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The Effectiveness of Continuous Versus Sequential Chelation in the Removal of Smear Layer and Their Influence On Push-Out Bond Strength of Bio-C Sealer (An in vitro study)

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Research Article

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ABSTRACT

Objectives: Successful endodontic treatment outcome requires effective shaping and cleaning of root canals. This study aims to evaluate the smear layer removal after continuous chelation (CC) (NaOCl\HEDP) and sequential chelation (SC) (NaOCl\EDTA) and their influence on the push-out bond strength (POBS) of Bio-C sealer.

Material and Methods: Palatal roots of the maxillary first molar (n=72) were divided into four groups (n=18) as follows: 3% NaOCl, SC: 3% NaOCl followed by 17% EDTA, CC: 3% NaOCl \9% HEDP and Distilled water. Thirty-two roots (n=8/group) were split longitudinally for smear layer evaluation using SEM. Forty roots were obturated with Guttapercha and Bio-C sealer using a single cone technique. Three sections were taken horizontally from the coronal, middle, and apical third (1.5±0.1 mm thickness) for the push-out test using a universal testing machine. The Kruskal-Wallis and Mann-Whitney tests were used to analyze the SEM data, while the One-way analysis of variance (ANOVA) test and the Tukey test were used to analyze POBS data. Z test to compare failure mode.

Results: There was no difference between SC and CC in the smear layer removal at all thirds (p>0.05). The POBS in CC was significantly higher than SC in all thirds (p<0.05). Failure mode distribution was 53.3% mixed, followed by 26.6% adhesive and 20% cohesive.

Conclusions: NaOCl\HEDP was as effective as NaOCl\EDTA in removing the smear layer. Neither of the two protocols could eliminate the smear layer completely. The bonding strength of Bio-C sealer was optimized by using NaOCl\HEDP combination.

Key words: Bio-C sealer, dual rinse HEDP, EDTA, push-out, smear layer.

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Introduction

The goal of a root canal treatment is three-dimensional obturation with a complete seal of the root canal system.[1] Root canal irrigation is the most important factor in the healing of periapical tissues.[2] Root canal irrigation is used to eliminate the smear layer that contains organic and inorganic debris.[3] Endodontic instruments during the shaping procedure causes smear layer to form and this layer may affect the success of endodontic therapy.[4,5] By removing the smear layer, the root canal walls may be cleaned and disinfected more thoroughly, and the root canal filling materials can adapt more effectively.[6]

Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are often used to clean the root canal and remove the smear layer.[7] Because NaOCl and EDTA cannot be used together; this rinse requires two separate irrigations known as "sequential chelation" Still, it has significant drawbacks, including that active chlorine is rapidly eliminated when NaOCl contacts EDTA.[8] In addition, extended EDTA exposure to root dentin diminished flexure strength of dentin.[9]

Etidronic acid or ("1- hydroxyethane-1, 1-diphosphonic acid") (HEDP or less commonly HEBP) is nitrogen-free biphosphonate weak chelator that can be mixed with NaOCl to create a single irrigant solution during mechanical preparation and as a final irrigation and eliminating sequential rinses with chemically incompatible solutions. Etidronate is a salt of etidronic acid where cations are linked to the anion of HEDP (usually Disodium etidronate “Na_2HEDP” or Tetrasodium etidronate “Na_4HEDP”).[10]

Zehnder *et al.*[10] was the first investigator who used HEDP for smear layer removal. HEDP\NaOCl mixture was found to minimize smear layer production during instrumentation, rather than removing an existing smear layer, hence the term "continuous chelation.” was used.[11] Based on this chemistry, Dual Rinse HEDP (Medcem, GmbH, Weinfelden, Switzerland) is a clinically certified product. It comes in a capsule (0.9 g etidronate), which must be mixed with 10 mL of sodium hypochlorite solution.[12]

Keeping the root canal's integrity and seal is dependent greatly on the endodontic sealers' ability to adhere to dentin.[13,14] Bio-C sealer (Angelus, Londrina, PR, Brazil) is a newly introduced hydraulic tricalcium silicate sealer. Manufacturers stated that it has a short setting time, is effective at sealing the complex root canal system, and maintains its volume.[15]

