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Comparison of Various Irrigation Techniques for the Removal of Silicone Oil-Based Calcium Hydroxide Intracanal Medicament from the Apical Third: An SEM Study

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Abstract

Objective To evaluate the efficacy of various irrigants (5.25% sodium hypochlorite [NaOCl] followed by 17% ethylenediaminetetraacetic acid [EDTA] and 0.2% chitosan nanoparticle [NP]) and different irrigation techniques (conventional and EndoVac) for the removal of Metapex from the apical third of the root canal by scanning electron microscopy (SEM) analysis.
Materials and Methods Forty extracted single-rooted human premolars were instrumented using a rotary ProTaper file system up to F3. The canals were dried and Metapex was placed inside the root canal. The access cavity was sealed with a cotton pellet and Cavit followed by the storage of specimens at 37°C at 100% humidity for 1 week. Cavit was removed and the samples were divided into: Group 1 (conventional irrigation using 5.25% NaOCl and 17% EDTA, $n = 10$), Group 2 (conventional irrigation using 0.2% chitosan NP, $n = 10$), Group 3 (EndoVac using 5.25% NaOCl and 17% EDTA, $n = 10$), and Group 4 (EndoVac using 0.2% chitosan NP, $n = 10$). After irrigant activation, the roots were divided in half, and the apical third of one-half of each tooth was subjected to SEM analysis. A five-graded scoring scale was used to evaluate dentinal wall cleanliness. Data were statistically analyzed using one-way analysis of variance.
Results There were statistically significant differences in mean canal cleanliness scores observed between the four groups. Chitosan NPs showed lower mean canal cleanliness scores at the apical one-third of the root canal indicating better cleanliness when compared with the other irrigants and techniques.
Conclusion None of the irrigation techniques was successful in entirely removing the calcium hydroxide intracanal medicament from the apical third.

Keywords
- calcium hydroxide
- cetrimide
- chitosan
- EndoVac
- intracanal medicament

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Introduction

Root canal treatment (RCT), the primary goal of endodontic treatment, is to prevent and control pulpal and periradicular infections. Given the importance of microbes in the etiology of these infections, it is evident that reducing or eliminating them is critical to the success of endodontic therapy.[1] While mechanical preparation of infected root canals may remove microorganisms, some bacteria hide in isthmuses, apical deltas, dentinal tubules, and ramifications due to the root canal system's complexity. Thus, intracanal medicaments could help eliminate these hidden bacteria.[2]

Calcium hydroxide ($Ca(OH)_2$) is currently the most extensively used intracanal medication. Due to its well-documented antibacterial efficacy against most endodontic infections, it is extensively used as a root canal dressing between treatment sessions. The highly alkaline environment created by $Ca(OH)_2$ is unfavorable to most bacteria.[3]

$Ca(OH)_2$ can be injected inside the root canal using a variety of carriers, including water, carboxymethyl cellulose, glycerin, and silicone oil. The physical and chemical properties of $Ca(OH)_2$ are affected by the type of vehicle used. Calcium and hydroxyl ions are released quickly in aqueous vehicles, whereas ionic dissociation takes longer in oily vehicles.[4]

Despite the efficacy of $Ca(OH)_2$ as an intracanal medicament, certain bacterial species have been observed to be resistant to total eradication, including *Enterococcus faecalis*.[2] In these scenarios, $Ca(OH)_2$ paste with a silicone oil-based carrier containing 38% iodoform (Metapex [Meta Biomed, Korea]) is preferred over $Ca(OH)_2$ alone because it disinfects dentinal tubules infected with *E. faecalis* better.[5]

Before starting the obturation, the $Ca(OH)_2$ medicament should be removed to avoid any interference with the obturating material and to permit maximum adherence between the sealer and the root canal wall.[6] Silicone oil-based $Ca(OH)_2$ is more difficult to remove than aqueous-based $Ca(OH)_2$ due to lesser penetration of the irrigant through the silicone oil layer.[7]

It has been demonstrated that eliminating $Ca(OH)_2$ intracanal medication requires sodium hypochlorite (NaOCl) (5.25%) followed by a final rinse of ethylenediaminetetraacetic acid (EDTA) (17%).

