliness.$^{[2]}$

- **Score-1:** 80%–100% removal of Ca(OH)$_2$ (total cleanliness)
- **Score-2:** 60%–80% removal of Ca(OH)$_2$ (great cleanliness)
- **Score-3:** 40%–60% removal of Ca(OH)$_2$ (partial cleanliness)
- **Score-4:** 20%–40% removal of Ca(OH)$_2$ (light cleanliness)
- **Score-5:** 0%–20% removal of Ca(OH)$_2$ (no cleanliness).

pH of chelator solutions was measured using Litmus paper (Universal Full-range Litmus test) and pH meter (PH-009[I] pen-type pH meter, RoHs, Brand Apex, India).
**Statistical analysis**

Kruskal–Wallis ANOVA was employed to compare scores among the three groups. Pair-wise comparisons were done using Mann–Whitney U-test [Table 1]. Significance was established at $P < 0.05$ level. Data were analyzed by IBM SPSS-Statistics (Version 19.0 for Windows, Armonk, NY: IBM Corp. 2010).

**RESULTS**

The results were obtained in the form of a total of 135 scanning electron micrographs ($\times 1500$) [Figure 1]. A $10 \times 10$ grid was superimposed over the micrographs using Microsoft® Office PowerPoint® (Version 14.0. Redmond, WA: Microsoft Corp. 2010). Counting was done in the horizontal direction. Percentage cleanliness was determined using the formula: 

$$\text{% cleanliness} = \frac{\text{number of open dentinal tubules}}{\text{total number of dentinal tubules}} \times 100.$$ 

Scores of Ca(OH)$_2$ removal are shown in Table 2. Inter-group comparison showed that in the coronal-third, etidronate (Group-III) showed highest cleanliness scores, followed by EDTA (Group-I). In the middle-third, EDTA (Group-I) and etidronate (Group-III) showed highest cleanliness scores. In the apical-third, Group-III performed as poorly.

**Table 1: Comparison of scores for dentinal wall cleanliness**

| Group | Root section | Intra-group comparison ($P<0.05$)* | Interpretation of scores | Comparison between groups ($P<0.05$)* |
|-------|--------------|-------------------------------------|---------------------------|-------------------------------------|
|       |               | Mean±SD† | Median |               | Coronal | Middle | Apical |
| I     | Coronal       | 2.53±1.06* | 2      | Great cleanliness | a       | a      | a      |
|       | Middle        | 3.07±1.33  | 3      | Partial cleanliness | a       | a      | a      |
|       | Apical        | 3.80±1.15b | 4      | Light cleanliness | b       | b      | b      |
| II    | Coronal       | 4.80±0.41  | 5      | No cleanliness   | b       | b      | b      |
|       | Middle        | 4.93±0.26  | 5      | No cleanliness   | b       | b      | b      |
|       | Apical        | 5.00±0.0   | 5      | No cleanliness   | a       | a      | a      |
| III   | Coronal       | 1.53±0.74a | 1      | Total cleanliness | c       | a      | -      |
|       | Middle        | 2.40±1.24a | 2      | Great cleanliness | a       | a      | a      |
|       | Apical        | 4.47±0.99c | 5      | No cleanliness   | a       | a      | -      |

*Statistical comparison between thirds of the same group and between groups. Different letters denote significant differences. †SD: Standard deviation.

**Figure 1:** Representative samples from each of the three experimental groups. C = Coronal-third; m = Middle-third; a = Apical-third.
as Group-II. Comparison of the thirds within the groups showed that group-I demonstrated greater cleanliness in coronal versus apical-third; Group-II performed poorly in all thirds; Group-III showed statistically significant difference in terms of cleanliness among all the thirds [Table 1 and Figure 2].

Table 2: Calcium hydroxide medicament removal scores

| Group | Root section | Scores |
|-------|--------------|--------|
|       |              | 1  | 2  | 3  | 4  | 5  |
| I     | Coronal      | 2  | 7  | 2  | 4  | -  |
|       | Middle       | 2  | 4  | 2  | 5  | 2  |
|       | Apical       | -  | 2  | 5  | 2  | 6  |
| II    | Coronal      | -  | -  | -  | 3  | 12 |
|       | Middle       | -  | -  | -  | 1  | 14 |
|       | Apical       | -  | -  | -  | -  | 15 |
| III   | Coronal      | 9  | 4  | 2  | -  | -  |
|       | Middle       | 4  | 5  | 3  | 2  | 1  |
|       | Apical       | -  | 1  | 2  | 1  | 11 |

pH of experimental chelators such as EDTA, citric acid, and etidronate was 6.8, 1.4, and 0.3, respectively.