Little information in the literature about the impact of Dual Rinse HEDP irrigation solution on smear layer removal and the Push-out bond strength (POBS) of calcium silicate-based sealers. No studies have been conducted to date to assess the effect of HEDP on the POBS of Bio-C sealer to root canal dentin. Thus, this study aimed to compare the influence of sequential chelation (3% NaOCl followed by 17% EDTA) and continuous chelation (3% NaOCl\9% HEDP) on the smear layer removal and its influence on POBS of Bio-C sealer at the different thirds of the root canal. The null hypothesis stated that there is no difference in smear layer removal after irrigation with NaOCl\HEDP and NaOCl\EDTA. Also, there is no difference in POBS of Bio-C sealer in root canals irrigated with NaOCl\HEDP and NaOCl\EDTA.

Material and Methods

The preferred reporting Items for Laboratory studies in Endodontics (PRILE) 2021 recommendations were followed in writing the study's manuscript.[16] The PRILE 2021 flowchart summarizes the important aspects of this study (Appendix 1). The materials utilized in this study are described in Table1.

The sample size calculation was performed using G*Power 3.1.9.4 (Heinrich Heine University, Düsseldorf, Germany) based on the results from previous studies[17,18] with an effect size of 0.8, power 0.95, α error 0.05, thus requiring sixteen teeth for each group. Eighteen teeth were assigned for each group.

Ethical approval from The Research Ethics Committee of the College of Dentistry, University of Baghdad (Project No. 511522, Ref. No.511) was gained for the use of extracted human teeth. Seventy-two extracted human maxillary first molars were included. Each tooth should have a straight palatal root, a round form canal, the length of palatal root (12 mm), and without cracks, external root resorption, or fracture. Calcified debris on the teeth were removed by using ultrasonic scalers. Then, the teeth were immersed in a thymol solution 1% (sigma –Aldrich, steinheim, Germany) for 48 hours for disinfection and stored in distilled water up to the experiment time. The palatal roots were sectioned and a K-file size 10 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted in each root until the file was observable through the apex, and the working length was established by subtracting 1 mm from this measurement (11mm).

The samples were randomly divided using the website (https://www.randomizer.org/s) based on irrigation protocols into four groups (n=18):

I. Positive control "NaOCl": 2 mL 3% NaOCl for 1 minute after each instrument, as a final rinse 5 mL 3% NaOCl for 1 minute. Then, 5 mL of distilled water for 1 minute.

II. Sequential chelation "NaOCl\EDTA": 2mL 3% NaOCl for 1 minute after each instrument, as a final rinse 5 mL 17% EDTA for 1 minute. Then, 5 mL of distilled water for 1 minute.

III. Continuous chelation "NaOCl\ HEDP": 2mL 3% NaOCl/9%HEDP for 1 minute after each instrument, as a final rinse 5 mL of 3% NaOCl/9% HEDP for 1-minute. Then, 5 mL of distilled water for 1 minute.

IV. Negative control "Distilled water": 2mL distilled water for 1 minute after each instrument and a final rinse of 5mL distilled water for 1 minute.

Immediately before instrumentation, the NaOCl/HEDP mixture was prepared by mixing one capsule (0.9 g etidronate powder) with 10 mL of the 3% NaOCl. ProTaper NEXT system (Dentsply Maillefer, Ballaigues, Switzerland) was used for the canals' instrumentation up to size X3 (30\.07), and an X-smart plus Endo-motor (Dentsply Maillefer, Ballaigues, Switzerland) was used with a rotation speed of 300 RPM and 200 gcm torque. A 30-gauge needle (United dental group, China) was used for the irrigation, which was inserted 2 mm shorter than the working length. All the procedures were performed by single operator.

SEM examination

Eight specimens from each group (32 samples) were chosen for SEM examination. Then, using a diamond disc and water irrigation, longitudinal grooves were created on the buccal and palatal surfaces. To prevent contamination during the splitting procedure, an X3 master cone was passively introduced into the root canals.

The roots were separated into two halves using a blade n° 15 and a hammer. The specimens were sputter coated with gold and analyzed by scanning electron microscope (Axia Chemisem, Thermo Scientific Fisher, USA,2021) in the center of the apical, middle, and coronal of the canal at (2.5, 6.5, and 10.5 mm from the apex respectively) at 1500x.

