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In Vitro Quantification of Residual Calcium Hydroxide Mixed with Different Carriers After Root Canal Irrigation with Three Different Techniques

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Abstract

Background and Aim: This study quantified the amount of residual calcium hydroxide (CH) mixed with saline, propylene glycol (PG), nano-silver (NS), and 0.2% chlorhexidine (CHX) carriers after root canal irrigation with the conventional needle (CN), safe-end needle (SEN), and ultrasonic irrigation (UI).

Materials and Methods: In this in vitro study, the root canals of 190 single-rooted premolars were instrumented with Mtwo, and longitudinally sectioned in half. After smear layer removal, the root canals were filled with CH mixed with either saline, PG, 0.2% CHX, or NS. CH was removed with CN irrigation, SEN irrigation, or UI. The residual CH was quantified in the coronal, middle, and apical thirds using a stereomicroscope. The penetration depth of CH into dentinal tubules was measured under a scanning electron microscope (SEM). Data were analyzed using three-way and one-way ANOVA, and Tukey's test (alpha=0.05).

Results: The effects of carrier type (P=0.0001), irrigation technique (P=0.0001), and distance from the apex (P=0.0001) were significant on the residual CH percentage. The efficacy of UI was slightly superior to other techniques in saline and CHX groups in the coronal third, and the NS group in the middle third. The irrigation technique had a significant effect on residual CH in the coronal and middle thirds, and no significant effect on the penetration depth of CH. The penetration depth of CH was not significantly different among different carrier groups (P>0.05).

Conclusion: The efficacy of UI in removal of CH was variable, depending on the carrier type and distance from the apex.

Keywords: Calcium Hydroxide; Needles; Root Canal Therapy; Therapeutic Irrigation; Ultrasonics

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Introduction

A major goal of root canal therapy is to prevent or treat periapical injury by eliminating or minimizing bacteria and their byproducts in the root canal system [1,2]. To achieve this, efficient root canal irrigation and application of intracanal medicaments have been proposed in

addition to mechanical cleaning and shaping of the root canal system [3,4].

Calcium hydroxide (CH) is the most commonly used intracanal medicament [5,6] due to its optimal properties such as antibacterial activity [7], biocompatibility [8], highly alkaline pH, and anti-inflammatory properties [2]. CH degrades the bacterial cell wall and bacterial protein structures [9], preventing reinfection in the root canal system by impairing the nutrient supply of residual microorganisms through the dissemination of hydroxyl ions [3]. However, CH cannot be used as a universal intracanal medicament for all cases as it has been reported to fail in eradicating enterococci and Candida albicans [5,9,10].

CH should be delivered into the root canal system in sufficient amounts to be effective [2]. However, complete elimination of CH from the root canal system before obturation is essential as CH residues in the root canal system can limit the penetration depth of disinfecting agents and sealers into dentinal tubules, decrease the flow, setting time, and bond strength of sealer to dentin, and increase the apical leakage of endodontically treated teeth, impairing optimal root canal sealing. CH particularly interferes with zinc oxide eugenol sealer, making it fragile and granular [11,12].

Elimination of CH from the root canal system depends on the smoothness of canal walls, root canal morphology, type of carrier, and the irrigation technique employed to remove residual CH [13,14]. Several techniques have been proposed for this purpose such as the use of endodontic hand files [15], active sonic irrigation [16], passive ultrasonic irrigation (UI) [17], canal brush system [18], nickel-titanium rotary instruments [19,20], and application of irrigants such as sodium hypochlorite and EDTA [21,22]. Denna et al. [23] reported lower residual CH levels in the canal when canal brush, sonic, and UI were used. However, no consensus exists regarding the superiority of one technique over the others for more efficient elimination of residual CH, and no technique has been able to completely eliminate the residual CH [24,25].

