ASSESSMENT OF CALCIUM HYDROXIDE REMOVAL USING BIOAKT ENDO ROOT CANAL IRRIGATION UNDER A SCANNING ELECTRON MICROSCOPE (IN-VITRO STUDY)

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ABSTRACT

BACKGROUND: Calcium hydroxide is widely used in endodontics when single-visit treatment isn't possible, but its removal can be challenging. Effective removal requires thorough mechanical instrumentation and copious irrigation.

Aim: This study aimed at assessing the efficacy of BioAkt Endo in removal of $Ca(OH)_2$ under a scanning electron microscope.

MATERIALS AND METHODS: Forty-four single-rooted lower premolars were divided into four groups to evaluate the effectiveness of different irrigation techniques for removing $Ca(OH)_2$ after one week. Group I used 0.5% BioAkt Endo, Group II used 0.5% BioAkt Endo with XP-endo Finisher activation, Group III used 2.5% NaOCl + 17% EDTA with XP-endo Finisher activation, and Group IV used 2.5% NaOCl + 17% EDTA without XP-endo Finisher. The residual $Ca(OH)_2$ was assessed using SEM and a scoring system to evaluate residue in the canal.

RESULTS: Based on $Ca(OH)_2$ removal along the whole length of the root canal; group III (2.5% NaOCl followed by 17% EDTA with the XP-endo Finisher) achieved the highest efficacy, followed by group II (BioAkt Endo + XP Finisher) with median score values 2.00 (minimum = 1.00, maximum = 2.00), 3.00 (minimum = 1.00, maximum = 4.00) respectively, while group I and group IV demonstrated the lowest efficacy in $Ca(OH)_2$ removal, displaying the same median score value of 4.00 with the same range from a minimum of 3.00 to a maximum of 4.00. A statistically significant difference was found between group III with the highest efficacy and group I and IV that showed the lowest efficacy (median score values: 2.00, 4.00, 4.00 respectively) (p value <0.0001*), and between group I and II with superiority to group II (p value < 0.040*).

CONCLUSION: BioAkt Endo alone is not competent for proper removal of $Ca(OH)_2$. However, BioAkt Endo and NaOCl/EDTA, when used in conjunction with XP-endo Finisher, yield promising results in removing $Ca(OH)_2$ with superiority to NaOCl/EDTA.

KEYWORDS: BioAkt Endo, Calcium hydroxide, Scanning electron microscopy, XP-endo Finisher.

RUNNING TITLE: Assessment of BioAkt Endo effectiveness in removing calcium hydroxide.

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INTRODUCTION

A primary objective of root canal therapy is the removal of microorganisms from the root canal space and creation of a sealed environment to avoid re-infection and promote complete healing of surrounding tissues (1). In 1920, Hermann introduced calcium hydroxide $Ca(OH)_2$ as a medication used within the root canal in the field of endodontics. Besides its excellent biocompatibility and antimicrobial activity, it is typically recommended for use in treating contaminated root canals or when it is not possible to complete the treatment in a single visit. The ideal time frame for the medication to be efficient has been suggested to be1-4 weeks (2).

The capacity of the root canal filling to form an adequate seal can be compromised if $Ca(OH)_2$ is not entirely washed out from the root canal walls (3,4). Furthermore, the dentinal tubules may not be able to be sealed because the sealer would harden quickly when it comes into touch with any residual $Ca(OH)_2$ on the root canal walls (5). Denna J et al., (2020) revealed that calcium hydroxide residues present on the walls of the root canal can interact with the endodontic sealer and alter its characteristics. The alterations involve raising its viscosity and reducing its flow, thereby impeding the sealer's penetration and attachment to the dentinal tubules. These alterations weaken the adhesive bond between the endodontic sealer and the dentinal tubules. Moreover, the solubility of calcium hydroxide within the root canal might create air bubbles at the contact between the dentin and filling, which can promote the proliferation of bacteria. Also, Kim and Kim found that a large amount of residual calcium hydroxide affects the zinc oxide-eugenol sealer and results in increased apical leakage after obturation (6). Consequently, the elimination of $Ca(OH)_2$ is a crucial step and has been examined with various methods and materials (7).

The goal of several researches has been to establish the optimal irrigation protocol for eliminating calcium hydroxide. Interestingly, when combined with filing, irrigation has been found to be much better in terms of root canal cleaning (8).

