Original Article

Comparative evaluation of removal of oil-based calcium hydroxide intracanal medicaments with two calcium chelators: An *in vitro* cone-beam computed tomography volumetric analysis

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Abstract

Background: Intracanal medicament (ICM) eliminates remaining bacteria and their toxins that were not removed by chemomechanical preparation during endodontic treatment.

Aim: The aim of this study was to compare and evaluate the removal of ozonated oil-based, silicone oil-based, and distilled water-based ICM with two calcium chelators, i.e., 0.2% chitosan and 17% ethylenediaminetetraacetic acid (EDTA).

Materials and Methods: A total of 54 mandibular permanent premolars were included and randomly allocated into 3 groups after cleaning and shaping along with thorough irrigation. Group 1 - ozonized calcium hydroxide group; Group 2 - Metapex group; Group 3 - $Ca(OH)_2$ with distilled water group. Using an irrigant for removal, the teeth in each group were distributed at random to two subgroups – (A) 0.2% chitosan solution; (B) 17% EDTA solution. All the samples were ultrasonically agitated and the volume remaining in each tooth after retrieval was estimated using additional cone-beam computed tomography scans. The result was analyzed using one-way analysis of variance, following *post hoc* Tukey test.

Results: The mean percentage of ozonized $Ca(OH)_2$, aqueous calcium hydroxide, and Metapex removed by 0.2% chitosan had greater values in comparison to 17% EDTA. When eliminating aqueous-based calcium hydroxide, both chelators showed similar effectiveness ($P > 0.05$). In contrast, 0.2% chitosan outperformed 17% EDTA in retrieving oil-based $Ca(OH)_2$ ($P < 0.05$).

Conclusion: The aqueous-based form of $Ca(OH)_2$ was removed more easily compared to the oil-based form. A combination of 0.2% chitosan and ultrasonics proved to be more effective than 17% EDTA in eliminating oil-based calcium hydroxide.

Keywords: Cone-beam computed tomography; chitosan; ethylenediaminetetraacetic acid; ozonized calcium hydroxide; retrieval

INTRODUCTION

Root canal treatment essentially aims to prevent pulp and periradicular tissue pathologies. The success of treatment relies on eliminating microorganisms.[1] Even after undergoing a thorough cleaning and shaping regimen, there is a possibility that not all bacterial habitats are eliminated. Bacterial growth between appointments can be reduced using an appropriate intracanal medicament (ICM). ICM eliminates remaining bacteria and their toxins that were not eliminated by chemomechanical instrumentation.[2]

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Date of submission : 07.08.2024
Review completed : 09.09.2024
Date of acceptance : 16.09.2024
Published : 11.11.2024

Access this article online	
Quick Response Code:	Website: https://journals.lww.com/jcde
	DOI: 10.4103/JCDE.JCDE_561_24

How to cite this article: Bajaj H, Nair R, Ganorkar O, Dole S, Parakh S, Singh ND. Comparative evaluation of removal of oil-based calcium hydroxide intracanal medicaments with two calcium chelators: An *in vitro* cone-beam computed tomography volumetric analysis. J Conserv Dent Endod 2024;27:1163-7.

Various intracanal medications are used in dental procedures, with calcium hydroxide ($Ca[OH]_2$) being the most preferred option. It has been associated to various biological properties, including its tissue disintegration ability, antimicrobial effects, prevention of tooth resorption, and promotion of hard tissue development during the repair process.[1]

The selection of vehicle for $Ca(OH)_2$ plays a significant role in its dissociation into calcium and hydroxyl ions, as it determines the ionic dissociation rate, thereby affecting the solubilization and absorption rates of the paste.[3] Recently, ozonated olive oil has been used as a vehicle with calcium hydroxide to increase its antimicrobial properties. The increased viscosity of ozonated olive oil allowed slower ionic liberation with gradual release of calcium ions even after 15 days.[4,5]

Before obturation, ICM should be completely eliminated from the canal walls by copious irrigation with the ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl), as the presence of any residual $Ca(OH)_2$ affects both sealer penetration and the strength of the dentinal bond.[6] Lambrianidis *et al*. found that $Ca(OH)_2$ still covered 45% of the surface area of the canal after the ICM was removed.[7] Therefore, the use of various calcium chelators has been investigated for more effective cleaning of the canal.