Horizontal marks were made at the middle of the coronal, mid-root, and apical sections on the cut/split dentine surface outside the root canal, using a permanent marker. This was to objectively locate the center of each sections when examined under the SEM.

Photomicrographs were scored by two calibrated evaluators according to Hülsmann *et al.*[19] criteria as follows: 1= indicated that the dentinal tubules were entirely open with no smear layer, 2= indicated that more than 50% of the dentinal tubules were open, 3= indicated that less than 50% of the dentinal tubules were open, and 4= indicated that more than 75% of the dentinal tubules were covered by the smear layer.

Canal filling

The remaining 40 roots (n=10/group) were obturated using the single cone obturation method using Guttapercha and Bio-C sealer (Angelus, Londrina, PR, Brazil). The master cone was inserted into the canal after the sealer had been injected at 4mm from the apex. Radiographs with mesiodistal and buccolingual directions were taken to verify correct root canal obturation without any voids. Then, all the teeth were kept with gauze moistened in Phosphate-Buffered Saline (Chemical point, Egham, United Kingdom) at 37°C for one week for the complete set of sealer.

Push-out test

Each root was inserted in clear cold cure acrylic (Duralay; Reliance Dental, Alsip, IL, USA) and sectioned perpendicular to the long axis using low speed saw (Isomet; Buhler, Ltd Lake Bluff, NY) with a diamond disk (0.5mm) under continuous water cooling to gain three slices of (1.5 ±0.1mm) thickness for each root third at the following distance from the apex (2-3.5, 6-7.5 and 10-11.5mm) respectively. The thickness of each slice was verified using a digital caliper.

A digital microscope (Q-scope, Netherlands) captured images of each slice's apical and coronal sides. Push-out test was performed using a universal testing machine (Tinius Olsen, United Kingdom) in the apico-coronal direction at a crosshead speed of 0.5 mm/min. The area calculation was performed using the formula:[18]

Area (mm^2) =D1+D2\2×π×h

D1, and D2 represent the largest and the smallest canal diameter, respectively, π is the constant 3.14, and h represents the root slice thickness. Three plunger sizes (0.7, 0.5, and 0.4 mm) were used calculated as 90% of canal diameter in the apical aspect of each root slice and the plungers were provided complete coverage over the GP without touching the canal walls and sealer.

The following equation was used to determine the POBS in megapascals and the maximal force (F) in Newtons: POBS (Mpa)=Force (N)\ surface area (mm^2). Failure mode analysis was performed using a digital microscope at 60 X magnification. The failure mode was categorized into the following types: Adhesive failure, Cohesive failure, and Mixed failure.[20]

Statistical analysis

The SPSS software version 26 (SPSS Inc., Chicago, IL, USA) was used for data analysis, with a significance level of ($p \leq 0.05$). Statistical tests included: The Kruskal-Wallis, Mann-Whitney U, Kappa, ANOVA, and Tukey post hoc tests. Z test to compare the failure mode.

Results

SEM examination

The Kappa test value was (0.89), indicating high agreement among the observers. The results of the smear layer scoring, including median and mean rank, are presented in [Table 2]. Representative SEM photomicrographs of all thirds are presented in [Figure 1].

There was no significant difference in smear layer scores between all thirds in the NaOCl and distilled water groups *(P>0.05),* whereas there was a significant difference in smear layer scores between thirds in the experimental groups *(P<0.05).* In the NaOCl \EDTA group, the apical third showed a significantly higher mean rank of smear layer scoring than the coronal third *(P<0.05).* while in the NaOCl\HEDP group, the apical third showed a significantly lower mean rank than the coronal third *(P<0.05),* as shown in [Table 2].

There was a significant difference in smear layer scores between the groups at each third *(P< 0.05).* There was no significant difference between NaOCl\EDTA and NaOCl\HEDP groups in the mean ranks of the smear layer scores at all thirds *(P>0.05).* Both NaOCL\EDTA and NaOCl\HEDP groups showed significantly lower mean ranks of smear layer scores compared to the control groups *(P< 0.05)*, as shown in [Table 2].