The XP-endo Finisher, developed by (FKG Dentaire in La Chaux-de-Fonds, Switzerland), is a Nickel Titanium (NiTi) instrument that features a unique design. The file has a C-shaped configuration in its apical portion and is known for its ability to effectively clean the root canal while at the same time keeping the dentine intact. Additionally, it can be utilized after any root canal preparation technique that achieves an apical size of 25 or larger.

This particular file has a diameter of ISO 25 and no taper (25/.00). However, it can expand up to 6 mm in diameter, which is a hundred times greater than a corresponding-sized file. In contrast to traditional NiTi instruments, the XP-endo Finisher's shape changes based on temperature due to its unique metallurgy. The root canal's temperature causes the file transition from its martensitic (M-phase) to its austenitic (A-phase) state. The file's A-phase shape allows it to access and clean parts of the canal with a unique configuration when in rotational mode. Once the file has cooled, it can be easily bent back into its original shape (M-phase). According to some theories, this instrument's special design has the potential to improve the level of contact with dentin compared to conventional NiTi instruments (9,10).

In terms of Calcium hydroxide removal from the root canal; EDTA solution, saline, sodium hypochlorite and their combinations were used as a gold standard irrigating solutions for washing out the intra-canal medication. In addition to acids such as citric acid which is considered a chelating agent and also showed improvement in $Ca(OH)_2$ removal as demonstrated in a previous study (11).

A recent endodontic irrigant called BioAkt Endo (New Tech Solutions s.r.l., Brescia, Italy) has been intended for clinical application, which contains 0.003% silver ions in 4.846% citric acid. This new two-in-one solution was developed as a substitute for EDTA. Research has indicated that acidic solutions are more efficient in dissolving the smear layer (12) and exhibit better antimicrobial behavior within the root canal system (13) compared to EDTA solutions. Although there is evidence that the irrigant is non-toxic and biocompatible (14). However, far to our knowledge this material has not been assessed for its ability to remove $Ca(OH)_2$. Consequently, this research aimed to examine the efficiency of BioAkt Endo solution in eliminating calcium hydroxide from extracted human teeth. The null hypothesis of this study was that no statistically significant difference would be found between BioAkt Endo and other irrigation protocol in removal of $Ca(OH)_2$.

MATERIALS AND METHODS

The Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 standards were used to do this study (15,16). The flowchart for conducting laboratory studies based on PRILE 2021 guidelines is presented in Figure (1).

The research was conducted as an in vitro study, 44 mandibular premolars extracted for orthodontic or periodontal reasons, were obtained from the oral surgery department, Alexandria University. The patient consented to the utilization of their teeth for this study. The ethics committee at the Faculty of Dentistry, Alexandria University, accepted the research plan (IORG 0785-30/2023).

Sample size estimation

The sample size was determined using Rosner's approach (17). Calculated by G*Power 3.1.9.7 (18). A sample size of 80% was determined with an alpha error of 5%. The overall mean scores of calcium hydroxide removal were 3.13 ± 1.18 using 17% EDTA and 4.91 ± 0.22 using citric acid that was assumed to have similar effect to silver citrate (19). Using a maximum SD = 1.18 based on the difference between independent means to guarantee sufficient study power, a sample of 9 samples per group is required, yielding an effect size of 1.508. This was enlarged to 11 samples to make up processing errors. Total sample = Number per group × Number of groups = 11 × 4 = 44 samples.

Selection criteria

Human extracted single rooted permanent mandibular premolars, criteria included teeth with straight and mature apices and free of visible root caries, selected teeth were recruited and periapically radiographed buccolingually and mesiodistally to confirm root structure. Teeth with root cracks, fractures, resorptive defects, or previous root canal treatment were excluded (20,21).

Specimen preparation

All teeth were soaked in 5.25% of NaOCl solution for 10 minutes and then thoroughly cleaned by a hand scaler to remove soft and hard deposits, and finally stored in distilled water until being used within three months (22).

Teeth were decoronated with a low-speed diamond disk while being cooled by water to achieve a consistent root length of 14 ± 1 mm. A #10 K-file was inserted into the canal until it could be seen through the apical foramen. The WL was determined by subtracting 1 mm from the established length (19,22,23).

Root canal instrumentation was performed using Hyflex CM file system (COLTENE, USA) in crown down manner according to the manufacture instructions starting from size 25/0.08 up to size 40/0.04. Sodium hypochlorite 2.5% solution was used as an irrigating solution during instrumentation in all groups (24). Canals were dried with paper points of equivalent size (40/0.02).