Several carriers are available for mixing with CH, such as saline, propylene glycol (PG), 99% nano-silver (NS) colloidal solution, and 0.2% chlorhexidine (CHX) [26,27]. These carriers affect the penetration depth of CH into dentin [28] and its antibacterial activity [29]. For instance, mixing CH with CHX [11] or 99% NS increased its antimicrobial activity [30]. Bacteria can infiltrate into dentinal tubules, and their penetration depth depends on the pH. Thus, the depth of intracanal medicaments' penetration is crucial in eliminating bacteria from root dentinal tubules [31]. Considering all the above, this study aimed to quantify the amount of residual CH mixed with saline, PG, NS, and 0.2% CHX carriers after root canal irrigation with the conventional needle (CN), safe-end needle (SEN), and UI. The penetration depth of CH into dentinal tubules was also measured.

Materials and Methods

This in vitro experimental study was conducted on 190 single-rooted, single-canal premolars extracted due to periodontal disease. The teeth were free from caries, fracture, crack, resorption, or curvature, had no history of previous endodontic treatment, and had similar cross-sections. The study protocol was approved by the ethics committee of the university (IR.TUMS.DENTISTRY.REC.1397.04) and was in accordance with the PRILE checklist.

Sample size:

The sample size was calculated to be 35 in each group using the ANOVA sample size calculation formula, assuming intermediate dispersion of the means:

$$F = d \times \frac{1}{2}\sqrt{\frac{(k+1)}{3(k-1)}}.$$

Specimen preparation:

Soft and hard tissue debris was removed; the teeth were disinfected in chloramine T solution and were then stored in saline at 37°C. To ensure presence of one single straight canal and similar morphology, all teeth underwent periapical radiography from the buccolingual and mesiodistal directions. A total of 190 teeth were selected by convenience sampling, and randomly assigned to the following groups (35 teeth in each carrier group, and 50 control teeth, including 10 positive controls for each carrier type and 10 negative controls) [12]:

1. CH (Golchadent, Karaj, Iran) + saline carrier (n=35)
   1.1. Positive control group with saline (n=10)
2. CH + PG (Maron, Tabriz, Iran) (n=35)
   2.1. Positive control group with PG carrier (n=10)
3. CH + 0.2% CHX carrier (Donyaye Behesht, Tehran, Iran) (n=35)
   3.1. Positive control group with 0.2% CHX carrier (n=10)
4. CH + NS colloidal solution (Sharif, Tehran, Iran) (n=35)
   4.1. Positive control group with NS colloidal solution (n=10)
5. Negative control group (n=10)

The teeth were decoronated with a diamond disc and high-speed handpiece such that the remaining root length was standardized at 15 mm. The working length (WL) of each tooth was radiographically determined 1 mm shorter than the apex. The pulp chamber was filled with 5% sodium hypochlorite (NaOCl; Clorox Bleach; Clorox, Oakland, CA) and a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canals to the WL to ensure patency. The root canals were cleaned and shaped with Mtwo rotary instrument (VDW, Munich, Germany) according to the manufacturer's instructions using Maraton endo-motor (Saeyang Microtech, Daegu, Korea). The sequence of the files used was #10/0.04, #15/0.05, #20/0.06, #25/0.06, #30/0.05, and #35/0.04. The rotary files were adjusted to the WL and used in the canal with RC-Prep lubricant (Spident, Incheon, Korea) with up-and-down movements with a short range until the file became utterly passive. When instrumentation was complete, a final irrigation protocol (30 G Max-i-Probe needle; Dentsply-Rinn, Elgin, IL) 1 mm short of the WL was performed for all canals using 10 mL of 5% NaOCl for 5 minutes followed by 5 mL of 17% EDTA (Pulpdent Corporation, Watertown, MA) for 2 minutes. Any remaining solution from the canals was removed, and then the canals were dried with paper points (Dentsply Tulsa Dental Specialties). The apex of all samples was sealed with sticky wax, covered in a saline-saturated cotton cloth, and stored in an incubator at 37°C and 100% humidity for 7 days. Afterwards, a putty impression was made from the roots. The roots were then removed from the impression and longitudinally sectioned in half with a disc with 0.17 mm thickness. Then, they were reattached with sticky wax and placed back in the impression. The root canal space in all groups, except for the negative control group, was filled with CH paste with a creamy consistency, which was prepared by mixing the CH powder (Golchadent, Karaj, Iran) with different carriers according to the manufacturer's instructions, as follows:

- saline group: 0.9% saline + CH powder with 50% ratio.
- CHX group: 0.2% CHX + CH powder with 50% ratio.
- NS group: 99% colloidal NS + CH powder in 30% powder/70% carrier ratio.
- PG group: PG + CH powder in 30% powder/70% carrier ratio.

