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Original Article

Comparative Effects of Continuous and Sequential Chelation Combined with Various Agitation Techniques on Smear Layer Elimination and Dentin Integrity

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ABSTRACT

The purpose of this in vitro study was to investigate how sequential versus continuous chelation affects the removal of the smear layer and the hardness of root canal dentin, and to evaluate the influence of different agitation methods. Sixty-four palatal roots of maxillary first molars were prepared to size X3 using Protaper Next files. Samples were divided into two irrigation protocols: sequential chelation (SC) with 3% NaOCl followed by 17% EDTA, and continuous chelation (CC) using a dual-rinse solution (3% NaOCl/9% HEDP). Each protocol was further subdivided according to agitation technique: conventional needle (CN), EndoActivator (EA), ultrasonic (UAI), and Er.Cr.YSGG 2780 nm laser (n = 8 per subgroup). Smear layer removal was evaluated by scanning electron microscopy (SEM), and Vickers microhardness measurements were taken at depths of 50 and 100 µm. Statistical analysis included Kruskal–Wallis, Wilcoxon, and Mann–Whitney U tests, with significance set at $p < 0.05$.

Continuous chelation with ultrasonic or laser activation resulted in greater smear layer reduction in the apical and coronal regions, respectively, compared to sequential chelation ($p < 0.05$). No notable differences between SC and CC were observed for conventional needle or EndoActivator groups ($p > 0.05$). Overall, CC preserved dentin microhardness better than SC, with the exception of specific sections in CN, EA, and UAI groups at certain depths. Continuous chelation provides similar efficacy in smear layer removal as sequential chelation, while exerting a less negative effect on dentin microhardness.

Keywords: Dentin microhardness, Smear layer removal, Chelation, Root canal irrigation, Agitation techniques

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Introduction

The primary goal of root canal therapy is to treat or prevent apical periodontitis, which arises as an immune response to microbial invasion within the root canal system [1]. This is achieved by eliminating or inhibiting microbial growth through cleaning, shaping, and three-dimensional obturation of the canal [2]. During mechanical instrumentation, a layer of organic and inorganic debris, termed the “smear layer,” forms on the dentin surface [3]. Evidence suggests that removing this layer improves the seal of root canal fillings and enhances the penetration of antimicrobial agents, sealers, and intracanal medications into the dentinal tubules [4]. Common irrigants employed for this purpose include sodium hypochlorite (NaOCl) for organic matter and ethylenediaminetetraacetic acid (EDTA) for inorganic components [5]. These solutions are typically applied in succession, a process referred to as “sequential chelation” [6]. Nevertheless, drawbacks such as the rapid depletion of NaOCl’s active chlorine when it contacts EDTA can occur [7].

Additionally, prolonged exposure to strong chelators like EDTA may compromise the mechanical properties of root dentin, reducing microhardness and flexural strength [8]. Sequential application is designed to prevent direct mixing of irrigants, which could otherwise intensify chemical interactions [9].

Etidronic acid (1-Hydroxyethylidene-1,1-bisphosphonate, HEBP, or HEDP) is a biocompatible, weak chelator that can be combined directly with NaOCl to provide simultaneous deproteinization, antimicrobial activity, and chelation [10]. Continuous chelation involves the use of this single solution throughout instrumentation, preserving the antibacterial and proteolytic properties of NaOCl [11]. Currently, dual-rinse HEDP (Medcem GmbH, Weinfelden, Switzerland) represents the only commercially approved continuous chelation product for clinical endodontic use. It is supplied as a 0.9 g etidronate capsule intended for dissolution in 10 mL of NaOCl [12, 13].