Oily based Metapex plus $Ca(OH)_2$ (Meta Biomed, Korea) was injected to fill the prepared canal, and then a temporary filling was used as a dressing material to the canal orifice MD-TempoPlus (Meta Biomed, Korea), specimens were incubated for seven days at 100% relative humidity by using an incubator (BST 50 20,VEB MLW Dentalfabrik Leipzig, Germany (1990)).

Grouping of samples

Four groups were randomly selected from the samples (n=11) based on $Ca(OH)_2$ removal protocol:

All groups were irrigated for three continuous minutes. Group I: 5 ml of BioAkt Endo without mechanical activation (26). Group II: 5 ml of BioAkt Endo activated with XP-endo Finisher file in rotation motion with 800 rpm and 1 N/cm torque (27). Group III: 5 ml of 2.5% NaOCL followed by 5 ml of 17 % EDTA solution and activated by XP-endo Finisher file (28). Group IV: 5 ml of 2.5% NaOCl followed by 5 ml of 17% EDTA solution without any mechanical activation.

In all groups, irrigation was achieved using a 30-gauge side venting irrigation needle attached to 5 ml disposable syringe.

To neutralize the effects of the different irrigating solutions, as a last rinse, 5 ml of distilled water were utilized. The root canals were then dried using paper points (29).

Using Microtome (Micracut 150, Metkon Metallography Bursa, Turkey), roots were split longitudinally. The chosen specimens were gradually dehydrated by exposing them to increasing concentrations of 90% water-based ethanol for a period of 24 hours at each concentration. Following this dehydration, the samples were subsequently placed in a vacuum chamber and plated with a 30nm layer of gold using a sputter-coating technique (30).

SEM Evaluation

Root canals were examined in the apical, middle, and coronal portions by using a scanning electron microscope (JSM-IT 200, JEOL, Japan) with 1000x magnification Figure (2). A customized five-category scale method was applied to evaluate the calcium hydroxide residues inside root canals (30).

The scores were presented in the following manner:

Score 0: No remnants.

Score 1: Minimal residual amounts (up to 25% covers the surface).

Score 2: Moderate amounts of remnants (25% to 50% covers the surface).

Score 3: Large amounts of remnants (50% to 75% covers the surface).

Score 4: Full of remnants (75% to 100% covers the surface).

Two examiners, independently, and blindly examined SEM specimens for assessment of $Ca(OH)_2$ residues. For the purpose of calibration, ten specimens were evaluated separately and were not included in the samples used for the study. The scores went through comparisons, and in case a discrepancy was discovered, the examiners collaboratively reviewed the sample and its scoring, ending up with a mutually accepted score. Intra-examiner reliability was assessed using Kappa statistics.

Statistical Analysis

The Shapiro-Wilk test and Q-Q plots were employed to evaluate the data's normality. Due to the lack of regular distribution of the data, median, minimum, and maximum were the primary means of presentation in addition to mean and standard deviation (SD). Frequency and percentage were used to summarize the distribution of the scores. The groups were assessed utilizing the Kruskal-Wallis test, and Dunn's post hoc test with Bonferroni correction was used to address any alpha error that might have resulted from multiple testing. Kappa Statistics was used to evaluate the agreement between two raters. Every test had two tails, and p value<0.05 was used as the significance level.

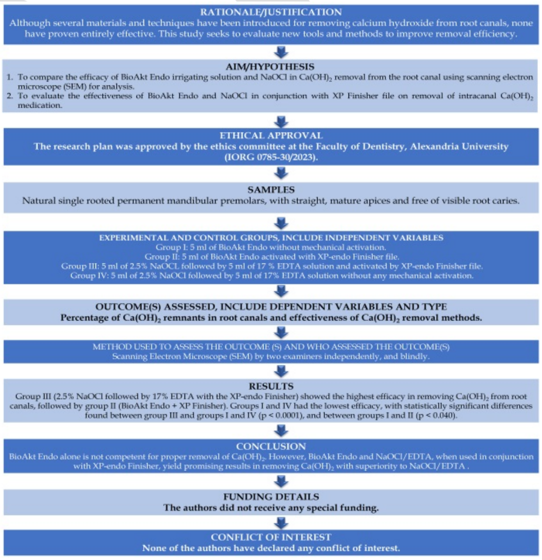

Figure (1): Flowchart based on PRILE 2021 guidelines.