For the removal of the remaining $Ca(OH)_2$ from the root canal, a variety of root canal irrigants, including saline, NaOCl, and EDTA, can be used alone or in conjunction with several techniques, including EndoVac, EndoActivator, CanalBrush, Max-i-Probe needle, and passive ultrasonic irrigation in conjunction with hand instrumentation.[8]

Chitosan nanoparticle (NP) is a nontoxic, biocompatible, biodegradable, and bioadhesive natural polysaccharide produced by deacetylating chitin.[5] Similar to EDTA and citric acid, it displays chelation characteristics but with significantly fewer negative effects.[9] The present study's purpose was to compare the efficacy of two irrigants (5.25% NaOCl followed by a final rinse of 17% EDTA, 0.2% chitosan NPs) and two techniques (conventional irrigation, EndoVac) for the removal of silicone oil-based $Ca(OH)_2$ medicament (Metapex) from the apical third of the root canal.

Materials and Methods

Sample size was determined based on a pilot study, by calculating mean and standard deviation. Ethical clearance was provided by institutional ethical committee (ABSM/EC66/2019) dated October 15, 2019. Forty extracted single-rooted human premolars extracted for orthodontic purposes were collected. The teeth included were caries free and with a completely formed apex. The specimens were cleaned of soft tissue and calculus using an ultrasonic device. A diamond disc (Confident Dental Equipments Ltd., India) was used to decoronate each of the 40 samples to produce a standardized root length of 15 mm. The study of Alturaiki et al formed the basis for the methodology. Root canal patency was established with a No. 15 K file (Dentsply Maillefer, Switzerland).[10] The working length (WL) was kept 1 mm short of the apex. The root canals were prepared using ProTaper rotary files till F3 (Dentsply Maillefer).

Canals were routinely cleaned with 5 mL of 17% EDTA (Anabond Stedman, Chennai, India), 5 mL of distilled water, and 5 mL of 5.25% NaOCl (Coltene, Switzerland) between each instrument. The needle was moved up and down inside the apical third during irrigation. After the canal had been dried with paper points, the access cavity was sealed with a cotton pellet and Cavit. The Metapex was then inserted into the root canal until it came out from the apex. The samples were kept for a week at 37°C and 100% humidity.

Chitosan NP was dissolved in 100 mL of 1% acetic acid, yielding a 0.2% chitosan NP solution. The provisional restoration was removed and the samples were randomly divided into four groups:

- Group 1 (conventional irrigation using 5.25% NaOCl and 17% EDTA, $n = 10$): The removal of CH was performed with a conventional irrigation needle with 5 mL of 5.25% NaOCl followed up by 5 mL of 17% EDTA. For 60 seconds, a continuous flow was applied while moving the needle up and down within the apical third. By positioning the rubber stopper 1 to 2 mm short of the operating length, the needle depth was standardized for each canal.
- Group 2 (conventional irrigation using 0.2% chitosan NPs, $n = 10$): The CH removal was done with 5 mL of 0.2% chitosan NPs and a conventional irrigation needle with the same technique as described earlier.
- Group 3 (EndoVac using 5.25% NaOCl and 17% EDTA, $n = 10$): Using the EndoVac device, the canals were irrigated for 30 seconds with each solution, 5 mL of 5.25% NaOCl and 5 mL of 17% EDTA.
- Group 4 (EndoVac using 0.2% chitosan NPs, $n = 10$): CH removal was performed using 0.2% chitosan NPs in combination with the EndoVac system for 60 seconds.

The decoronated teeth are longitudinally sectioned before scanning electron microscopy (SEM) analysis following pretreatment and Metapex implantation. A similar methodology is used in this investigation as well. It is claimed that longitudinal sectioning will result in a more precise measurement of the root canal residuals.[11]