Chemo-mechanical preparation significantly reduces bacterial populations by targeting canal walls and promoting the penetration of disinfectants into dentinal tubules [14]. To further enhance irrigation efficiency and eliminate resistant microorganisms, multiple agitation strategies have been developed [15], including hand files, gutta-percha cones, plastic instruments, sonic and ultrasonic devices, apical negative pressure irrigation, and photon-induced photoacoustic streaming [16]. Sonic agitation with polymer tips generates hydrodynamic effects and intracanal waves in a filled canal [17], whereas passive ultrasonic irrigation (PUI) transmits energy via oscillating metal files, producing acoustic streaming and cavitation to improve fluid dynamics within the canal [18]. Er,Cr:YSGG (2780 nm) and Er:YAG (2940 nm) lasers can also remove smear layers through direct ablation of dentin [19], and laser-assisted irrigation protocols enhance fluid movement and pressure against canal walls, aiding in debris and smear layer removal even in minimally prepared canals [20].

While combining chelators with NaOCl may offer therapeutic benefits, it can negatively affect the dentin matrix. Prior research has reported decreases in dentin microhardness, flexural strength, fracture resistance, and erosion following NaOCl irrigation combined sequentially with EDTA or HEDP [9].

Limited studies have investigated the effects of dual-rinse HEDP with various agitation techniques on smear layer removal and dentin microhardness [21]. Therefore, the present study aimed to compare the efficacy of sequential chelation (SC: 3% NaOCl followed by 17% EDTA) versus continuous chelation (CC: 3% NaOCl/9% HEDP) using conventional needle, EndoActivator, ultrasonic, and laser agitation techniques. The null hypothesis was that no significant differences would be observed in smear layer removal or dentin microhardness among the tested chelation methods and agitation protocols.

Materials and Methods

Tooth selection

The study utilized 64 extracted maxillary first molars from patients aged 18–35 years to limit variability in dentin hardness. Sample size calculations determined that eight teeth per subgroup would provide 95% statistical power with an alpha of 0.05 and an effect size of 1.16 [22]. Ethical clearance was obtained from the College of Dentistry, University of Baghdad (Project No. 851523; Ref. No. 851, 23 November 2023). Only teeth with straight palatal roots and free from fractures, external resorption, or cracks were included.

Preparation of samples

Teeth were initially cleaned of calculus using an ultrasonic scaler. They were then stored in a 1% thymol solution (Sigma–Aldrich, Germany) for 48 hours, followed by deionized water until use. Palatal roots were standardized to a length of 12 mm. Working length was established by inserting a size 10 K-file (Dentsply Maillefer, Switzerland) until it was visible at the apex, and subtracting 1 mm. The same K-file size was used to ensure proper canal access.

Root canal shaping was carried out using Protaper Next® rotary files (X3, 30/0.07) with an Endo Motor (E-CONNECT, Changzhou Sifary Medical Technology, China) set at 300 RPM and 2 Ncm torque, following the manufacturer's protocol. Irrigation between files was delivered using a 30-gauge, double-sided needle (SinaliDent, China) positioned 2 mm short of the working length.

Irrigation protocols

The teeth were randomly divided into two groups (n = 32) based on the irrigation regimen:

- *Sequential chelation (SC):* 3% NaOCl (2 mL) applied for 1 min after each file, followed by 17% EDTA (5 mL) for 1 min, and a final rinse with 5 mL distilled water [6].
- *Continuous chelation (CC):* 3% NaOCl combined with 9% HEDP (2 mL) for 1 min after each file, followed by 5 mL of the same solution for 1 min, and a final rinse with 5 mL distilled water [6]. The NaOCl/HEDP solution was freshly prepared by mixing 10 mL 3% NaOCl with 0.9 g of etidronate powder immediately before use.