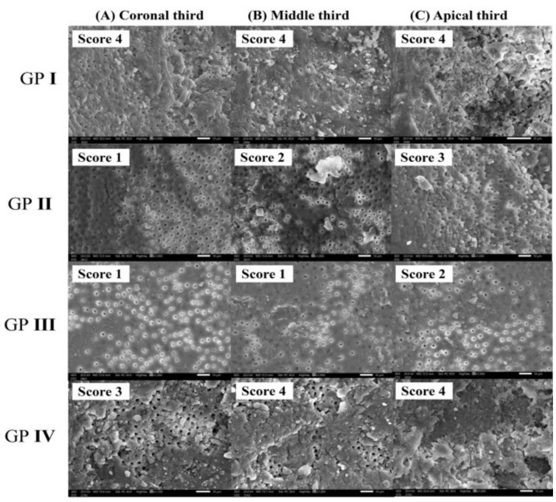

Figure (2): SEM micrography with magnification X1000. of the root canal wall represented one sample in each group and showed its scores at the (A) coronal third, (B) middle third and (C)apical third.

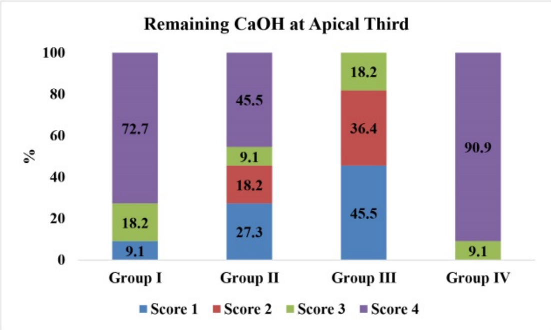

Figure (3): Remaining CaOH at Apical Third.

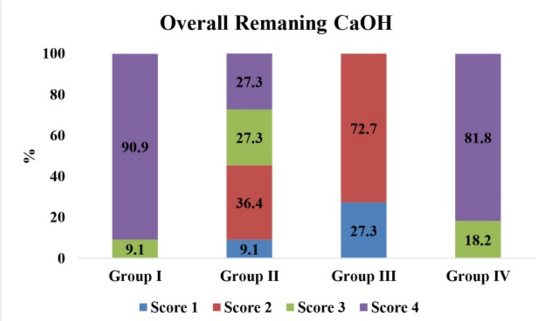

Figure (4): Remaining CaOH overall scores.

RESULTS

There was a substantial agreement between the two raters where the kappa values for the three thirds of teeth were more than 80%.

In the coronal third, group III (NaOCl/EDTA + XP) showed significantly better results compared to group I (BioAkt Endo) ($p = 0.0001^*$) and group IV (NaOCl/EDTA) ($p = 0.013^*$) in cleaning effectiveness of $Ca(OH)_2$ paste. Group II (BioAktEndo + XP) was also more effective than group I (BioAkt Endo) and showed a statistically significant difference ($p = 0.008^*$). In the middle portion of the canal, a statistically significant difference was found between group III (NaOCl/EDTA + XP) and both group I (BioAkt Endo) ($p < 0.0001^*$) and group IV (NaOCl/EDTA) ($p < 0.0001^*$) in favor of group III for removing $Ca(OH)_2$.

In the apical area, all groups recorded less canal cleanliness in comparison to coronal and middle thirds, (with median scores of 4.00, 3.00, 2.00, and 4.00 for groups I, II, III, and IV respectively). Group III (NaOCl/EDTA + XP) demonstrated superiority over the other groups and highlighted a statistically significant difference with both group I (BioAkt Endo) ($p < 0.003^*$) and group IV (NaOCl/EDTA) ($p < 0.0001^*$). Table (1) and Figure (3).

Table (2) and Figure (4) show the overall comparison of the remaining $Ca(OH)_2$ at the entire length of the canals regarding the four study groups. Remaining $Ca(OH)_2$ in median score was 4.00, 3.00, 2.00, and 4.00 for groups I, II, III, and IV respectively. Group III (2.5% NaOCl followed by 17% EDTA with the XP-endo Finisher) achieved the highest efficacy, followed by group II (BioAkt Endo + XP Finisher) with median score values 2.00 (minimum = 1.00, maximum = 2.00), 3.00 (minimum = 1.00, maximum = 4.00) respectively, while group I and group IV demonstrated the lowest efficacy in $Ca(OH)_2$ removal, displaying the same median score value of 4.00 with the same range from a minimum of 3.00 to a maximum of 4.00. A statistically significant difference was found between group III with the highest efficacy and group I and IV that showed the lowest efficacy (median score values: 2.00, 4.00, 4.00 respectively) (p value $<0.0001^*$), and between group I and II with superiority to group II (p value $< 0.040^*$).