CH paste was delivered into the canals using a #25 Lentulo spiral (Micro Mega Finger, Besancon, France) with a low-speed handpiece. Lentulo was introduced into the canal to 2 mm shorter than the apex. Radiographs in the mesiodistal and buccolingual directions were obtained to confirm complete filling of the root

canals. Next, each group was divided into four subgroups for removal of CH with the following methods:

1. CN (Ava Pezeshk, Tehran, Iran)
2. SEN (Ava Pezeshk, Tehran, Iran)
3. UI (Osada Electric Co, Tokyo City, Tokyo, Japan).
4. No irrigation (positive control)

The experiment involved inserting a 30-gauge irrigation needle into the root canals to 1-2 mm shorter than the WL, and calibrating it with a rubber stop in all experimental groups. Each group was subjected to a total irrigation time of 2 minutes using 6 mL of irrigant volume, except for the control groups. Ultrasonic activation was performed for 1 minute using the ENAC piezoelectric ultrasonic system, with 25 kHz fixed frequency of the oscillating instrument (#30 Flexofile) after removing the CH paste using 2 mL of 2.5% NaOCl solution in the UI group. All teeth were then irrigated with 5 cc of saline and up-and-down movement. The positive control group had CH-obturated root canals without any irrigation, while the negative control group did not have any CH filling in the instrumented root canals. All procedures were performed by an endodontist.

Afterward, the tooth halves were separated by mildly heating the sticky wax connecting them. The coronal, middle, and apical thirds underwent stereomicroscopic assessment to calculate the residual CH's surface area and the canal's total surface area under a stereomicroscope (Hund, Wetzlar, Germany) at x40 magnification, and images were transferred to AutoCAD software 2019 (Autodesk Inc, San Rafael, CA, USA). The surface area percentage of the residual CH relative to the entire canal surface area was then calculated and reported. Only dentinal tubules were visible in the negative control group due to the absence of CH. As a result, the residual percentage of CH in each carrier group was calculated and compared with each other and with the control group to determine the success rate of each irrigation method in CH removal.

To measure the penetration depth of CH mixed with different carriers into dentinal tubules, each tooth half was gold sputter-coated (400 nm) and inspected by two calibrated endodontists independently. They analyzed the amount of residual CH on the photographs in a blind manner under a scanning electron microscope (SEM; Optica Italia, Alicante, Spain) at x400 magnification. The depth of CH penetration into dentinal tubules was measured using MountainsSEM® software premium version 1.2 (Digital surf, Besançon, France) in different areas (in micrometers) and compared with each other and with the control group to determine the efficacy of each irrigation method for CH removal [15].

Statistical analysis:

Data were analyzed using SPSS version 22 (SPSS Inc., IL, USA). The effects of the type of carrier, irrigation method, and area (distance from the apex) on the amount of residual CH were analyzed by three-way ANOVA. One-way ANOVA was used to evaluate the effect of carrier type on CH penetration depth, and to compare the amount of residual CH and its penetration depth among the groups. Tukey's test was used for pairwise comparisons. $P<0.05$ was considered statistically significant.

Results

Amount of residual CH:

Table 1 presents the measures of central dispersion for the residual amount of CH with different carriers in different parts of the root canal system in different irrigation protocols.

CH with NS carrier: The amount of CH in the coronal third was the highest, followed by the middle and apical thirds. In the coronal and middle thirds, the percentage of residual CH in the UI and SEN irrigation groups was lower than that in the CN group. The difference between SEN and UI was insignificant in the coronal ($P=0.9$) and middle ($P=1$) thirds. However, the difference between SEN and CN with UI was

significant (P<0.05). In the apical third, residual CH was the lowest in UI, followed by SEN and the CN group. Only the difference between the UI and CN was significant (P<0.05).