Irrigant agitation techniques

Each main group was subdivided into four subgroups (n = 8) based on the activation method used:

1. *Conventional needle (CN):* A 30-gauge double-vented needle was inserted 2 mm short of working length and moved in an up-and-down motion during irrigation.
2. *Sonic activation (EndoActivator, EA):* A medium-sized polymer tip (25/0.04) was activated at 10,000 RPM for 60 s and positioned 2 mm from the working length, without exerting pressure.
3. *Passive ultrasonic irrigation (UAI):* A 20/0.02 silver tip was coupled to an ultrasonic unit (Ultra X, Eighteeth, China) operating at 45 kHz. The tip was held 1 mm from working length, performing apical–coronal motions over a 2–3 mm range for 60 s.
4. *Laser activation (Er:Cr:YSGG):* A 200 µm radial-firing tip (25 mm) was used with the laser set at 2780 nm, 1.25 W, 50 Hz, 25 mJ, and 60 µs pulse duration. The fiber was positioned 2 mm from the apex and moved helically from apex to coronal in three 20-second cycles (total 60 s).

For SC groups, agitation was performed using EDTA, while for CC groups, the NaOCl/HEDP solution was used. A single irrigation cycle was applied before agitation for all samples. Detailed irrigation volumes and sequences are summarized in **Table 1**.

Table 1. The protocols of irrigation and agitation used in each group of the study.

Group	Irrigation and Agitation Protocol Sequence
SC Group	1. Apply 2 mL of 3% NaOCl for 1 minute after each instrument change. 2. **SC + CN subgroup**: Use 5 mL of 17% EDTA for 1 minute. **SC + EA, SC + UAI, SC + Laser subgroups**: Use 5 mL of 17% EDTA for 1 minute with three 20-second agitation cycles. 3. Final rinse with 5 mL of distilled water for 1 minute.
CC Group	1. Apply 2 mL of 3% NaOCl combined with 9% Dual Rinse HEDP for 1 minute after each instrument change. 2. **CC + CN subgroup**: Use 5 mL of 3% NaOCl/9% Dual Rinse HEDP for 1 minute. **CC + EA, CC + UAI, CC + Laser subgroups**: Use 5 mL of 3% NaOCl/9% Dual Rinse HEDP for 1 minute with three 20-second agitation cycles. 3. Final rinse with 5 mL of distilled water for 1 minute.

SC = sequential chelation, CC = continuous chelation, CN = conventional needle, EA = Endoactivator, UAI = ultrasonic activated irrigation.

Root sectioning and preparation for SEM and microhardness testing

Each palatal root was initially sectioned using diamond discs (22 × 0.4 mm, Komet Dental, Lemgo, Germany) under 3.5× magnification with dental loupes. Longitudinal notches were carefully created on the buccal and palatal surfaces with continuous water irrigation to guide controlled splitting. To preserve canal integrity and avoid debris contamination during the separation process, a master cone of size X3 was inserted into the canal. The roots were then split using a number 11 surgical blade and a light tapping technique.

For scanning electron microscopy, the bisected samples were dehydrated and coated with a thin layer of gold using a vacuum sputter coater. Observations were performed with an Axia Chemisem SEM (Thermo Scientific Fisher, Waltham, MA, USA, 2021) at an accelerating voltage of 30 kV and a magnification of 2500×. Imaging was conducted in the coronal, middle, and apical thirds of the canals. To ensure consistency in evaluation, horizontal reference lines were drawn at the midpoint of each section, designating a 50 µm region of interest on the canal wall. Two independent, calibrated examiners assessed the images using the Hulsmann scoring system, which categorizes the smear layer according to the percentage of open dentinal tubules. A score of one indicated complete absence of smear layer with fully open tubules, while a score of four reflected extensive coverage of the tubules by the smear layer. Scores two and three represented partial coverage, corresponding to more than 50% and fewer than 50% of tubules remaining open, respectively.

For microhardness evaluation, the remaining halves of the roots were embedded horizontally in autopolymerizing acrylic blocks. The dentin surfaces were ground flat with progressively finer carbide papers (800, 1200, and 2400 grit) and subsequently polished with a 50 µm alumina suspension using a composite polishing kit (Shofu Dental, Kyoto, Japan) on a rotating felt disc. Vickers hardness measurements were performed using a HVS-1000 tester (Jinan, Shandong, China; 220 V, 50 Hz). At least six indentations were made on each sample, distributed across the coronal, middle, and apical thirds, at 50 µm and 100 µm depths from the canal wall surface. The complete experimental workflow, including sample preparation, SEM analysis, and microhardness testing, is summarized in **Figure 1**.