The natural polysaccharide chitosan, found in the shells of crabs or shrimps resulting from the deacetylation of chitin, possesses qualities such as biocompatibility, bioadhesion, biodegradability, and nontoxicity.[8] Chitosan exhibits chelation properties similar to citric acid and EDTA with fewer adverse effects, emphasizing its potential as a valuable tool in endodontics.[9,10]

Several techniques, including the use of irrigation syringes, vibratory files, canal brushes, EndoVac, sonics, and passive ultrasonics, were demonstrated to aid in the recovery of calcium hydroxide. Passive ultrasonics has proven to be the most successful of these methods, resulting in the cleanest canals.[11]

Many researchers have evaluated the retrievability of calcium hydroxide; however, there is limited literature available related to the retrievability of ozonated oil-based $Ca(OH)_2$. Therefore, this investigation was carried out to evaluate the removal efficacy of ozonated oil-based, silicone oil-based, and distilled water-based $Ca(OH)_2$ ICMs using two calcium chelators, namely, 0.2% chitosan and 17% EDTA along with ultrasonic irrigation. The analysis of the volume of calcium hydroxide removed was evaluated using cone-beam computed tomography (CBCT).

In the present study, the null hypothesis stated that there is no difference in residual volumes when using two calcium chelators for the removal of water- and oil-based calcium hydroxide.

MATERIALS AND METHODS

Institutional ethical committee approval was obtained for this *in vitro* study, ref: CDCRI/DEAN/ETHICSCOMMITTEE/CONS-03/2022. The study included 54 extracted lower premolars with a single root and canal that were extracted due to orthodontic reasons. The samples were collected from the department of oral and maxillofacial surgery. Teeth with defects such as cracks or fractures were not included. All the teeth samples were separated from their crowns to form standardized root samples with an average length of 14 mm. To confirm the patency of the canal, #10 K-file was inserted slightly beyond the apex, after which 1 mm was subtracted from this measurement to obtain the working length. To prepare the canals, ProTaper rotary file was used (Densply-Mailiefer, Ballaigues, Switzerland) till F4 (40, taper 0.06) with crown down technique. After each instrument, irrigation was done by 2 ml of 5.25% NaOCl and then by 5 ml of 17% EDTA which was kept for at least 1 min to irrigate the root canals after final preparation. Paper points (Densply-Mailiefer, Ballaigues, Switzerland) were used to dry the canals after the final rinse with 10 ml of normal saline.

The samples were divided according to vehicles employed for carrying Ca(OH)2. Three groups of 18 samples each were chosen.

- Group 1 - Ozonized Ca(OH)2 group-prepared by combining 1 mg of calcium hydroxide (ProDent, India, Woodpecker) with 2 mL of ozonated oil (ADC INC. Dentozoneindia, Navi Mumbai), barium sulfate (Burgoyne Burbidges and Co, Mumbai, Maharashtra, India) was added for radiopacity (BaSO4:Ca(OH)2 ratio 1:8). The material was then placed into the canals using a lentulospiral until the point where it extruded from the apical foramen
- Group 2 –Metapex group (Meta Biomed, Korea) - material was injected until it was forced out through the apical foramen
- Group 3 – Calcium hydroxide with distilled water group-prepared by mixing distilled water with 8 mg of calcium hydroxide and 1 mg of barium sulfate. The material was then placed in the canals using a lentulospiral until it extruded from apical foramen.

Excess material was removed using wet cotton. A cotton pellet and temporary restorative material (Dental Avenue, Mumbai, India) were used for sealing the access cavities. Following that, the samples were stored for 7 days in an environment with 100% relative humidity, at 37° C. For CBCT imaging, teeth were randomly embedded in U-shaped wax rims, with 2 mm of modeling wax surrounding each tooth to simulate soft tissue and enhance image contrast.