Push-out test

The mean values and standard deviation of POBS (MPa) are summarized in [Table 3]. NaOCl\HEDP group has the highest mean values at all thirds, followed by the NaOCl\EDTA group. There was a statistically significant difference in POBS among the groups in all thirds *(P< 0.05).* The NaOCl\HEDP group showed significantly higher POBS than the NaOCl\EDTA and control groups at all thirds *(P< 0.05).* NaOCl\EDTA group showed significantly higher POBS than the distilled water group at all thirds *(P<0.05)*, however, there was no difference in POBS between NaOCl\EDTA and NaOCl groups in all thirds *(P> 0.05).*

There was a significant difference in POBS mean values among thirds in all groups *(P< 0.05)*. In all groups, the coronal third showed significantly higher POBS as compared to the apical third *(P< 0.05),* while there was no difference in POBS between the middle and apical thirds *(P> 0.05).*

The distribution of failure mode is illustrated in [Figure 2]. The percentages of failure mode were 53.3% mixed, followed by 26.6% adhesive and 20% cohesive. An example of each failure mode is illustrated in [Figure 3]. Z-test revealed that there was a significantly higher cohesive failure in the NaOCl\HEDP group compared to NaOCl\EDTA (z = -2.10, p= 0.034), both NaOCl and distilled water groups didn't exhibit cohesive failure. Also, there was significantly higher mixed failure in the NaOCl \EDTA group compared to NaOCl\HEDP and distilled water (z = -2.10, p= 0.034) and (z = -2.6, p= 0.009), respectively and no difference to the NaOCl group (z = -1.6, p= 0.1). No significant difference was observed in the adhesive mode of failure between NaOCl and distilled water groups (z = 1.03, p= 0.29).

RATIONALE/JUSTIFICATION

A sequential chelation protocol of sodium hypochlorite followed by EDTA is recomended for the efficient removal of smear layer .According to the literature, this protocol has many disadvantages and negative effect on dentin .Continuous chelation by using soft chelator HEDP combined with sodium hypochlorite may exert minimal detrimental effect on dentin . There is minimal evidence concerning the effects of Dual rinse HEDP irrigation solution on smear layer removal and on the bond strength of calcium silicate based sealers. Up to now, no studies have evaluated the effect of continuous chelation by Dual rinse HEDP on POBS of bio c sealer to root canal dentin.

AIM/HYPOTHESIS

The aim of this study was to compare and assess the influence of sequential chelation protocol (3% NaOCl followed by 17% EDTA) and continuous chelation (3% NaOCl\9% HEDP) on the removal of the smear layer from the instrumented root canals and its influence on POBS of Bio c sealer at the different thirds of the root canal.

ETHICAL APPROVAL

Ethical approval was obtained for the collection of extracted human teeth in this study from The Research Ethics Committee of the College of Dentistry, University of Baghdad (Ref No. 511).

SAMPLES

72 freshly extracted human maxillary first molars were used. The teeth were selected according to the following criteria: Straight palatal root, Round shape canal, Palatal root length at least 12 mm and maximum apical diameter of ISO size #15, Patent and centrally located apical foramen and without cracks, external root resorption or fracture.

EXPERIMENTAL AND CONTROL GROUPS, INCLUDE INDEPENDENT VARIABLES

I. Positive control (NaOCl): (n=18) 2 mL 3% NaOCl for 1 minute after each instrument change, 5 mL 3% NaOCl for 1-minute and final rinse of 5 mL distilled water for 1 minute.

II. Sequential chelation (NaOCl\EDTA):(n=18) 2mL 3% NaOCl for 1 minute after each instrument change, 5 mL 17% EDTA for 1-minute and final rinse of 5 mL distilled water for 1 minute.

III. Continuous chelation (NaOCl\ HEDP)(n=18): 2mL 3% NaOCl/9%HEDP for 1 minute after each instrument change, 5 mL 3% NaOCl/9% HEDP for 1-minute and final rinse of 5 mL distilled water for 1 minute.

IV. Negative control (Distilled water):(n=18) 2mL distilled water for 1 minute after each instrument change and final rinse of 5mL distilled water for 1 minute.