Table 1. Measures of central dispersion for the residual amount of CH with NS, saline, PG and CHX carriers in different parts of the root canal in different irrigation protocols

Carrier type	Irrigation protocol	Area	Mean	Std. deviation	Minimum	Maximum
Residual amount of CH with **NS** carrier	CN	Coronal	32.98	6.14	24.00	42.20
		Middle	44.00	8.28	28.30	54.00
		Apical	40.00	7.40	31.00	50.00
	SEN	Coronal	22.70	6.99	13.00	31.1
		Middle	34.00	6.77	24.20	43.0
		Apical	45.00	6.31	33.70	54.40
	UI	Coronal	24.00	6.78	16.00	35.30
		Middle	30.00	6.33	21.00	38.10
		Apical	36.00	6.19	27.00	45.00
	Control	Coronal	78.66	1.52	77.00	80.00
		Middle	62.66	4.04	58.00	65.00
		Apical	27.66	4.16	23.00	31.00
Residual amount of CH with **saline** carrier	CN	Coronal	33.60	5.97	23.80	43.00
		Middle	22.00	6.35	12.00	30.10
		Apical	25.00	5.93	16.00	35.00
	SEN	Coronal	22.00	6.15	12.20	30.00
		Middle	20.00	5.49	13.00	28.30
		Apical	44.00	5.87	36.00	53.30
	UI	Coronal	20.99	5.98	12.00	30.30
		Middle	18.60	7.03	8.20	27.00
		Apical	19.00	8.89	1.10	29.00
	Control	Coronal	86.00	4.00	82.00	90.00
		Middle	67.66	1.15	67.00	69.00
		Apical	44.00	5.29	40.00	50.00
Residual amount of CH with **PG** carrier	CN	Coronal	22.20	6.69	12.60	32.00
		Middle	9.30	4.02	4.10	18.00
		Apical	26.00	7.01	10.00	35.00
	SEN	Coronal	23.00	6.01	14.70	32.00
		Middle	9.95	5.40	1.60	19.00
		Apical	20.00	4.51	12.00	26.00
	UI	Coronal	20.00	7.00	11.00	29.00
		Middle	7.00	3.70	2.00	14.60
		Apical	14.00	6.98	4.50	23.00
	Control	Coronal	65.00	2.00	63.00	67.00
		Middle	43.66	2.08	42.00	46.00
		Apical	57.00	29.33	29.00	30.00
Residual amount of CH with **CHX** carrier	CN	Coronal	33.00	7.59	23.10	43.50
		Middle	22.00	5.92	13.00	30.00
		Apical	25.00	6.22	16.30	33.00
	SEN	Coronal	22.20	9.39	12.00	37.00
		Middle	20.00	6.02	11.00	29.00
		Apical	43.90	7.23	34.00	53.00
	UI	Coronal	19.99	9.25	1.10	30.00
		Middle	17.00	5.94	8.40	26.00
		Apical	19.80	5.91	13.10	29.00
	Control	Coronal	73.00	2.00	71.00	75.00
		Middle	57.00	52.33	52.00	53.00
		Apical	34.66	2.08	33.00	37.00

In the CN irrigation, the amount of residual CH was the lowest in the coronal third, followed by the apical and middle thirds. However, only the difference between the coronal and middle thirds was significant (P<0.05). Applying SEN and UI, the residual CH was the lowest in the coronal third, followed by the middle and apical thirds. Significant differences were found between all areas in all pairwise comparisons (P<0.05). In comparison with the positive control group, the percentage of residual CH was significantly lower in the coronal (P=0.000), middle (P=0.000), and apical (P=0.002) thirds in the NS group.

CH with saline carrier: The residual CH was the highest in the coronal third, followed by the middle and apical thirds. In the coronal and middle thirds, the residual CH was lower in the UI and SEN groups than the CN group. The difference between the UI and SEN groups was insignificant in the coronal (P=0.9) and middle (P=0.15) thirds. However, in the CN group, the difference between the SEN and UI was significant (P<0.05). The difference among the three irrigation protocols was insignificant in the middle third (P=0.4). In the apical third, the percentage of residual CH in the UI and CN groups was lower than that in the SEN group. The difference between the UI and CN was not significant (P=0.15). However, SEN had significant differences with the UI and CN groups (P<0.05).