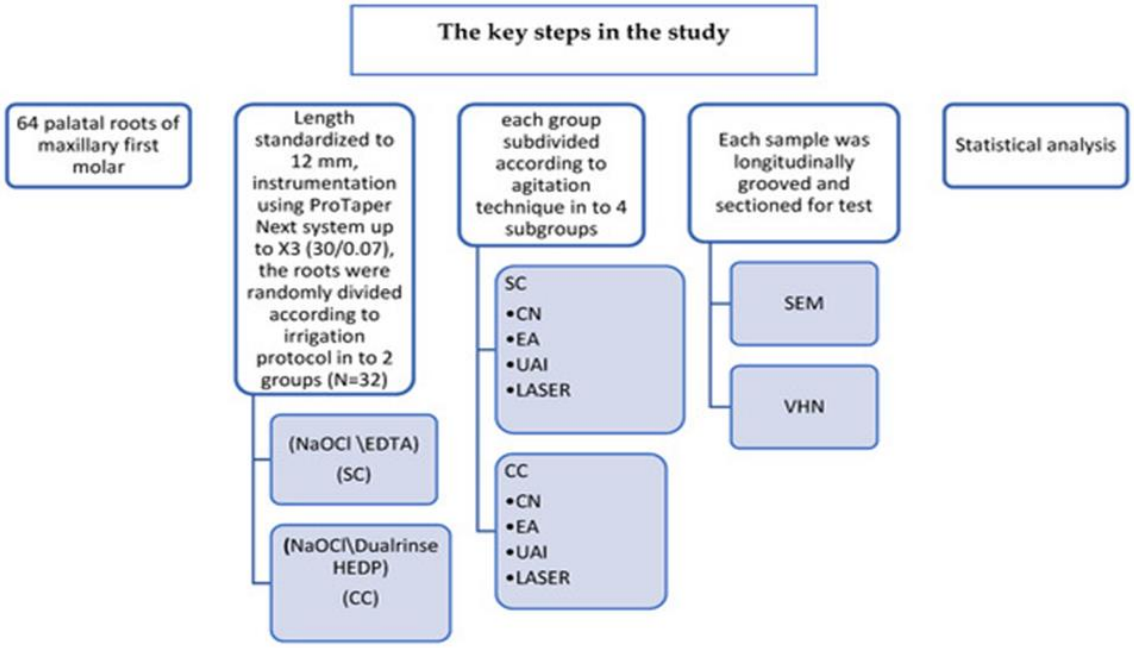


Figure 1. Flow chart summarizing the key steps in the study: SC sequential chelation, CC continuous chelation, CN conventional needle, EA Endoactivator, UAI ultrasonic activated irrigation.