DISCUSSION

Ca(OH)₂ is the most commonly used intracanal medicament in between sessions of root canal therapy. In spite of its numerous favorable benefits,[7] fact remains that it must be removed from the root canals after serving its purpose. Medicament residue on the root canal walls promotes formation of calcium carbonate particles and hinders sealer penetration into dentinal tubules.[1,8] Ca(OH)₂ also interacts with the zinc oxide eugenol containing sealer, causing gradual reduction in viscosity, manifested as rapid setting reaction. In addition, the set sealer will be poorly cohesive and granular due to the formation of calcium eugenolate, with unreacted residual eugenol. Such remnants in critical (apical) areas affect clinical performance of the
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It is possible that etidronate is a weak chelator that requires extended contact duration in smear layer removal. However, smear layer removal efficiency may not translate into a Ca(OH)₂ removal efficiency – this may be explained by the fact that the chelation property of citric acid action is dependent not only on the direct physical chelating action, but also on the solution pH. Citric acid is an ineffective chelator at neutral pH. It is possible that the highly alkaline pH of Ca(OH)₂ raises citric acid pH toward neutrality, thus limiting the effectiveness of citric acid in Ca(OH)₂ removal. In the future, a titration study may reveal how rapidly or effectively a chelator (such as citric acid) gets neutralized by highly alkaline medicament such as Ca(OH)₂. On the other hand, EDTA remains effective even at neutral pH. Thus, the highly alkaline pH of Ca(OH)₂ may not efficiently inhibit EDTA chelation.

The second observation, albeit a cautious one, is that dentinal erosion is evident in the scanning electron micrographs of EDTA group (Group-I) and more so in the etidronate group (Group-III). Nandini et al. used volumetric analysis by spiral computed tomography to assess Ca(OH)₂ removal. In spite of the magnitude of data (three-dimensional volume measurements of amount of Ca(OH)₂ packed in the canal without root sectioning), this study utilized SEM to reveal the condition of the dentinal tubules (occluded, open, and eroded). Prolonged exposure to strong chelators such as EDTA or citric acid may weaken root dentin. In stark contrast, literature states that etidronate is a weak chelator that requires extended duration of action in smear layer removal (5 min.). Its Ca(OH)₂ removal efficiency remained untested. It is reasonable to assume that smear layer and Ca(OH)₂ removal efficiency of a chelator are not same due to different nature of substrates (smear layer = organic + inorganic; Ca(OH)₂ = purely inorganic).

The 18% etidronate solution used in this study was found to be strongly acidic (pH = 0.3). Gubler et al. (2008) as cited by Lottanni et al. found that the high acidity of peracetic acid caused calcium of root dentin to stay in solution and prevented its re-precipitation. These researchers explained that the decalcification kinetics differed between EDTA and peracetic acid – despite the weak chelating power of peracetic acid, similar amounts of calcium were eluted from root canal as compared to EDTA, which is a much stronger chelator but can only be in solution at slightly alkaline pH. A similar connection may be made with etidronate – its high acidity compensates for its apparent weak chelation. These factors explain the dentinal erosion in Group-III. Thus, in terms of contact duration of etidronate irrigant required for Ca(OH)₂ removal, it may be safe to suggest that a 5 min period is longer than required.

In coronal and middle-third, etidronate performed on par with EDTA. However, in apical-third, etidronate performed as poorly as Group-II in terms of cleanliness. Paqué et al. found that etidronate could reduce but not completely prevent hard tissue debris accumulation during rotary root canal instrumentation. In this context, the poor scores of Group-III/apical-third may be attributed to the deposition of dentin debris (from erosion).

Evaluating the scanning electron micrographs of the root canal thirds must be performed with caution. In the micrographs published in their study, Lottanti et al. explained that although there is no smear layer, tubules appear “occluded” with few tubular openings in middle and apical root sections due to sclerosis. This is in contrast to instrumented areas that have a homogeneous smear layer.

Analyzing the study results, the third observation was that the apical-third, irrespective of the experimental group, had lowest scores. The thirds (coronal, middle, and apical) of each of the root samples were scored separately for cleanliness because irrigant/chelator efficiency decreases significantly from coronal part toward apical. This is in agreement with previous studies. The crudest of the irrigation techniques – conventional syringe irrigation – remains the most commonly practiced method of irrigation due to its simplicity, easy control of needle penetration depth, and irrigant volume flushed through the canal. The results of this study reiterate the need for more sophisticated methods of irrigation such as ultrasonic irrigation systems.

**CONCLUSION**

In case of citric acid (Group-II) and etidronate (Group-III), the solution pH impacts their Ca(OH)₂ removal efficiency in vast different ways: The highly alkaline pH of Ca(OH)₂ may increase citric acid pH toward neutrality, where it becomes an inefficient chelator; on the contrary, high acidity of etidronate may compensate for its weak chelating ability.
Since smear layer and Ca(OH)₂ medicament are substrates of a different nature, etidronate may not require 5 min contact duration as an irrigant in Ca(OH)₂ removal. Dentinal erosion may be more likely for this strongly acidic chelator if permitted longer contact duration with root canal walls.

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Conflicts of interest

There are no conflicts of interest.

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