In the UI and CN groups, the percentage of residual CH in the middle third was the lowest, followed by the apical and coronal thirds. In the CN group, the residual CH in the coronal third significantly differed from that in other areas (P<0.05). However, in the UI group, the difference among different areas was insignificant (P=0.7). In the SEN group, the residual CH was the lowest in the middle third, followed by the coronal and apical thirds. Apical third had significant differences with other areas in the amount of residual CH (P<0.05).

In comparison with the positive control group, the percentage of residual CH was significantly lower in the coronal (P=0.000), middle (P=0.000), and apical (P=0.000) thirds in the CH and saline group.

CH with PG carrier: The amount of residual CH in the coronal third was the highest, followed by the apical and middle thirds. No significant difference was noted in the coronal (P=0.5) and middle (P=0.3) thirds among the three methods. In the apical third, the residual CH was the lowest in the UI, followed by the SEN and CN groups. Only the difference between the UI and CN groups was significant (P<0.05). In the CN group, the residual CH mixed with PG was the lowest in the middle third, followed by the coronal and apical thirds. The residual CH in the middle third significantly differed from that in the coronal and apical thirds (P<0.05). In the SEN group, the residual CH was the lowest in the middle third, followed by the apical and coronal thirds. The middle third had a significant difference with other areas in this regard (P<0.05). In the UI group, the residual amount of CH was the lowest in the middle third, followed by the apical and coronal thirds. The middle third had a significant difference with other areas in this regard (P<0.05).

In comparison with the positive control group, the percentage of residual CH was significantly lower in the coronal (P=0.000), middle (P=0.000), and apical (P=0.000) thirds in the CH and PG groups.

CH with CHX carrier: The amount of residual CH in the coronal third was the highest, followed by the middle and apical thirds. In the coronal and apical thirds, the residual CH was the lowest in the UI, followed by the SEN and CN groups. Only the difference between the UI and CN groups was significant (P<0.05). In the middle third, the residual CH was the lowest in the UI, followed by the SEN and CN groups. All pairwise comparisons revealed significant differences (P<0.05). In the CN and UI groups, the residual

CH was the lowest in the middle third, followed by the apical and coronal thirds. The difference in residual CH in the coronal third in the CN group was significant with other areas (P<0.05). However, in the UI group, the difference among different areas was insignificant (P=0.6). In the SEN group, the residual CH was the lowest in the middle third, followed by the coronal and apical thirds.

Penetration depth into dentinal tubules:

Table 2 shows the penetration depth of CH mixed with different carriers into dentinal tubules. The results indicated no significant difference in penetration depth between CH mixed with different carriers (P>0.05). ANOVA revealed that the effects of carrier type and distance from the apex, and their interaction effect were not significant on the penetration depth of CH. Similarly, there was no significant difference in penetration depth of CH among different groups in the coronal, middle, or apical thirds (P>0.05).

Figure 1 shows the SEM photomicrographs, and Figure 2 shows the stereomicroscopic photomicrographs of the root dentinal walls in different groups.

Table 2. Penetration depth of CH mixed with different carriers into dentinal tubules (control group)

Carrier	Area	Mean	Std. deviation	Minimum	Maximum	P-value
NS	Coronal	348	26	370	380	0.63
	Middle	328.66	87.76	277	430	0.06
	Apical	237	72.09	190	320	0.06
Saline	Coronal	330	25.14	220	267	0.06
	Middle	66.328	8	230	246	0.06
	Apical	287	20	229	251	0.07
PG	Coronal	267.33	2.08	265	269	0.06
	Middle	263.33	12.09	254	277	0.08
	Apical	211.33	9.60	201	220	0.07
CHX	Coronal	281	82.08	156	302	0.06
	Middle	257.66	181.08	171	490	0.06
	Apical	207.33	72.56	203	340	0.07