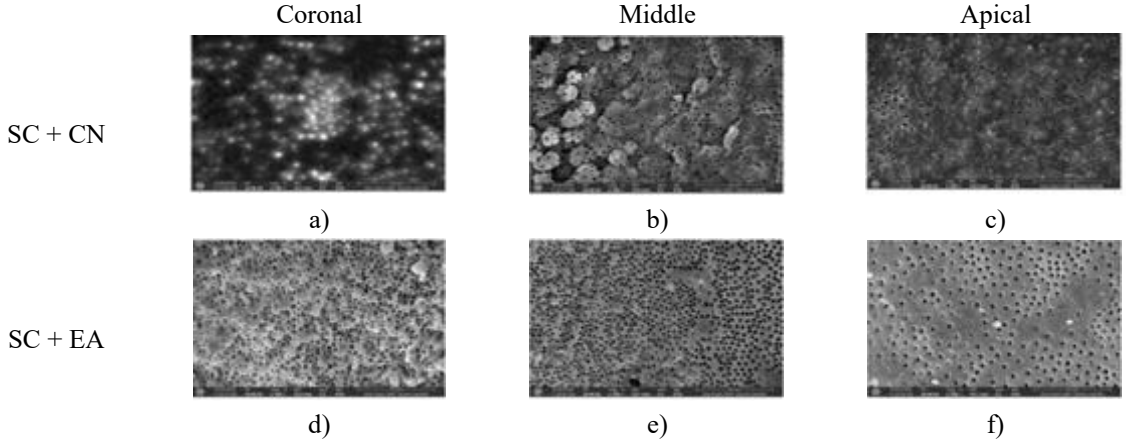
Statistical analysis

All statistical analyses were performed using SPSS software version 26 (SPSS Inc., Chicago, IL, USA). The Shapiro–Wilks test was applied to evaluate the distribution normality of the dataset. Comparisons across groups, canal sections, and measurement depths were conducted using non-parametric tests, specifically the Kruskal–Wallis test, Wilcoxon signed-rank test, and Mann–Whitney U test. A threshold of $p < 0.05$ was considered indicative of statistical significance. Sample size estimation was conducted to ensure 95% study power, with an alpha level of 0.05 and an effect size of 1.16, resulting in a minimum requirement of eight specimens per group [22]. Inter-examiner reliability for the smear layer evaluation was assessed using weighted kappa, yielding a high level of agreement ($\kappa = 0.89$).

Results and Discussion

Smear layer evaluation

The consistency between the two evaluators was substantial, with a weighted kappa of 0.89. **Table 2** summarizes the smear layer scores, including both median values and mean ranks. Representative SEM images illustrating the coronal, middle, and apical thirds of the canals for the sequential chelation (SC) groups are presented in **Figure 2**, while those for the continuous chelation (CC) groups are shown in **Figure 3**. Kruskal–Wallis analysis demonstrated significant differences among the tested groups ($p < 0.05$).



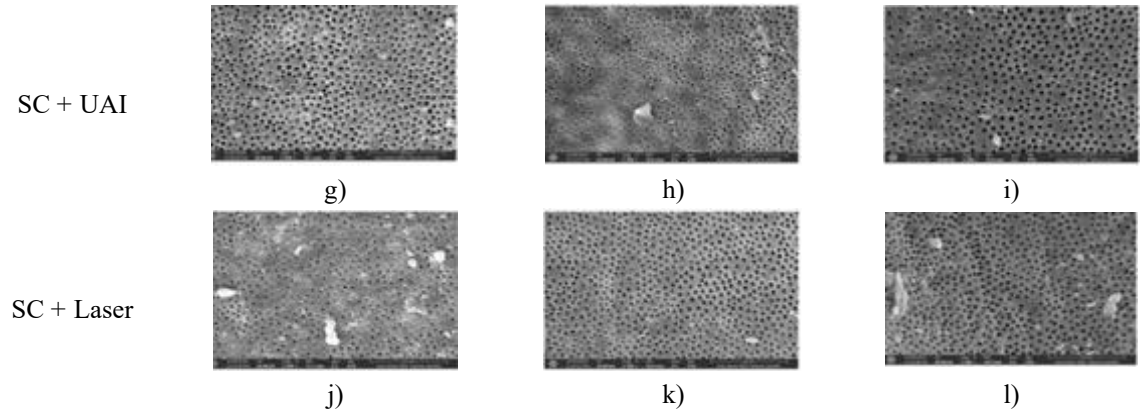


Figure 2. Representative SEM images (magnification 2500×) showing root canal lumen at coronal, middle, and apical sections using SC (sequential chelation) + CN (conventional needle irrigation, EA (EndoActivator), UAI (ultrasonic activated irrigation), and Er:Cr:YSGG laser.