Using OnDemand 3D software, the amount of filled material in the tooth was determined following CBCT imaging (Cybermed Inc., Korea) [Figure 1]. Then, the intracanal medication was removed by ultrasonic agitation of the irrigants using a #15U file (Mani, Japan), using the endomode of an ultrasonic unit (Woodpecker Uds E Led Ultrasonic Scaler, China). For irrigation, a 30-gauge single-side vented needle that was 2 mm shorter than the working length was utilized.

Using an irrigant for removal, the samples in each group were distributed at random to two subgroups.

1. Group 1A – Ozonized calcium hydroxide was removed with 1 ml of 0.2% chitosan solution
2. Group 1B – Ozonized calcium hydroxide was removed with 1 ml of 17% EDTA solution
3. Group 2A – Metapex was removed with 1 ml of 0.2% chitosan solution
4. Group 2B – Metapex was removed with 1 ml of 17% EDTA solution
5. Group 3A – Calcium hydroxide was removed with 1 ml of 0.2% chitosan solution
6. Group 3B – Calcium hydroxide was removed with 1 ml of 17% EDTA solution.

All the samples were ultrasonically agitated for 1 min, then, final rinsing was done with 1 ml of distilled water. The volume remaining in each tooth after retrieval was estimated using additional CBCT scans [Figure 2], performed with the same approach as previously described.

Outcome assessment

The calcium hydroxide volume was measured in cubic millimeters. Retrievability was determined by the formula ([$a - b$]/a] $\times$100, here, "a" represents the initial volume of the material in the canal, while "b" denotes the remaining volume of the material. Analysis of the result was done using one-way analysis of variance, following *post hoc* Tukey test.

RESULTS

Results were expressed in terms of mean and standard deviation. Statistical significance was set at $P < 0.05$. The mean percentage of ozonized $Ca(OH)_2$, aqueous calcium hydroxide, and Metapex removed by 0.2%