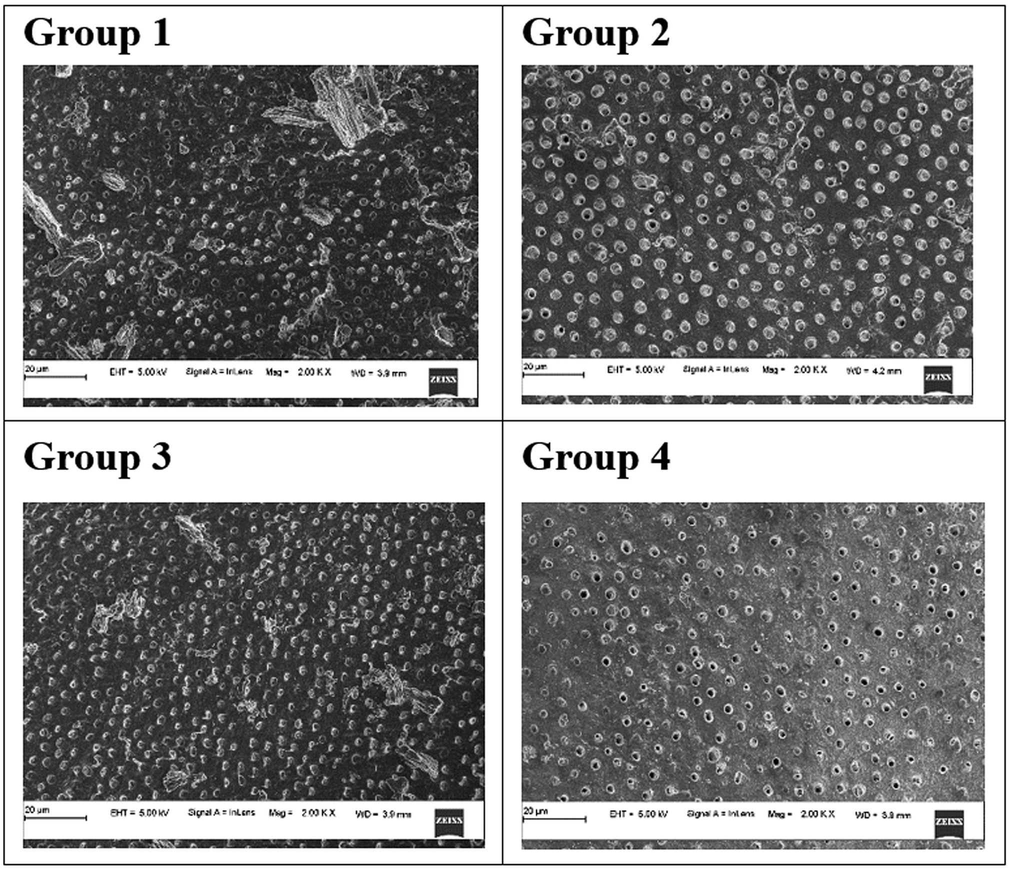

Fig. 1 Scanning electron microscopy images of the apical third of the root canal at ×2,000 magnification.

After irrigant activation, the roots were divided into two halves buccolingually without perforating the root canal and SEM analysis was performed for one half of each tooth at a magnification of ×2,000 (Carl Zeiss Sigma FESEM 03-81) at the apical third (►**Fig. 1**). For SEM analysis, the samples were dehydrated, then fixed on aluminum stubs, and finally coated with gold sputtering. Two endodontists who were blinded to the samples being tested evaluated the SEM photomicrographs for cleanliness. The degree of $Ca(OH)_2$ medication removal and dentinal wall cleaning was assessed using a five-grade scoring system.[10] They are as follows: Score 1: 80 to 100% CH removal (total cleanliness); Score 2: 60 to 80% CH removal (great cleanliness); Score 3: 40 to 60% CH removal (partial cleanliness); Score 4: 20 to 40% CH removal (light cleanliness), and Score 5: 0 to 20% CH removal (no cleanliness).

Results

The study followed the protocol of double blinding during the whole procedure, where each sample was examined by two people blindly and evaluated. Sample size was 10 per group. As four groups were included in this study, it gave an overall sample size of 40. Where between group variance was 0.83, power was 80% and an α error of 5%, which gave the required sample size of 40, where sample size per group is 10.

Since this was a qualitative analysis, chi-square test was done. The *p*-value associated with the chi-square test is less than 0.001, which is highly significant (►**Table 1**). To compare the difference between each group, one-way analysis of variance was done. On comparison of the degree of $Ca(OH)_2$ removal, the highest mean is seen in Group 1 (4.3) followed by Group 2 (3.5), Group 3 (3), and the least value in Group 4 (1.7). This parameter is statistically significant with a *p*-value of <0.001. To compare each group, post hoc analysis was done which indicates significant differences between Groups 1 and 3, Groups 1 and 4, Groups 2 and 4, Groups 3 and 4 with a *p*-value of less than 0.05 for each.

Chitosan NPs showed the least mean canal cleanliness scores at the apical one-third of the root canal when compared with the other irrigants along with EndoVac method (1.7 ± 0.675). A significant difference in mean canal cleanliness scores was observed in the four groups ($p = 0.00$).

The conventional needle irrigation method showed higher scores of canal cleanliness in the apical area when compared with the EndoVac method with the irrigating solutions used and a statistically significant difference was observed between the methods at $p < 0.05$.