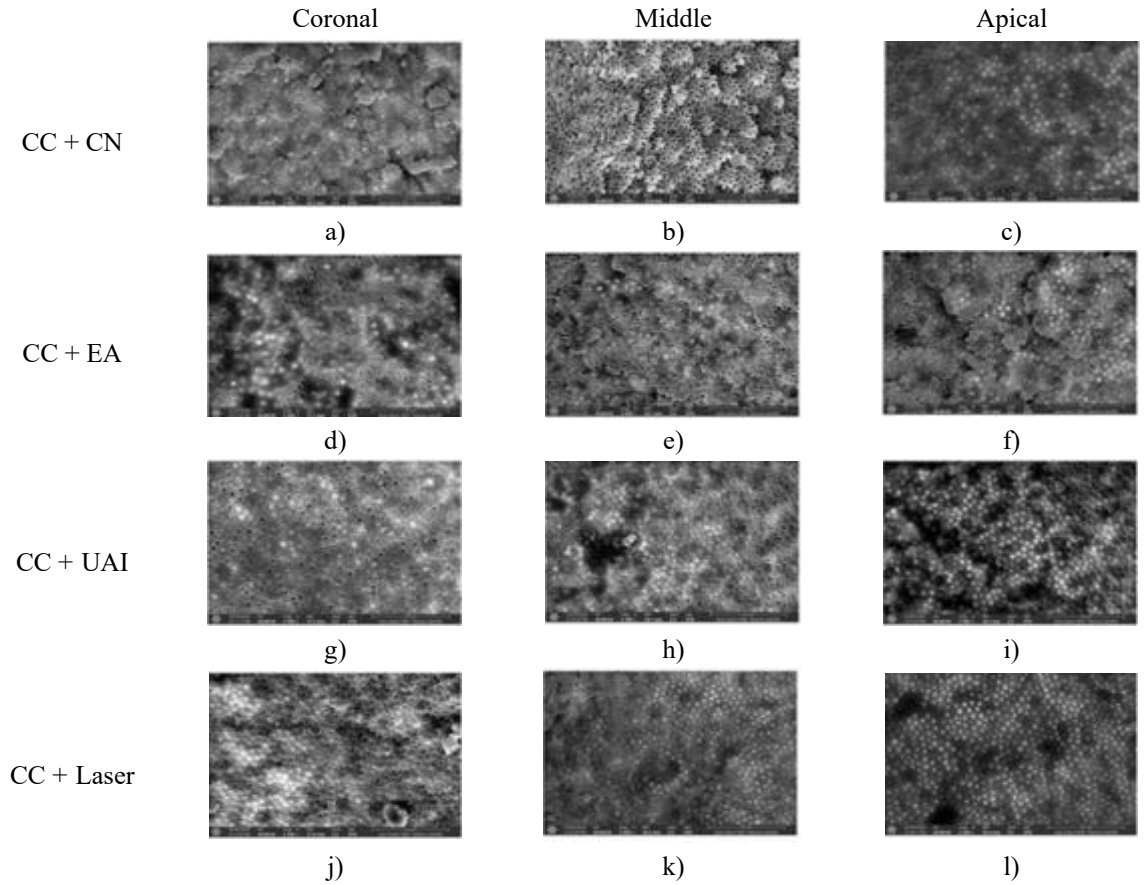


Figure 3. Representative SEM images (magnification 2500×) showing root canal lumen at coronal, middle, and apical sections using CC (continuous chelation) + CN (Conventional needle irrigation, EA (EndoActivator), UAI (Ultrasonic activated irrigation), and Er:Cr:YSGG laser.

Table 2. Median and mean rank of smear layer scoring in the coronal, middle, and apical thirds of the root canal for all groups.

