Retrievability of calcium hydroxide intracanal medicament with three calcium chelators, ethylenediaminetetraacetic acid, citric acid, and chitosan from root canals: An in vitro cone beam computed tomography volumetric analysis

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Abstract

Aim: This study compared the amount of aqueous-based and oil-based calcium hydroxide remaining in the canal, after removal with two different chelators 17% EDTA, 20% Citric acid and 0.2% Chitosan in combination with ultrasonic agitation.

Methods and Material: Cleaning and shaping of root canals of 28 mandibular premolar was done and canals were filled either with Metapex or Ca(OH)$_2$, mixed with distilled water. Volumetric analysis was performed utilizing cone beam-computed tomography (CBCT) after seven days of incubation. Ca(OH)$_2$ was removed using either 17% EDTA, 20% Citric acid or 0.2% Chitosan in combination with ultrasonic agitation.

Statistical analysis used: Volumetric analysis was repeated and percentage difference was calculated and statistically analyzed using Kruskal-Wallis and Mann-Whitney U test.

Results: All the three chelators failed to remove aqueous-based as well as oil-based Ca(OH)$_2$ completely from the root canal. Aqueous-based Ca(OH)$_2$ was easier to be removed than oil-based Ca(OH)$_2$. 0.2% Chitosan in combination with ultrasonics performed better than 17% EDTA and 20% citric acid in removal of Ca(OH)$_2$.

Conclusion: Combination of 0.2% Chitosan and ultrasonic agitation results in lower amount of Ca(OH)$_2$ remnants than 17% EDTA, 20% Citric acid irrespective of type of vehicle present in the mix.

Keywords: Calcium hydroxide; chitosan; cone beam computed tomography; ethylenediaminetetraacetic acid; intracanal medicament; retrieval; ultrasonics

INTRODUCTION

Endodontic treatment is essentially directed towards the prevention and control of pulpal and periradicular infections. Given the relevance of microorganisms in the pathogenesis of these lesions, it is clear that the outcome of endodontic therapy depends on their reduction or elimination.¹

Chemomechanical preparation of the root canal significantly reduces the number of microorganisms in the infected root canals. However, the eradication of microorganisms from canal irregularities is enhanced by intracanal medicaments

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that prevent the proliferation of residual strains, as well as recontamination.\(^2\)

Currently, calcium hydroxide is the most popular intracanal medicament. It is widely used as a root canal dressing between treatment sessions due to its well documented antibacterial activity against most of the endodontic pathogens. Most bacteria are unable to survive the highly alkaline environment provided by calcium hydroxide.\(^7\)

As a medicament, calcium hydroxide can be placed within the root canal using different vehicles such as water, carboxymethyl cellulose, glycerin and silicone oil. The type of vehicle used affects the physical and chemical properties of calcium hydroxide. Aqueous vehicles cause rapid release of calcium and hydroxyl ions, while oily vehicles prolong the ionic dissociation.\(^4\)

Prior to obturation, the calcium hydroxide medicament must be eliminated from the root canal system as residual calcium hydroxide can negatively influence the sealing qualities of the root canal fillings.\(^2\)

This is routinely achieved by copious irrigation using sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) in combination with hand filling. However, this technique is not efficient as in vitro studies have reported that this method still leaves over 40% calcium hydroxide residues within the root canal.

Since the combination of sodium hypochlorite and EDTA alone has not been sufficient to eliminate calcium hydroxide, the role of calcium chelators such as citric acid, maleic acid, and peracetic acid has been investigated. Reports suggest that they are more effective in removing calcium hydroxide from the root canals.

Chitosan, a natural polysaccharide prepared by the deacetylation of chitin, is endowed with the properties of biocompatibility, biodegradability, bioadhesion, and no toxicity.\(^5\) Its chelation activities are similar to EDTA and citric acid, while it has very less detrimental effects. Hence, in this study, chitosan, EDTA, and citric acid were used.

Recently, several other techniques have been recommended for more efficient elimination of calcium hydroxide medicament such as the use of vibratory file, canal brush, irrigation syringe, EndoVac, sonics, and passive ultrasonics. Of all these methods, passive ultrasonics has been found to produce cleaner canals.\(^6\) This is accomplished with a small file vibrated in a previously shaped root canal to produce acoustic streaming that transfers its energy to the irrigant inside the canal.

Various methods have been employed to measure the remaining calcium hydroxide residues in the root canal. These are stereomicroscopy, scanning electron microscopy, spiral computed tomography (CT), etc.\(^7\) Recently, cone beam computed tomography (CBCT) has been found to be more superior. In this method, three-dimensional volume measurements are possible without sectioning the specimens, thus avoiding the loss of specimens during the process. Hence, it is a more accurate and faster method.

The aim of this in vitro study was to assess the efficiency of three calcium chelators, 17% EDTA solution, and 0.2% chitosan in combination with ultrasonic agitation against calcium hydroxide medicaments mixed with two different vehicles. The two formulations used in the study were the commercially available calcium hydroxide paste in silicone oil and freshly mixed Ca(OH)\(_2\) powder in distilled water. The volume of Ca(OH)\(_2\) removed was analyzed with CBCT.