OUTCOME(S) ASSESSED, INCLUDE DEPENDENT VARIABLES AND TYPE

I. smear layer removal(ordinal variable)

II. push-out bond strength values (continuous variable)

III. mode of bond failure(nominal variable).

METHOD USED TO ASSESS THE OUTCOME (S) AND WHO ASSESSED THE OUTCOME(S)

Smear layer removal were examined using Scanning Electron Microscope and the photomicrographs at 1500 x magnification were scored independently by two blinded observers .The push-out bond strength were tested using universal testing machine at cross head speed 0.5mm\min.The mode of failure was assesed using digital microscope at 60 x magnification

RESULTS

There was no difference between SC and CC in removing the smear layer at all thirds ($p> 0.05$) . The bond strength in the coronal third was higher than that in the middle and apical ($p<0.05$). The highest mean of push-out bond strength was recorded in the CC (5.175±0.93 Mpa), while the lowest mean was found with distilled water (0.574±0.28 Mpa). The bond strength in NaOCl\HEDP group was significantly higher than NaOCl\EDTA at all thirds.

CONCLUSION(S)

The ability of NaOCl\ HEDP to remove the smear layer was as effective as NaOCl\EDTA and neither of the two protocols could render all root canals free of smear layer. HEDP\NaOCl mixture optimized the bond strength of Bio c sealer to dentin as compared with NaOCl\EDTA.Continuous chelation protocol may be considered an alternative to the commonly used irrigation with NaOCl\EDTA.

CONFLICT OF INTEREST

The authors deny any conflicts of interest related to this study.

Figure 1. *PRILE 2021 Flowchart*

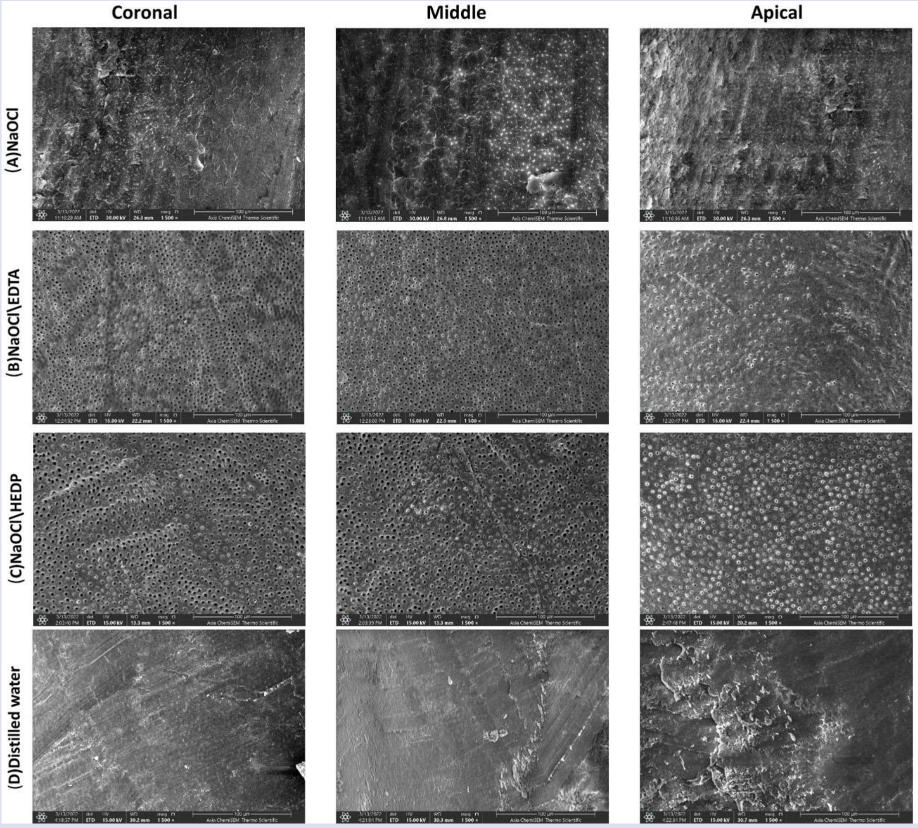

Figure 1. *Scanning electron microscope photomicrographs at 1500x showing; root specimens irrigated with NaOCl (A), NaOCl \EDTA (B), NaOCl\HEDP(C), and Distilled water (D) at different thirds of root canal (Coronal, middle, and apical).*