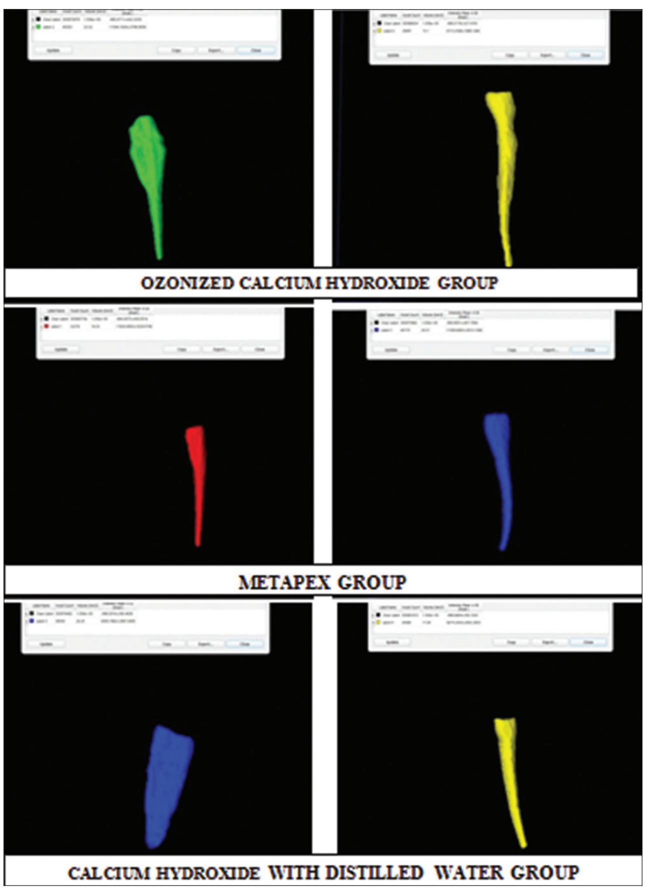

Figure 1: Initial volume calculation of $Ca(OH)_2$

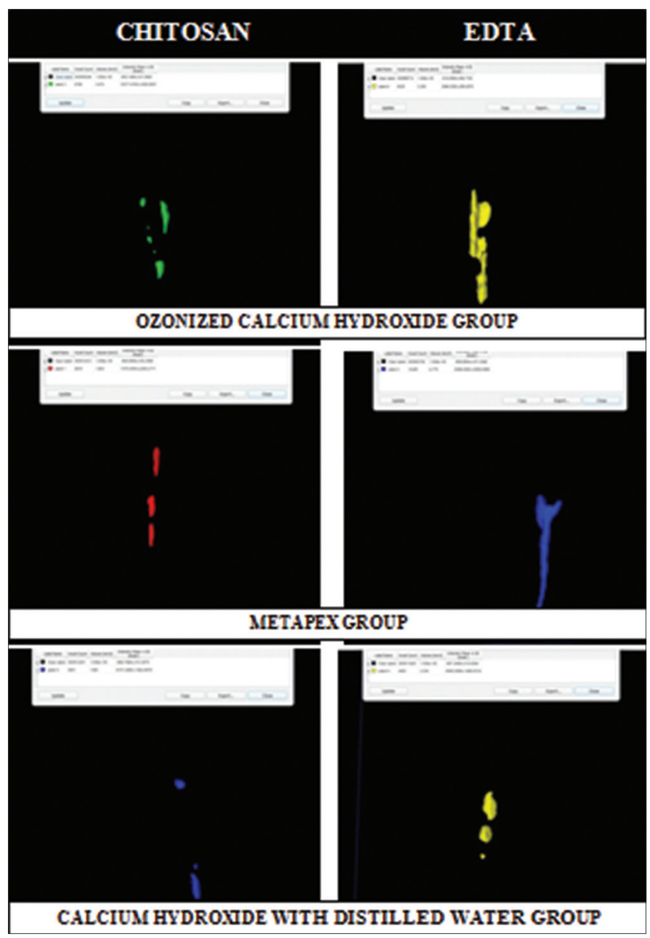

Figure 2: Volume calculation after retrieving $Ca(OH)_2$

Chitosan had greater values in comparison to 17% EDTA. When eliminating aqueous-based calcium hydroxide, both chelators showed similar effectiveness ($P > 0.05$). In contrast, 0.2% chitosan outperformed 17% EDTA in retrieving oil-based $Ca(OH)_2$ ($P < 0.05$) [Tables 1 and 2]. Moreover, ozonized calcium hydroxide showed slightly better retrievability compared to Metapex.

DISCUSSION

ICM aims to eliminate remaining bacteria, inhibit the growth of bacteria in between treatments, and function as physiochemical barrier to prevent canal reinfection and the supply of nutrients to any remaining bacteria.[12] Due to their prolonged presence compared to irrigants, medicaments can effectively target bacteria in inaccessible areas of root canals.[13]

In the present study, the null hypothesis was rejected as there was a difference in residual volumes of ozonized calcium hydroxide, Metapex, and water-based calcium hydroxide when retrieved with 17% EDTA and 0.2% chitosan.

As calcium hydroxide is the most frequently used ICM, thus it was selected for the study. For calcium hydroxide to be effective, it should be appropriately compacted within the canal space, typically in conjunction with a suitable carrying vehicle.[14] The vehicle that is employed to mix calcium hydroxide significantly affects the rate of ionic dissociation, but its impact on retrievability is uncertain.[3] Therefore, the search for the best vehicle has led to the use of ozonized olive oil, silicone oil (Metapex), and distilled water.

Table 1: Mean and standard deviation obtained from various groups

	n	Mean	SD	SE	95% CI for mean	
					Lower bound	Upper bound
Group 1A	9	84.68	3.07	1.02350	82.3276	87.0480
Group 1B	9	69.68	9.36	3.12206	62.4904	76.8893
Group 2A	9	81.35	4.82	1.60741	77.6521	85.0654
Group 2B	9	64.79	5.69	1.89726	60.4240	69.1741
Group 3A	9	95.37	2.81	0.93875	93.2065	97.5361
Group 3B	9	90.65	4.02	1.34147	87.5581	93.7450