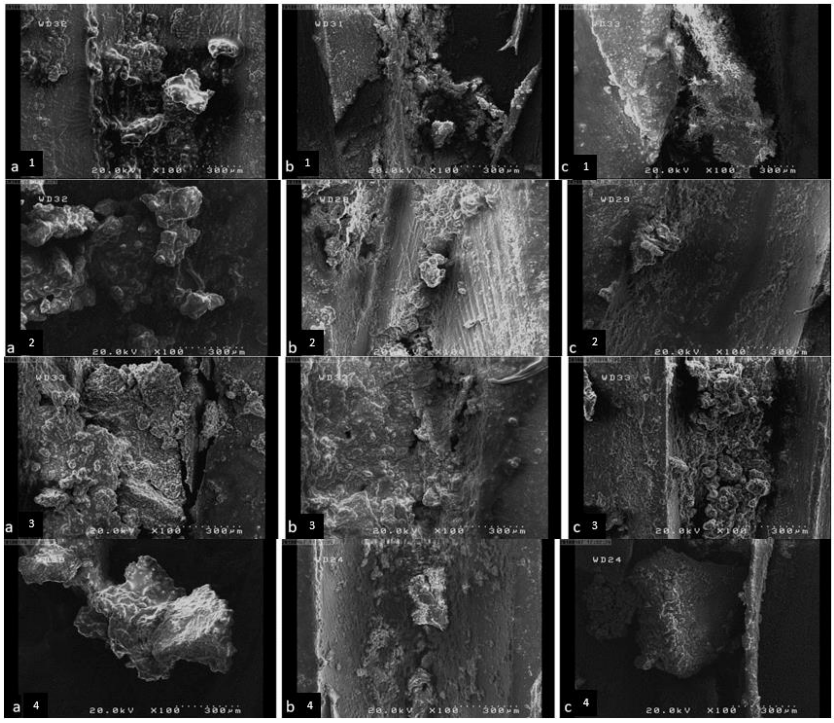

Figure 1. Residual CH mixed with saline (1), NS (2), PG (3), and CHX (4) on root canal walls after (a) CN irrigation, (b) UI, and (c) SEN irrigation

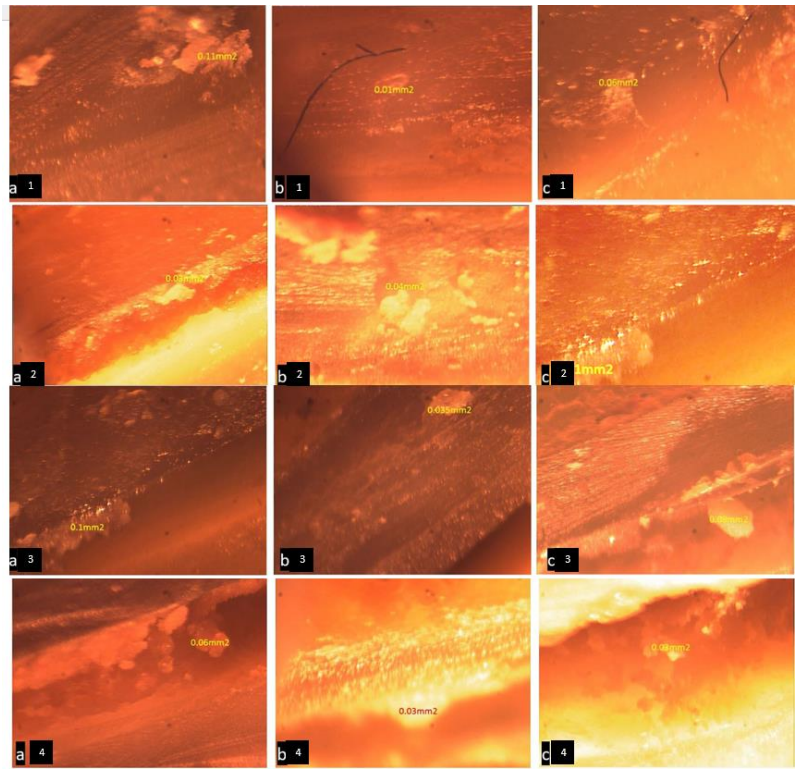

Figure 2. Post-experiment stereomicroscopic micrograph of CH mixed with saline (1), NS (2), PG (3), and CHX (4) following (a) CN irrigation (coronal third), (b) UI (apical third), and (c) SEN irrigation (middle third)

Discussion

This study quantified the amount of residual CH mixed with saline, PG, NS, and 0.2% CHX carriers after root canal irrigation with the CN irrigation, SEN irrigation, and UI. The penetration depth of CH into dentinal tubules was also measured. The results showed that none of the irrigation protocols could completely eliminate the residual CH from the root canal system, irrespective of the type of carrier, which was in agreement with the results of previous studies [26,32,33].