Results of the study indicated that there is statistical difference between the values of the group with significant *p*-value. $Ca(OH)_2$ removal with EndoVac using 0.2% chitosan

Table 1 Depicting degree of calcium hydroxide removal

Degree of calcium hydroxide removal			Group				Total
			Group 1	Group 2	Group 3	Group 4	
	Score 1	Count	0	0	0	4	4
		% within group	0%	0%	0%	40%	10%
	Score 2	Count	0	0	2	5	7
		% within group	0%	0%	20%	50%	17.5%
	Score 3	Count	2	5	6	1	14
		% within group	20%	50%	60%	10%	35%
	Score 4	Count	3	5	2	0	10
		% within group	30%	50%	20%	0%	25%
	Score 5	Count	5	0	0	0	5
		% within group	50%	0%	0%	0%	12.5%
Total		Count	10	10	10	10	40
		% within group	100%	100%	100%	100%	100%

NPs has shown better results as compared with all the other groups.

Discussion

Intracanal medicaments reduce bacterial growth in the canal and prevent the spread of infection within the root canal by acting as a physiochemical barrier. $Ca(OH)_2$ is proven to be an effective intracanal medicament due to its excellent antimicrobial properties, neutralization of bacterial endotoxins, and in periapical tissue repair. The complete removal of intracanal medicaments might be impracticable many times due to the morphological variations of the root and canal wall irregularities. Therefore, in the current study, the extracted single-canal human premolars with straight roots were used.[8,12]

The residual intracanal medicament may serve as an irritant causing failure of endodontic treatment.[13] Additionally, when residual $Ca(OH)_2$ interacts with zinc oxide and eugenol-based sealers, calcium eugenolate is created, which is brittle in consistency and has a granulated structure due to which the sealer penetration is hindered.

Moreover, the interaction of residual $Ca(OH)_2$ with zinc oxide and eugenol-based sealers leads to the formation of calcium eugenolate. The former, being brittle with a granular structure, impedes the sealer penetration into the dentinal tubules, which can eventually impact the treatment outcome.[14] Hence, complete removal of the residual intracanal medicament before obturation of the root canals has been suggested.[15]

Aqueous vehicles are the most often utilized carriers for $Ca(OH)_2$ intracanal medication because they encourage quick ion liberation, while viscous and oily vehicles release calcium and hydroxyl ions over longer periods, minimizing the number of dressing-changing sessions. However, the use of intracanal medications with oily vehicles may leave a residue on the root canal walls that will make it harder for the sealer to attach to the walls, which will affect the treatment outcome.[16] A study done by Sokhi et al, to evaluate the effect of three CH-based intracanal medicaments on the apical sealing ability of AH Plus—gutta-percha obturation, concluded that the vehicle used to carry $Ca(OH)_2$ intracanal medication may significantly influence the apical sealing ability of gutta-percha—AH Plus obturated canals.[17,18]

Numerous studies have suggested the method of $Ca(OH)_2$ removal from the root canal walls.[8,10] The most commonly used clinical technique for the removal of $Ca(OH)_2$ intracanal medicament is by using the master apical file combined with numerous irrigants such as normal saline, NaOCl, EDTA, alone and in combination.[18,19]

In the present study, the objective was to assess the efficacy of 5.25% NaOCl followed by a final rinse of 17% EDTA and 0.2% chitosan NPs used with conventional irrigation and EndoVac for the removal of Metapex from the apical third of the root canal by SEM analysis. The outcomes showed that none of the techniques was successful in eliminating the $Ca(OH)_2$ medication from the apical third.

NaOCl has been commonly used in endodontics in various concentrations as the primary root canal irrigant due to its antibacterial activity against a wide array of microorganisms, including *Enterococcus*, *Actinomyces*, and *Candida* species, which are difficult to eliminate from the root canals. Based on the previous studies, the best irrigation protocol for the removal of $Ca(OH)_2$ intracanal medicament is by using 5.25% NaOCl and 17% EDTA.[8] During the canal preparation method, alternating between NaOCl and EDTA solutions decreases debris formation and results in cleaner canals, according to various studies.

Chitosan's chelating mechanism on dentin has not been well documented. This bioactive biopolymer, on the other hand, is commonly employed as a chelating agent to remove heavy metals from wastewater. The chelating mechanism of chitosan has been explained using two theories. To begin, the bridge model proposes that two or more chitosan amino groups are attached to the same metal ion. Second, the pendant model implies that just one amino group is used in the binding, and the metal ion is suspended from the amino group.[20] The chelation of calcium ions in dentin, which results in the loss of inorganic materials from the smear layer, could be caused by any of the two methods. A study, done to investigate the ability of bioactive chitosan NPs to remove the smear layer and inhibit bacterial recolonization on dentin, concluded that it could be used as a final irrigant during RCT with the dual benefit of removing the smear layer and inhibiting bacterial recolonization on root dentin.[21] Another study concluded that 17% EDTA is a potent chelating agent that can successfully remove the smear layer but compromises the Ca/P ratio of dentin. However, 0.2% chitosan and its NPs have comparable chelating effects and induce remineralization of the root canal dentin.[22]