**SUBJECTS AND METHODS**

Twenty-eight extracted single canaled mandibular premolars free of fracture, cracks, or any other defects were selected. Access cavities were prepared and the root canals were subjected to chemomechanical preparation with the crown down technique using ProTaper files (Densply-Mailiefer, Ballaigues, Switzerland) till F4 and 2 ml of 5.25% NaOCl was used as an irrigant after each instrument and 5 ml of 17% EDTA for final flush. Canals were dried with paper points (Densply-Mailiefer, Ballaigues, Switzerland).

Two formulations of Ca(OH)\(_2\), with different vehicles were selected. Metapex (Meta Dental Corp. Ltd., Elmhurst, NY, USA) a commercially available product is composed of Ca(OH)\(_2\), silicone oil, and iodoform. The chemically pure (95%) Ca(OH)\(_2\) powder (Merck India Ltd., Mumbai, India) was mixed with distilled water in the 1:1 ratio and bismuth trioxide was added in the ratio of 1:8 by weight for radiopacity.

The teeth were divided into two groups of 14 teeth each:

1. **Group 1:** Metapex was injected into the root canal until the material extruded through the apex
2. **Group 2:** Ca(OH)\(_2\) mixed with distilled water was placed into the canals with lentulo spiral until the material extruded through the apex.

Teeth were held in wet sponge during placement of Ca(OH)\(_2\) formulations. Excess material was wiped off with moist cotton. The access cavities were temporarily sealed with a cotton pellet and Cavit and were stored at 37°C and 100% relative humidity for 7 days. Subsequently, the teeth were mounted in a modeling wax for the purpose of CBCT.

After CBCT imaging, the volume of the filled material in each tooth was estimated in the coronal section using the OnDemand 3D software (Cybermed Inc., Korea). The teeth in each group were held in the wet sponge and further randomly divided into three subgroups on the...
basis of irrigant used for removal. A 30 gauge endodontic needle was used for irrigation at 2 mm from the working length. Ultrasonic agitation was performed with a Satelec ultrasonic endodontic tip K15 Sonofile (Dentsply, Tulsa, OK, USA) in endomode of ultrasonic unit (Woodpecker Dte-D5 ultrasonic scaler, China).

The teeth in each group were further randomly divided into three subgroups on the basis of irrigant used for removal.

1. Group 1A (n = 6): Metapex retrieved with 1 ml of 17% EDTA + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water
2. Group 1B (n = 2): Metapex retrieved with 1 ml of Citric acid + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water
3. Group 1C (n = 6): Metapex retrieved with 1 ml of 0.2% Chitosan solution + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water
4. Group 2A (n = 6): Ca(OH)$_2$ retrieved with 1 ml of 17% EDTA + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water
5. Group 2B (n = 2): Ca(OH)$_2$ retrieved with 1 ml citric acid + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water
6. Group 2C (n = 6): Ca(OH)$_2$ retrieved with 1 ml of 0.2% Chitosan solution + ultrasonic agitation for 1 min + final rinse with 1 ml of distilled water.

For the preparation of 0.2% chitosan solution, 0.2 g of chitosan (Acros organics, 90% degree of deacetylation) was diluted with 100 ml of 1% acetic acid and the mixture was stirred for 2 h using a magnetic stirrer. The pH of the solution was measured using a digital pH meter and was adjusted to 3.2.

A second CBCT was done and the volume of remaining material in each tooth was estimated as before.

**Outcome assessment**

The calculation of Ca(OH)$_2$ volume in each specimen was performed using the OnDemand 3D software (Cybermed Inc., Korea). Volume of Ca(OH)$_2$ was expressed as a cubic mm. The removal efficiency was calculated as \( \frac{|a-b|}{a} \times 100\% \), where “a” was the volume of the material packed in the root canal and “b” was the volume remaining after retrieval. The data were statistically analyzed using independent t-test and Mann–Whitney U-test.

**RESULTS**

The removal efficacy of aqueous and oil-based Ca(OH)$_2$ removed by 0.2% chitosan was more (95% and 92%) than for 20% citric acid (86% and 79%) followed by 17% EDTA (74% and 76%) [Figures 1 and 2]. All the three chelators (17% EDTA, 20% citric acid, and 0.2% chitosan) removed the aqueous-based Ca(OH)$_2$ significantly better \((P < 0.05)\) than oil-based Ca(OH)$_2$.

**DISCUSSION**

Thorough instrumentation supported by irrigation reduces the number of microorganisms in the infected root canal (Bystrom and Sundqvist 1985). However, the eradication of microorganisms from canal irregularities is enhanced by intracanal medicaments that prevents the proliferation of residual strains, as well as recontamination (Chong and Pitt Ford 1992).

Calcium hydroxide is the most popular intracanal medicament till date, owing to its well documented antibacterial activity against most of the strains identified in the root canal infections (Law and Massner 2004).

Prior to obturation, the canal space must be thoroughly debrided of calcium hydroxide as residual calcium hydroxide can negatively influence the outcome of obturation.