Table (1): Comparison of remaining calcium hydroxide at apical third among the study groups

	Group I BioAkt Endo (n=11)	**Group II BioAkt Endo + XP (n=11)**	**Group III NaOCl/EDTA + XP (n=11)**	**Group IV NaOCl/EDTA (n=11)**
Score 1: n (%)	1 (9.1%)	3 (27.3%)	5 (45.5%)	0 (0%)
Score 2: n (%)	0 (0%)	2 (18.2%)	4 (36.4%)	0 (0%)
Score 3: n (%)	2 (18.2%)	1 (9.1%)	2 (18.2%)	1 (9.1%)
Score 4: n (%)	8 (72.7%)	5 (45.5%)	0 (0%)	10 (90.9%)
Mean ±SD	3.55 ±0.93	2.73 ±1.35	1.73 ±0.79	3.91 ±0.30
Median	4.00	3.00	2.00	4.00
Min – Max	1.00 – 4.00	1.00 – 4.00	1.00 – 3.00	3.00 – 4.00
H test	21.256			
***p* value**	<0.0001*			

*Statistically significant at p<0.05, H test: Kruskal Wallis test

Table (2): Overall Comparison of remaining calcium hydroxide at the entire length of the canals among the study groups

	Group I BioAkt Endo (n=11)	**Group II BioAkt Endo + XP (n=11)**	**Group III NaOCl/EDTA + XP (n=11)**	**Group IV NaOCl/EDTA (n=11)**
Score 1: n (%)	0 (0%)	1 (9.1%)	3 (27.3%)	0 (0%)
Score 2: n (%)	0 (0%)	4 (36.4%)	8 (72.7%)	0 (0%)
Score 3: n (%)	1 (9.1%)	3 (27.3%)	0 (0%)	2 (18.2%)
Score 4: n (%)	10 (90.9%)	3 (27.3%)	0 (0%)	9 (81.8%)
Mean ±SD	3.91±0.30	2.73±1.01	1.73 ±0.47	3.82±0.41
Median	4.00	3.00	2.00	4.00
Min – Max	3.00 – 4.00	1.00 – 4.00	1.00 – 2.00	3.00 – 4.00
H test ***p* value**	31.734 <0.0001*			

*Statistically significant at $p<0.05$, H test: Kruskal Wallis test

DISCUSSION

From the results of this study, the null hypothesis was accepted as the comparison held between BioAkt Endo and the other irrigation protocol (2.5% NaOCl + 17% EDTA) did not show any statistically significant difference when performed with agitation (group II and III). Furthermore, there was no statistically significant difference between the groups (I and IV) performed without agitation.

Intra-canal medications are frequently utilized to temporarily fill the root canal space. Since calcium hydroxide has well-established antibacterial activity, therefore it is typically utilized as an intra-canal medication. However, it has been discovered that the final sealing of the canal is negatively impacted by the remaining amounts of $Ca(OH)_2$ paste on the root canal walls. For that reason, it is necessary to properly eliminate intra-canal calcium hydroxide by irrigant solutions before the final obturation of root canal spaces (31).

In this study, oily based Metapex plus $Ca(OH)_2$ was utilized, which is reported by Nandini et. al. to be more challenging to remove than powder form $Ca(OH)_2$ combined with distilled water (32).

To wash out the $Ca(OH)_2$ medicament, we used 2.5% sodium hypochlorite (NaOCI) and 17% EDTA for 3 minutes each, separately; which was demonstrated to be successful in removing dentinal debris. This agrees with a previous study that stated EDTA is important for more than just binding calcium ions from the dentinal walls; it also helps separate and remove $Ca(OH)_2$ molecules from the canal. Additionally, EDTA can neutralize any remaining $Ca(OH)_2$ residues, potentially preventing chemical reactions with the sealer cement (33).

Moreover, we used the XP-endo Finisher file, which has a high impact on cleaning the root canal morphologies and significantly removes more $Ca(OH)_2$ paste following irrigation. This is in agreement with previous findings reported by Uygun et al. 2016, Wigler et al. 2017 and Keskin et al. 2017 (34).

In this study, we used a recent root canal irrigating solution, BioAkt Endo, to assess its effectiveness in $Ca(OH)_2$ removal due to the ability of its citric acid component and chelation effect that can dissolve the mineralized tissue and expose the dentinal tubules at a slightly acidic environment, as reported in a previous study (12).