SE: Standard error, SD: Standard deviation, CI: Confidence interval

Table 2: *Post hoc* Tukey for multiple comparisons between the group

(I) Groups–(J) Groups	Mean difference (I−J)	Significant (*P*<0.05)
Group 1A–Group 2A	3.32904	0.783
Group 1A–Group 3A	−10.68353*	0.002
Group 2A–Group 3A	−14.01257	0.000
Group 1B–Group 2B	4.89079	0.408
Group 1B–Group 3B	−20.96172*	0.000
Group 2B–Group 3B	−25.85251*	0.000
Group 1A–Group 1B	14.997	0.000
Group 2A–Group 2B	16.559	0.000
Group 3A–Group 3B	4.719	0.448

*$P<0.05$ is significant

In the present study, Ozonized olive oil was selected as a vehicle, as it increases the alkalinity and antibacterial activity of calcium hydroxide. The reason for this is, firstly, the increase in viscosity leading to slower ionic liberation and secondly, the increased release of calcium ions suggesting higher mineralization activity postozonation.[5]

Several irrigants, such as, saline, EDTA, sodium hypochlorite, citric acid as well as malic acid are utilized to eliminate calcium hydroxide. 0.2% chitosan demonstrated chelation activity comparable to that of 10% citric acid and 15% EDTA.[15] Silva *et al*.[15] examined the effect of various concentrations of chitosan on the dentin surface and the eradication of smear layer and concluded that 0.2% chitosan showed best results. Thus, the present investigation examined 0.2% concentration of chitosan.

Furthermore, various methods are employed such as hand files, rotary files, canal brushes, irrigation syringes, sonics, Endovac, and passive ultrasonics to eliminate calcium hydroxide, with passive ultrasonics proving to be the most effective method.[11,16,17] As a result, passive ultrasonics was utilized in the research.

Techniques that were used to examine $Ca(OH)_2$ remnants are digital imaging, scanning electron microscopy, and longitudinal sectioning.[7,16,18] These techniques have the disadvantage of examining only the area covered by calcium hydroxide and not the volume. Nandani *et al*.,[19] Wiseman *et al*.,[17] and Ballal *et al*.[20] used CBCT as it allows for volumetric analysis and eliminates the need for sectioning; therefore, the current study calculated the residual calcium hydroxide using CBCT.

The current study shows that, regardless of the vehicle employed, none of the chelating agents investigated could totally remove the $Ca(OH)_2$. This is contrary to De Faria-Júnior *et al*.[21] and in accordance with Nandini *et al*.,[19] Kenee *et al*.,[16] and Kontakiotis *et al*.[22]

Both the chelators removed the water-based form of calcium hydroxide significantly better than the oil-based form. This might be ascribed to reduced penetration of chelating and irrigating solutions through the oil layer.[19] Oil-based calcium hydroxide showed significantly ($P < 0.05$) better retrievability with chitosan than EDTA. This may be due to better oil penetration of 0.2% chitosan, which facilitates chelation more effectively than 17% EDTA, which preferentially chelates calcium ions in water.[10] Furthermore, it was observed that ozonized calcium hydroxide showed slightly better ($P > 0.05$) retrievability than silicone oil-based calcium hydroxide. This can be due to the fact that the density of silicone oil (0.96 kg/m^3)[23] is more than that of ozonized olive oil (0.9343 kg/m^3).[24] Furthermore, the surface tension value of olive oil is 33.377 dynes/cm, which is in between the values of distilled water and silicone oil.[25] This may account for the intermediate retrieval values for ozonized olive oil-based $Ca(OH)_2$.

The limitation of this study was the selection of large, single-rooted mandibular premolars. The results may differ for posterior teeth with narrow, curved canals because it can be difficult to reach the apical third area of the canals. The radio-opacity detected in the CBCT was the only source of data used in the investigation and was not confirmed with SEM. Further research is needed to examine the retrievability of ozonized olive oil with different chelators.

CONCLUSION

Both the tested chelators were ineffective to remove $Ca(OH)_2$ completely from the canals, regardless of the vehicle used. The aqueous-based form of $Ca(OH)_2$ was removed more easily compared to the oil-based form. A combination of 0.2% chitosan and ultrasonics proved to be more effective than 17% EDTA in eliminating oil-based calcium hydroxide. In addition, ozonized calcium hydroxide demonstrated slightly better retrievability than Metapex.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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