*In vitro* studies have shown that remnant calcium hydroxide can:

- Hinder the penetration of sealers into the dentinal tubules
• Markedly increase the apical leakage of root canal treated teeth, and potentially interact with zinc oxide eugenol sealers and make them brittle and granular.[9-11]

Intracanal calcium hydroxide is usually removed from the root canal by the use of copious manual irrigation with either sodium hypochlorite combined with hand instrumentation and a final rinse with EDTA. However, these techniques are inefficient to completely eliminate calcium hydroxide.

Although EDTA helps in the removal of smear layer and it also chelates calcium from calcium hydroxide intracanal medicaments, studies have shown it to be more erosive on the dentin as compared to other chelating agents. Hence, other calcium chelators were used in this study, namely citric acid and chitosan along with passive ultrasonic irrigation.

Studies have shown that the type of vehicle used can affect the calcium hydroxide retrieval from the root canal.[12] While the aqueous form of calcium hydroxide medicament can be removed more efficiently, paste-based calcium hydroxide medicaments are reported to be more difficult to eliminate. Therefore, two different vehicles were used in the study, distilled water and silicone oil.

Various methods have been employed for assessing calcium hydroxide remnants after the removal of calcium hydroxide from the root canal. One such technique requires longitudinal sectioning of the specimen and digital imaging to measure the surface area covered by calcium hydroxide. Lambrianidis et al[12] and Kenee et al[13] used scanning electron microscopy for evaluation. The disadvantage of these techniques is that they analyze surface area covered with calcium hydroxide, not the volume. In addition, these require sectioning of tooth which may cause loss of material and may influence the results. In the present study, CBCT was used for the calculation of remaining calcium hydroxide as this technique not only enables volumetric analysis but also no sectioning is required and therefore no loss of material. This is also reported to be a superior method by various researchers.[14]

The results of the present study demonstrated that none of the chelating agents tested were able to remove calcium hydroxide completely irrespective to the type of vehicles used. This is accordance with Nandini et al., Kenee et al., and Kontakiotis et al.[15]

The result of the present study also emphasizes the fact that vehicle used to mix calcium hydroxide paste is important for complete retrieval. All the three calcium chelators 0.2% chitosan, 17% EDTA and 20% citric acid solution removed aqueous based Ca (OH)₂ more effectively than oil-based Ca(OH)₂.

Silicone oil which was the oily vehicle present might have restricted its dissolution and removal from the root canal by the tested chelators. The 20% citric acid performed better in comparison to 17% EDTA solution in the removal of Metapex. This probably could be because citric acid is able to penetrate the silicone oil better in comparison to EDTA and chelates the calcium ions. However, 0.2% chitosan performed better than 17% EDTA and 20% citric acid solution in the removal of both aqueous-based and oil-based Ca(OH)₂. This is in accordance to the studies by Nandini et al[7] and Ballal et al.[16] The remaining calcium hydroxide was found to be packed mainly in the apical thirds of about 2–3 mm.[7]

The chelating mechanism of chitosan on dentin has previously not been well documented. However, this bioactive biopolymer is widely used as a chelating agent to absorb heavy metals from waste water. Two theories have been used to explain the chelating mechanism of chitosan. First, the bridge model states that two or more amino groups of chitosan bind to the same metal ion. Second, the pendant model suggests that one amino group is utilized in the binding, and the metal ion is linked to the amino group like a pendant. Either of the two mechanisms could be responsible for the chelation of calcium ions in dentin resulting in the depletion of inorganic matter from the smear layer.[17]

Regardless of the vehicle used in the present study, remnants were found in the apical region and also on the root canal walls. Although an apical plug of calcium hydroxide has been advocated for its prolonged antimicrobial activity, after obturation (Holland 1984), it is preferable to remove it as it might enhance the apical leakage, due to dilution of tissue fluids.

The present study confirms the role of passive ultrasonic irrigation as a superior method of removing calcium hydroxide from the root canals. Since PUI is done with a small size file (#15 file), in an already enlarged canal space, the file moves freely allowing the irrigant to penetrate more easily into all regions of the root canal space. Due to active streaming of the irrigant, its potential to contact a greater surface area of the canal wall is enhanced. Moreover, a large volume of the irrigant allows better debridement. This could be the reason for the superior performance of passive ultrasonic irrigation as opposed to manual irrigation. This, which has also been supported by previous studies done by van der Sluis et al.[18] and Sandra et al.[19]

CONCLUSION

• None of the chelators in combination with ultrasonics was able to completely remove the calcium hydroxide. 0.2% Chitosan in combination with ultrasonics performed better than 17% EDTA and 20% citric acid in
removal of Ca(OH)$_2$.  
• Combination of ultrasonic agitation with chelator results in cleaner canal for both aqueous-based, as well as oil-based Ca(OH)$_2$.  
• The vehicle used to prepare calcium hydroxide influences its retrieval  
• Oil-based calcium hydroxide is more difficult to remove than powder form calcium hydroxide mixed with distilled water  
• Regardless of the vehicle used in the present study, remnants were found mainly in the apical region and also on the root canal walls.

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Conflicts of interest 
There are no conflicts of interest.

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