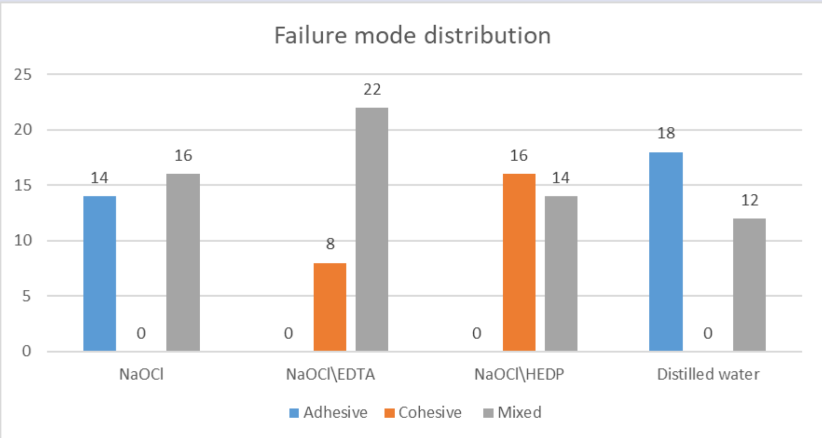

Figure 2. *Bar chart illustrating the failure mode distribution.*

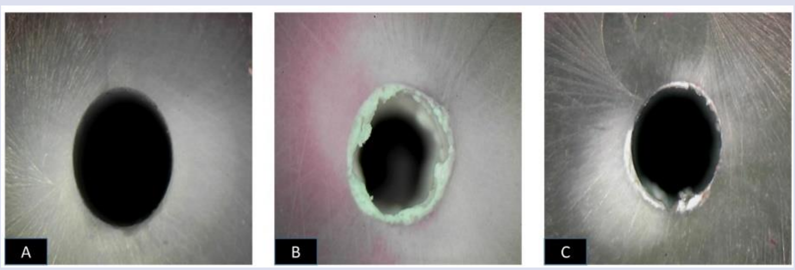

Figure 3. *Images were obtained by digital microscope at 60 x magnification for the examination of failure mode, (A) adhesive failure, (B) cohesive failure, and (C) mixed failure.*

Table 1. Materials' composition, manufacturers, and Lot numbers.

Materials	Composition	Manufacturer	Lot numbers
9%HEDP (Dual Rinse)	1-hydroxyethane 1,1-diphosphonic acid (etidronate powder)	(Medcem, GmbH, Weinfelden, Switzerland)	DR210419
Bio-C Sealer	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agents.	(Angelus, Londrina, Parana, Brazil)	60123
NaOCl 3%	Ionizes in water into Na+ and OCL-	(Modern medical equipment LLC, Dubai, UAE)	PK2122464
EDTA 17%	Disodium edetate (ethylene Diamine Tetra acetic acid 17%), water, and excipients.	(Cerkamed ,Stalowa wola ,Poland)	1408201
PBS (7.4 ±0.2 pH)	Sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate	(Chemical point, Egham, United Kingdom)	9066

NaOCl: Sodium hypochlorite; OCL-: hypochlorite ion; Na+: sodium; EDTA: ethylene Diamine Tetra acetic acid; PBS: Phosphate Buffered Saline.

Table 2. Smear layer (median, mean rank) of all groups in the coronal, middle, and apical thirds of the root canal.

Groups	Root canal thirds					
	Coronal		Middle		Apical	
	Median	Mean rank	Median	Mean rank	Median	Mean rank
NaOCl	4.00	$24.00^{a,b}$	4.00	$24.50^{a,b}$	4.00	$22.69^{a,b}$
NaOCl\EDTA	2.00	$7.50^{a,c,A}$	2 .00	$8.18^{a,c}$	3.00	$10.88^{a,c,A}$
NaOCl\HEDP	2.00	$9.50^{b,d,A}$	2 .00	$8.88^{b,d}$	3.00	$8.00^{b,d,A}$
Distilled water	4.00	$25.00^{c,d}$	4.00	$24.50^{c,d}$	4.00	$24.50^{c,d}$

NaOCl: sodium hypochlorite; EDTA: ethylene Diamine Tetra acetic acid; HEDP: 1-hydroxyethane 1,1-diphosphonic acid.
Identical superscript uppercase letters represent a statistically significant difference between thirds in each row (in each group). Identical superscript lowercase letters represent a statistically significant difference between groups in each column.