Considering the results in the positive control groups, all irrigating protocols significantly decreased the residual amount of CH, irrespective of the type of carrier. Of the three irrigation protocols, irrespective of the type of carrier and distance from the apex (coronal/middle/apical third), the highest CH residues were noted in CN irrigation; UI yielded the lowest CH residues, irrespective of the type of carrier and distance from the apex. The same results were reported by Volponi et al, [34] and Momenijavid et al [35]. Volponi et al. [34] used different irrigation protocols to remove CH from the root canal walls, including CN irrigation with a side-vented needle, manual dynamic agitation, sonic agitation with Endo-activator, and passive UI. None of the tested protocols could completely eliminate CH from the root canal walls. They showed that sonic agitation with Endo-activator had the highest efficacy, followed by passive UI, manual agitation, and CN irrigation with a side-vented needle.

Momenijavid et al. [35] evaluated the efficacy of different irrigation protocols, including laser irrigation, passive UI, and CN irrigation, for CH removal from artificially created standard grooves. None of the tested protocols could completely eliminate CH from all parts of the root canal system. However, passive UI and laser irrigation resulted in more significant removal of

CH than other methods. Greater elimination of CH from the root canal system by UI can be due to the generation of vibrations in the irrigants in the root canal system and the Eddy flow following activation, which are not present in the CN irrigation [24,36,37].

In the present study, in the passive UI group and when CH was mixed with CHX and saline, less CH residues were observed, which can be related to the small size of NS particles and high surface tension of PG.

The present study illustrated that irrespective of the type of carrier, the amount of residual CH in the coronal third was either significantly lower than that in other parts of the root canal, or had no significant difference, which is due to greater space and easier access of instruments for removal of CH from the coronal third. Moreover, irrespective of the type of carrier, the amount of residual CH in the application of SEN was greater in the apical third, and this difference was significant in some cases. Considering the unique design of this needle and its closed end, and subsequent release of irrigant only through the side vents, lower CH removal from the apical third was somehow expected.

Concerning the penetration depth of CH, no significant difference was noted in the present study among different carriers despite their differences in molecular properties, size of particles, and surface tension. Due to the nano-scale particle size [38], NS was expected to have greater penetration depth than other carriers. Also, PG, due to its high surface tension, was expected to have greater penetration depth; however, no significant difference was observed in this regard among the groups. Similarly, Kranz et al. [29] found no significant difference in penetration depth of CH with PG and distilled water carriers. Also, Teja et al. [39] reported similar penetration depth of CH, and nano-CH into dentinal tubules. The present study confirmed the results of the abovementioned two studies.

The present study found that the penetration depth of CH was greater in the coronal third compared to the middle and apical thirds. Additionally, the penetration depth was greater in the middle third compared to the apical third, possibly due to the larger diameter of dentinal tubules in the coronal and middle thirds, irrespective of the carrier type. Therefore, selecting the type of carrier should be based on the required antibacterial properties rather than the penetration depth into dentinal tubules.

It is important to note that while some of the CH that penetrated the dentinal tubules was removed by irrigation, the amount of CH before and after irrigation could not be quantified due to technical limitations. Only the penetration depth of CH into dentinal tubules was measured under SEM and reported. Also, this study did not assess the effectiveness of different irrigation protocols in removing CH from the dentinal tubule openings due to lack of equipment. Further studies are needed to address these topics by marking the CH to trace it. In future studies, other methods such as scoring could be used to quantify the amount of residual CH. Furthermore, the efficacy of the tested irrigation protocols should be compared with that of EndoActivator. Further investigations are also warranted to eliminate the confounding effect of the type of intracanal medicament in presence of dental debris.

Conclusion

UI was found to be slightly more effective than other techniques in removing CH from some parts of the root canal system. However, none of the irrigation techniques were able to completely eliminate CH from the entire root canal system. Other factors, such as the antibacterial activity of the carrier material, should be considered when selecting the type of

carrier for CH. Additionally, when using SEN irrigation, an alternative method should be used to ensure efficient cleaning of the apical third.

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