Various methods such as capturing images using a digital camera, stereomicroscope, micro-computed tomography (CT), and cone beam CT have been used to assess the cleanliness of the root canal walls. Most of the studies assessed the amount of residual intracanal medicament in the canal walls by measuring the surface area of the residues on the canal walls. The amount covered is then scored, and estimated through SEM, or by volumetric analysis using spiral CT.[9]

SEM is considered a standard and the most reliable approach to examine and evaluate canal cleanliness after the removal of $Ca(OH)_2$ using various recent technologies along with irrigants. Nandini et al used volumetric analysis with spiral CT to evaluate $Ca(OH)_2$ elimination. The present work used SEM analysis to emphasize the condition of the dentinal tubules despite the benefits of spiral CT, such as three-dimensional volume measurements of the remaining $Ca(OH)_2$ packed in the canal without root sectioning (open, eroded, and occluded).[11,23]

In the present study, the removal of Metapex from the apical third of the root canal using conventional irrigation technique and EndoVac technique with 5.25% NaOCl followed by a final rinse of 17% EDTA and 0.2% chitosan NPs was assessed. The comparison of the effectiveness of the irrigants, as well as the techniques, was evaluated using SEM in the apical third of the root canal. A five-grade scoring scale was used which determines the extent of cleanliness of the canal by the percentage of $Ca(OH)_2$ removed.[8,10]

Considering the irrigant used in the present study, 0.2% chitosan NPs was found to be the most effective using the EndoVac technique. EndoVac has the advantage of producing negative pressure and allowing for safe use up to the WL without irrigant extrusion past the apical constriction of the canal. It makes use of a suction device with a macro- or microcannula connected to it. A negative pressure is created by the cannula, which is attached to a high-speed suction. This draws the irrigant to the cannula's tip and pokes little holes in it to let the irrigation solution and debris escape. A study conducted to compare the debridement efficacy of EndoVac irrigation versus conventional needle irrigation in vivo concluded that EndoVac irrigation resulted in significantly less debris at 1 mm from WL compared with conventional needle irrigation.[24] Another study done to compare the smear layer removal efficacy of 17% EDTA, 0.2% chitosan NPs, and QMix 2in1 at apical third of root canal system, using EndoVac system concluded that the final irrigation with QMix 2in1 solution aids in better smear layer removal at the apical third of the root canal system, using EndoVac system irrigation system.[25] The current study's findings are supported by prior research that indicates the EndoVac system leaves substantially less debris behind than conventional irrigation techniques when used with both irrigants.[21,26]

Conventional syringe irrigation has been widely used for the delivery of irrigants due to the ease of control of the needle penetration depth into the canal space and the quantity of the irrigant that is flushed through the canal. An irrigant's hydrodynamic activation is enhanced by agitating the needle by moving it up and down within the canal. It also reduces the chance of apical extrusion of the irrigant. The needle placed within the canal should remain loose during irrigation to allow the backflow of the irrigant, which also helps in the coronal displacement of the debris and avoids the periapical extrusion of the irrigant.[27]

The most commonly used irrigation technology is still the conventional irrigation technique due to several reasons such as its simplicity, ease of controlling the depth of needle penetration, and irrigant volume flushed down through the canal. The results of this study demonstrate the necessity for more sophisticated irrigation techniques, such as EndoVac. Literature implies that using an agitation system can improve the action of the irrigant, thereby improving its efficacy in removing $Ca(OH)_2$.[8]

Limitations and Future Directions

While these in vitro findings are promising, they may not translate directly to clinical situations due to the complexities present. In vivo studies are crucial to validate these findings in a clinical context, as the current in vitro environment may not fully represent real-world conditions. Another limitation of the present study was less sample size, it can be done with a larger sample size. Future research should explore other optimal concentrations of the different agents used, different agitation techniques such as ultrasonic agitation, and potential synergistic effects with different irrigants to maximize the medicament removal and treatment efficacy.

Conclusion

The present study has limitations as it is an in vitro study and should be performed in a large number of samples in a simulated clinical environment. Within the limitations of this study, it can be concluded that the EndoVac technique along with 0.2% chitosan NPs proved to be the most effective in removing the silicone oil-based intracanal medicament from the apical third of the root canal.

Conflict of Interest
None declared.

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