Table 3. Means ± SD of push-out bond strength values (Mpa) in the study groups.

Group	Coronal	Middle	Apical
NaOCl	$1.911 \pm 0.552^{a,b,A,B}$	$1.264 \pm 0.646^{a,B}$	$0.865 \pm 0.410^{a,A}$
NaOCl\EDTA	$2.361 \pm 0.963^{c,d,A}$	$1.813 \pm 0.695^{b,c}$	$1.376 \pm 0.692^{b,A}$
NaOCl\HEDP	$5.175 \pm 0.932^{a,c,e,A,B}$	$3.519 \pm 1.113^{a,b,d,B}$	$2.832 \pm 0.906^{a,b,c,A}$
Distilled water	$0.995 \pm 0.399^{b,d,e,A,B}$	$0.636 \pm 0.271^{a,b,c,d,B}$	$0.574 \pm 0.281^{b,c,A}$

SD:standard deviation; MPa:MegaPascal; NaOCl: sodium hypochlorite; EDTA: ethylene Diamine Tetra acetic acid; HEDP: 1-hydroxyethane 1,1-diphosphonic acid. Identical superscript uppercase letters represent a statistically significant difference among thirds within each group in each row. Identical superscript lowercase letters represent a statistically significant difference between relevant groups in each column.

Discussion

This study aims to compare the effectiveness of CC protocol by a mixture of NaOCl\ HEDP to SC by NaOCl \EDTA on smear layer removal from dentinal tubules and their influence on POBS of Bio-c sealer. In this study, both protocols have comparable effectiveness in removing the smear layer at all thirds. Thus, the first hypothesis was accepted. Whereas, the second hypothesis was rejected because the CC protocol showed significantly higher POBS as compared to SC at all thirds.

Scanning electron microscopy (SEM) is the most widely used method for assessing cleaning efficiency as it aids in the examination of the whole area of the canal using a numeric evaluation score for the smear layer.

The findings of this study revealed that there was no significant difference between sequential chelation (NaOCl\EDTA) and continuous chelation (NaOCl\HEDP) in terms of the smear layer removal. SEM photomicrographs showed that in both groups clean dentin areas could be seen in the coronal third and middle thirds, and most of the dentinal tubules were opened, whereas in the apical third, most dentin areas were covered with a smear layer, and there were fewer opened dentinal tubules than in the coronal and middle thirds. These results agree with the previous study by kfir *et al.*[17] which revealed that both protocols have comparable effectiveness in removing the smear layer from dentinal tubules but neither of the two protocols evaluated could completely remove the smear layer from all root canal thirds.

Conversely, it was claimed that HEDP has shown a greater capacity for smear layer removal than EDTA.[21] Additionally, this may be explained by the fact that HEDP minimizes the formation of the smear layer and lowers the deposition of hard tissue debris during root canal instrumentation.[12]

On the other hand, at neutral pH, Deari *et al.*[22] found that HEDP was less effective than EDTA at removing the smear layer. HEDP's higher pH and lower stability constant compared to EDTA explained this finding. Because of the low concentration of HEDP and the short duration of application, Zehnder *et al.* [10] found that 17% EDTA is more effective than 3.5% Na4 HEDP\0.5% NaOCL over 1 minute in eliminating smear layer.

In this study, root samples irrigated using NaOCl alone showed a statistically significant heavy smear layer at all thirds as compared to the experimental groups. The ineffectiveness of NaOCl alone to remove the smear layer was previously reported[23] and this is because of its limited action that is restricted to the organic constituent of the smear layer. SEM photomicrographs revealed that both NaOCl and distilled water groups showed a significant amount of smear layer in all thirds (coronal, middle, and apical) and nearly all of the dentinal tubules were covered with the smear layer.