Based on results over the entire length of the root canal, this research revealed that using the XP-endo Finisher instrument either in conjunction with NaOCl followed by EDTA (group III) or with BioAkt Endo (group II) irrigating solutions, presented significantly better cleaning efficiency when compared to NaOCl followed by EDTA (group IV) and BioAkt Endo (group I) without using XP-endo Finisher in terms of removal of $Ca(OH)_2$. These findings might be attributed to the activation of the XP Finisher instruments and yielding irregular movements inside root canal which results in cleaning of the canals (35). A study conducted by Hamdan et al. (36) confirmed the effectiveness of the XP-endo Finisher tool in eliminating $Ca(OH)_2$ from the root canal.

The apical region of every group exhibited a higher quantity of $Ca(OH)_2$ residues in comparison to the middle and coronal regions. The reduced efficacy of irrigation solutions in the apical area may be due to the smaller diameter of the canal's apical part, rendering the removal of $Ca(OH)_2$ in this area more difficult (37).

The study's findings clearly show that the effectiveness of endodontic cleaning is greatly influenced by the selection of irrigation solutions and the usage of supplemental instruments such as the XP-endo Finisher. Group I, which used 0.5% BioAkt Endo without the XP-endo Finisher, exhibited the least performance among all groups. This further highlights the importance of the XP-endo Finisher in achieving optimal cleaning results.

Group II, employing 0.5% BioAkt Endo with the XP-endo Finisher, also yielded excellent results, though slightly inferior to those of Group III. This suggests that BioAkt Endo is better complimented with mechanical agitation to give better results.

Group III, which utilized 2.5% NaOCl followed by 17% EDTA with the XP-endo Finisher, demonstrated the best overall results in terms of cleaning efficacy. This combination proved superior to all other tested groups, highlighting the enhanced effectiveness of the XP-endo Finisher when used with these irrigants.

Finally, Group IV, which used 2.5% NaOCl followed by 17% EDTA without the XP-endo Finisher, showed poor performance in removing $Ca(OH)_2$. When compared to BioAkt Endo without the XP-endo finisher, it showed comparable results with superiority to NaOCl/EDTA. This could be due to the combination of two chemical irrigating solutions and the ability of EDTA to remove inorganic dentin components and its ability to chemically interact with calcium ions. Also, EDTA is commonly utilized as an effective chelating agent that has the ability to eliminate dentin debris and smear layer. However, NaOCl alone has limited ability to interact with calcium ions and dissolve the inorganic components (38). This combination enhanced the efficacy of $Ca(OH)_2$ removal and achieved better results (39).

Previous study compared higher concentration of 10% citric acid (CA) with 17% EDTA for removing calcium hydroxide and generally favored citric acid, though not by a significant difference (40).

However, in the present study, 17% EDTA proved more effective at removing $Ca(OH)_2$ from BioAkt Endo, which contains 4.846% CA in its composition. Several factors could explain this discrepancy: First, EDTA is used in combination with another irrigating solution, sodium hypochlorite (NaOCl), which also aids in $Ca(OH)_2$ removal and enhances EDTA's effectiveness. Second, the concentration of CA in BioAkt Endo is relatively low, which might affect its ability to chelate calcium as effectively as higher concentrations of CA. Third, methodology variations and application methods for chelating agents might influence the effectiveness of $Ca(OH)_2$ removal. These factors might have mostly or fully contributed to the observed differences in outcomes regarding the present study and other recent studies.

CONCLUSION

BioAkt Endo alone is not competent for proper removal of $Ca(OH)_2$. However, BioAkt Endo and NaOCl/EDTA, when used in conjunction with XP-endo Finisher, yield promising results in removing $Ca(OH)_2$ with superiority to NaOCl/EDTA.

CONFLICT OF INTEREST
None of the authors have declared any conflict of interest.
FUNDING STATEMENT
For this work, the authors did not receive any special funding.