Group	Coronal	Middle	Apical
	Median	Mean Rank	Median
SC + CN	2.00	23.00 a,*	2.00
SC + EA	1.50	17.00	1.50
SC + UAI	1.50	17.00	2.00

SC + Laser	1.00	9.00 a,*	1.50
CC + CN	2.00	17.63	2.00
CC + EA	2.00	16.13	2.00
CC + UAI	2.00	18.06	2.00
CC + Laser	2.00	14.19	2.00

* Kruskal–Wallis test. Identical superscript lowercase letters indicate significant differences among relevant groups in each chelator (Mann–Whitney U test for pairwise comparison). Identical superscript lowercase letters indicate significant differences among relevant groups in between chelators (Mann–Whitney U test for pairwise comparison ($p < 0.05$)). SC sequential chelation, CC continuous chelation, CN conventional needle, EA Endoactivator, UAI ultrasonic activated irrigation.

Higher smear layer scores reflected a greater accumulation of smear material and a lower proportion of open dentinal tubules. In the ultrasonic (UAI) groups, continuous chelation (CC) showed a significantly greater smear layer presence than sequential chelation (SC) in the apical third ($p < 0.05$). Similarly, in the laser-activated groups, CC samples exhibited higher median scores compared to SC in the coronal third ($p < 0.05$). Within SC-treated samples, the conventional needle (CN) subgroup had more pronounced smear layers than the laser subgroup in the coronal region ($p < 0.05$). For the CC groups, CN samples also demonstrated higher smear layer accumulation than those treated with EndoActivator (EA) or laser in the apical third. Furthermore, in the apical third, the UAI subgroup contained more residual smear layer than the laser-activated samples ($p < 0.05$) **(Figure 4)**.

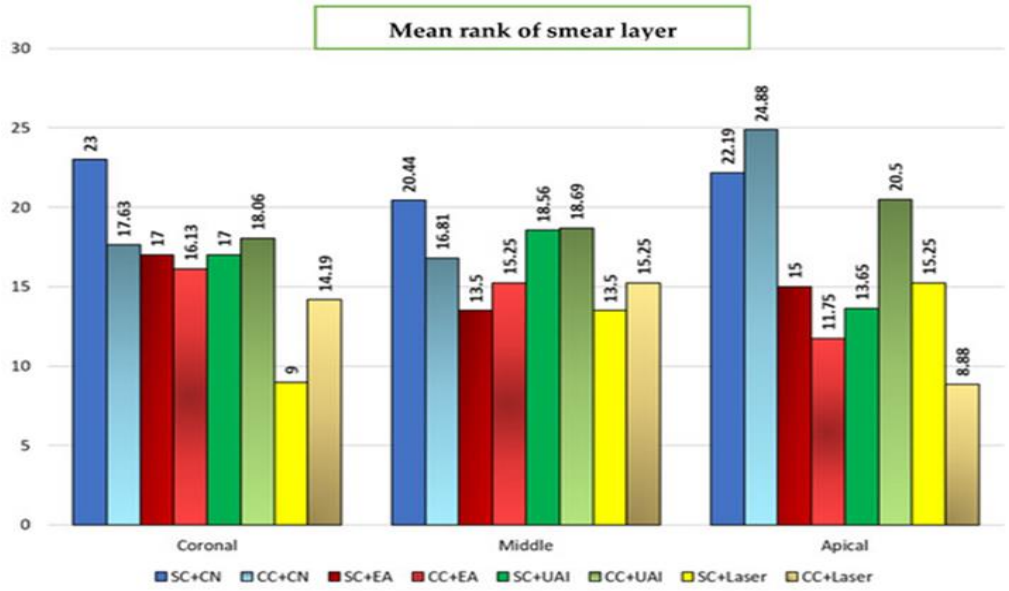


Figure 4. Bar chart illustrating the mean rank of smear layer after SC (sequential chelation) and CC (continuous chelation) in coronal, middle, and apical sections using CN (Conventional needle irrigation), EA (EndoActivator), UAI (Ultrasonic activated irrigation), and Er:Cr:YSGG laser.