Despite the non-statistically significant difference between EDTA and HEDP groups, HEDP removed slightly more smear layer in the apical third than EDTA. This contrasts with the results of the study done by Yadav *et al.*[24] who reported the ineffectiveness of etidronic acid in cleaning the apical third of the root canal and this might be because of its weak chelating action.[25]

In this study, the apical third showed the least smear layer removed compared to the middle and coronal, which was attributed to decreased permeability, and sclerotic nature of the dentin at this region.[26] Additionally, vapor lock formation hinders the circulation and action of the irrigating solutions.[27]

The POBS test is used to assess the bond strengths of root canal sealers to dentin.[28] The POBS of Bio-C sealer was significantly higher after irrigation with (NaOCl \HEDP) as compared to other groups. This result agrees with a previous investigation that reported that pre-conditioning dentin with NaOCl\ HEDP mixture improved the POBS of calcium silicate-based sealers.[18] The better performance of HEDP can be ascribed to a variety of reasons. Firstly, HEDP exhibited no negative effect on the hydration characteristics of calcium silicate types of the cement.[29] Secondly, the continuous chelation facilitated the dissolution of organic dentin components, conditioning of the inorganic component, resulting in a reduction of smear layer and smear plugs, enhanced micromechanical retention, and a greater bond strength to root canal dentin.[30] Thirdly, HEDP had deeper sealer penetration than (NaOCl/EDTA) and a higher proportion of sealer covering canal walls.[31] NaOCl\HEDP may provide better conditioning of root canal walls for root filling materials which in turn enhancing the bond strength of endodontic materials to root canal dentin with favorable prognosis for endodontic treatment.

The POBS in the group of EDTA was significantly lower than that of HEDP at all thirds. This could be linked to the effect of EDTA on apatite formed during the Bio-C sealer setting reaction. After chemomechanical preparation, Lee *et al.*[32] found that EDTA residues continued to chelate the ions of calcium that formed from MTA during hydration, preventing the precipitation of the hydrated product and reducing its microhardness. Atmeh *et al*.[33] hypothesized that calcium decrease at the sealer-dentin interface or degradation of the calcium silicate component in the sealer would impede the creation of the "mineral infiltration zone".

Another finding is that POBS decreases in a corono-apical direction, which agrees with Anju *et al.*[34] This is because apical dentin has fewer patent tubules than coronal dentin[7] and more sclerotic dentin, which is not favorable to sealer infiltration.

The low POBS in (NaOCl) group could be due to NaOCl only removing the organic portion of the smear layer and cannot dissolve the inorganic materials thereby reducing the bonding of Bio-C sealer to the root canal walls. This agrees with previous study[35] that reported a decrease in POBS of endodontic sealer following irrigation with NaOCl. Studies have also revealed that NaOCl interacts with calcium silicate cement, which can impact its adhesion.[36]

The lowest POBS values were in the distilled water group. This agrees with previous studies[29] that showed inferior smear layer removal of distilled water, indicating the negative effects of undisturbed smear layer on POBS values by preventing the penetration of sealer inside dentinal tubules.

Regarding the modes of failure, in this study the mixed mode was the predominant one and this finding was in agreement with Falakaloğlu and Gündoğar.[37] This related to Bio-C sealer's bioactivity, which encourages hydroxyapatite formation, and its excellent flow rate[15], which enables the sealer's penetration. Similarly, Caceres *et al*.[38] discovered that Bio-C sealer demonstrated uniform tubular penetration with few gaps and this may be attributed to its hydrophilic nature, which contains nanometric particles which enables deeper penetration.

The study is limited by in vitro environment. As a result, the obtained results cannot be generalized to clinical scenarios. The effectiveness of chelating irrigants may be affected by agitation devices. Also, digital image analysis can be used to score the smear layer. It takes less time, can measure other important characteristics including density and average dentinal tubule diameter, and can overcome evaluator bias. Furthermore, the applied load was static which is not resemble the dynamic load in the oral cavity, therefore the performance of the material in this in vitro study may differ from the clinical situation.

Conclusions

Continuous chelation did not vary from sequential chelation in smear layer removal. However, neither of the two protocols could eliminate the smear layer completely from root canals. The present study strengthens the fact that the POBS of calcium silicate-based sealers was differentially influenced by the irrigation protocol used. NaOCl\HEDP protocol improved the bond strength of the Bio-C Sealer.

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Conflicts of Interest Statement

All authors hereby deny any conflicts of interest in connection with this article.

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