REFERENCES

1. Thonai S, Kataki R, Das L, Begum F, Deka A, Borah DK. Comparative evaluation of calcium ion release, pH change, and dentinal tubule penetration of four different formulations of calcium hydroxide-based intracanal medicaments - An in vitro study. J Conserv Dent Endod. 2023;26:657-62.
2. Zou X, Zheng X, Liang Y, Zhang C, Fan B, Liang J, et al. Expert consensus on irrigation and intracanal medication in root canal therapy. Int J Oral Sci. 2024;16:23.
3. de Lima Dias-Junior LC, Castro RF, Fernandes AD, Guerreiro MY, Silva EJ, da Silva Brandão JM. Final endodontic irrigation with 70% ethanol enhanced calcium hydroxide removal from the apical third. J Endod. 2021;47:105-11.
4. Savaris JM, Isoton JC, Fluck BF, Tedesco M, Bortoluzzi EA, Garcia LD, da Silveira Teixeira C. Comparative Analysis of AH Plus Bond Strength to Root Canal Dentin and Adhesive Interface Quality after Calcium Hydroxide Removal using Different Irrigation Protocols. J Endod. 2024;50:659-66.
5. Er Karaoğlu G, Aydın ZU. The effect of different irrigation activation methods on the penetration of the irrigation solution into the dentinal tubules in teeth with calcium hydroxide applied and oval-shaped canals: Confocal laser scanning microscope analysis. Microsc Res Tech. 2024;87:2194-203.
6. Denna J, Shafie LA, Alsofi L, Al-Habib M, AlShwaimi E. Efficacy of the Rotary Instrument XP-Endo Finisher in the Removal of Calcium Hydroxide Intracanal Medicament in Combination with Different Irrigation Techniques: A Microtomographic Study. Materials (Basel). 2020;13:2222.
7. Donnermeyer D, Wyrsch H, Bürklein S, Schäfer E. Removal of calcium hydroxide from artificial grooves in straight root canals: sonic activation using EDDY versus passive ultrasonic irrigation and XPendo Finisher. J Endod. 2019;45(:322-6.
8. Singh AK, Ramanna PK, Kumari D, Poddar P, Shah M, Dixit A. In vitro assessment of intracanal calcium hydroxide removal using various irrigation systems: An SEM study. J Contemp Dent Pract. 2021;22:1003-7.
9. Bortoluzzi EA, Carlon D, Meghil MM, El-Awady AR, Niu L, Bergeron BE, et al. Efficacy of 3D conforming nickel titanium rotary instruments in eliminating canal wall bacteria

from oval-shaped root canals. J Dent. 2015;43:597-604.
10. Peters OA, Arias A, Paqué F. A Micro-computed Tomographic Assessment of Root Canal Preparation with a Novel Instrument, TRUShape, in Mesial Roots of Mandibular Molars. J Endod. 2015;41:1545-50.
11. Agrawal P, Garg G, Bavabeedu SS, Arora S, Moyin S, Punathil S. Evaluation of intracanal calcium hydroxide removal with different techniques: a scanning electron microscope study. J Contemp Dent Pract. 2018;19:1463-8.
12. Banode AM, Gade V, Patil S, Gade J, Chandhok D, Sinkar R. Comparative scanning electron microscopy evaluation of smear layer removal with 17% ethylenediaminetetraacetic acid, 10% citric acid and newer irrigantQMix: In vitro study. Indian J Oral Health Res. 2015;1:56-61.
13. Georgopoulou M, Kontakiotis E, Nakou M. Evaluation of the antimicrobial effectiveness of citric acid and sodium hypochlorite on the anaerobic flora of the infected root canal. Int Endod J. 1994;27:139-43.
14. Küçük M, Kermeoğlu F. Efficacy of different irrigation methods on dentinal tubule penetration of Chlorhexidine, QMix and Irritrol: A confocal laser scanning microscopy study. AustEndod J. 2019;45:202-8.
15. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr, et al. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: explanation and elaboration. Int Endod J. 2021;5:1491-515.
16. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr, et al. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: A consensus-based development. Int Endod J. 2021;54:1482-1490.
17. Rosner B. Hypothesis Testing: Two-Sample Inference. In: Fundamentals of biostatistics.7thed. Boston:Brooks/Cole: Nelson Education; 2015. 269-301.
18. Universität Düsseldorf. G*Power.2019. Available at: https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.
19. Chockattu SJ, Deepak BS, Goud KM. 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. J Conserv Dent. 2017;20:6-11.
20. Shaheen NA. The efficacy of different single file systems in cleaning oval shaped root canal. Tanta Dent J.2019;16:73-9.
21. Ahmed HM. A critical analysis of laboratory and clinical research methods to study root and canal anatomy. Int Endod J. 2022;55:229-80.
22. Kongkiatkool P, Puapichartdumrong P, Tantanapornkul W, Piyapattamin T, Wisithphrom K. Accuracy of digital periapical radiography and cone beam computed tomography for evaluation of root canal configuration in human mandibular first premolars. J Int Dent Medical Res.2020;13:80-5.
23. Radeva E, Radev R, Maznev M, Uzunov ST. Shaping ability of ProTaper Gold in preparation of moderate curved canals (in vitro study). IJSRP. 2021;11:2250-3153.
24. Zhou J, Liu T, Guo L. Effectiveness of XP-Endo Finisher and passive ultrasonic irrigation on intracanal medicament removal from root canals: a systematic review and meta-analysis. BMC oral health. 2021;21:294.
25. Chockattu SJ, Deepak BS, Goud KM. 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. J Conserv Dent. 2017;20:6-11.
26. Giardino L, Pedullà E, Cavani F, Bisciotti F, Giannetti L, Checchi V, et al. Comparative Evaluation of the Penetration Depth into Dentinal Tubules of Three Endodontic Irrigants. Materials (Basel) 2021;14:5853.
27. Denna J, Shafie LA, Alsofi L, Al-Habib M, AlShwaimi E. Efficacy of the Rotary Instrument XP-Endo Finisher in the Removal of Calcium Hydroxide Intracanal Medicament in Combination with Different Irrigation Techniques: A Microtomographic Study. Materials (Basel). 2020;13:2222.
28. Tonini R, Giovarruscio M, Gorni F, Ionescu A, Brambilla E, Mikhailovna IM, et al. In Vitro Evaluation of Antibacterial Properties and Smear Layer Removal/Sealer Penetration of a Novel Silver-Citrate Root Canal Irrigant. Materials (Basel) 2020;13:194.
29. Silva CC, Ferreira VMD, De-Deus G, Herrera DR, Prado MD, Silva EJNLD. Effect of Intermediate Flush Using Different Devices to Prevent Chemical Smear Layer Formation. Braz Dent J. 2017;28:447-52.
30. Gutmann JL, Saunders WP, Nguyen L, Guo IY, Saunders EM. Ultrasonic root-end preparation. Part 1. SEM analysis. Int Endod J. 1994;27:318-24.
31. Suleiman HT, El Kateb NM, Aboelseoud M, Sherif RA. Removal of calcium hydroxide intracanal medicament with four different techniques (A cone beam computed tomography in vitro study). Alex Dent J. 2022;47:147-54.

32. De Faria-Júnior NB, Keine KC, Só MV, Weckwerth PH, Guerreiro-Tanomaru JM, Kuga MC. Residues of calcium hydroxide-based intracanal medication associated with different vehicles: A scanning electron microscopy evaluation. Microsc Res Tech.2012;75:898-902.
33. Mohan S. A Sem Evaluation of Effect of Different Irrigating Solutions on the Removal of Calcium Hydroxide Intra Canal Medicament Using Ultrasonics, Rotary Files and Hand Files (Master's thesis, Rajiv Gandhi University of Health Sciences (India)).2016.
34. Silva EJNL, Belladonna FG, Zuolo AS, Rodrigues E, Ehrhardt IC, Souza EM, et al. Effectiveness of XP-endo Finisher and XP-endo Finisher R in removing root filling remnants: a micro-CT study. IntEndod J. 2018;51:86-91.
35. Marques-da-Silva B, Alberton CS, Tomazinho FSF, Gabardo MCL, Duarte MAH, Vivan RR, et al. Effectiveness of five instruments when removing calcium hydroxide paste from simulated internal root resorption cavities in extracted maxillary central incisors. IntEndod J. 2020;53:366-75.
36. Hamdan R, Michetti J, Pinchon D, Diemer F, Georgelin-Gurgel M. The XP-Endo Finisher for the removal of calcium hydroxide paste from root canals and from the apical third. J ClinExp Dent. 2017;9:e855-60.
37. Özlek E, Kadı G. Evaluation of Calcium Hydroxide Removal Efficiency of Different Concentrations and Forms of Sodium Hypochlorite. Eastern J Med. 2021;26:543-9.
38. Rödig T, Vogel S, Zapf A, Hülsmann M. Efficacy of different irrigants in the removal of calcium hydroxide from root canals. Int Endod J. 2010;43:519-27.
39. Nakano M, Yamamoto Y, Misawa A, Yamakawa S, Yoshida T, Yamazaki Y, Hosoya N. A comparative evaluation of irrigation methods for calcium hydroxide removal from the root canal: An in vitro study. Dental Research and Oral Health. 2021;4:25-33.
40. Wang Y, Guo LY, Fang HZ, Zou WL, Yang YM, Gao Y, Yang H, Hu T. An in vitro study on the efficacy of removing calcium hydroxide from curved root canal systems in root canal therapy. Int J Oral Sci. 2017;9:110-116.