Microhardness

Table 3 summarizes the Vickers microhardness (VHN) measurements (mean ± SD) obtained after the various irrigation protocols. Within the sequential chelation (SC) groups, significant differences in VHN were observed across the coronal, middle, and apical thirds at both 50 µm and 100 µm depths ($p < 0.05$). When comparing microhardness between the 50 µm and 100 µm depths within each subgroup, no significant differences were found for most groups ($p > 0.05$), except in the middle third of the laser-treated samples, where the VHN at 50 µm depth was significantly higher than at 100 µm ($p < 0.05$), suggesting increased dentin hardness closer to the canal surface.

Table 3. Average Vickers microhardness values (mean ± standard deviation) of dentin after different irrigating protocols.

Section and Depth	Group	Mean (VHN)	SD	Group	Mean (VHN)	SD
	Sequential Chelation			Continuous Chelation		

Coronal_50	SC + CN	58.84 ^{a,*}	18.69	CC + CN	79.45	6.87
	SC + EA	34.34 ^{a,b,*}	6.49	CC + EA	59.96 ^{a,*}	18.85
	SC + UAI	43.72	8.99	CC + UAI	71.06 ^{b,*}	25.56
	SC + Laser	59.08 ^{b,*}	7.61	CC + Laser	87.48 ^{a,b,*}	14.81
Middle_50	SC + CN	59.45 ^{a,*}	14.93	CC + CN	75.57	6.84
	SC + EA	33.04 ^{a,b,*}	9.87	CC + EA	61.15	22.87
	SC + UAI	43.39	6.71	CC + UAI	69.77	20.67
	SC + Laser	56.18 ^{b,*}	6.26	CC + Laser	85.40	8.62
Apical_50	SC + CN	60.29 ^{a,*}	16.79	CC + CN	78.66	0.51
	SC + EA	36.42 ^{a,b,*}	6.46	CC + EA	64.16 ^{a,*}	13.10
	SC + UAI	44.07	1.94	CC + UAI	70.56 ^{b,*}	23.00
	SC + Laser	58.76 ^{b,*}	10.36	CC + Laser	90.06 ^{a,b,*}	20.64
Coronal_100	SC + CN	51.66 ^{a,*}	14.81	CC + CN	75.44	3.08
	SC + EA	34.59 ^{a,b,*}	8.95	CC + EA	51.46 ^{a,*}	22.49
	SC + UAI	42.24	9.74	CC + UAI	58.97 ^{b,*}	24.97
	SC + Laser	53.34 ^{b,*}	7.23	CC + Laser	82.17 ^{a,b,*}	12.24
Middle_100	SC + CN	53.91 ^{a,*}	13.09	CC + CN	78.25	5.84
	SC + EA	35.64 ^{a,b,*}	4.76	CC + EA	54.52	25.22
	SC + UAI	40.08	11.24	CC + UAI	66.30	19.32
	SC + Laser	50.62 ^{b,*}	5.18	CC + Laser	79.70	10.34
Apical_100	SC + CN	50.52 ^{a,*}	11.10	CC + CN	76.62 ^{a,*}	1.41
	SC + EA	34.24 ^{a,b,*}	10.99	CC + EA	56.58 ^{a,b,*}	15.60
	SC + UAI	42.61	7.52	CC + UAI	66.11	21.94
	SC + Laser	52.84 ^{b,*}	5.05	CC + Laser	81.14 ^{b,*}	22.42

* Kruskal–Wallis test. Identical superscript lowercase letters represent significant differences among the relevant groups in each section and depth (Mann–Whitney U test ($p < 0.05$). SC sequential chelation, CC continuous chelation, CN conventional needle, EA Endoactivator, UAI ultrasonic activated irrigation.

In the CC groups, analysis of dentin microhardness revealed notable differences among the subgroups within the coronal and apical regions at both 50 µm and 100 µm depths ($p < 0.05$). No significant variation was observed in the middle third across any subgroup at either depth ($p > 0.05$). When comparing measurements at 50 µm and 100 µm within individual subgroups, the only significant increase in hardness was found in the apical portion of the CN and laser subgroups, with higher values at 50 µm, suggesting that dentin closer to the canal surface exhibits greater resistance.

When CC and SC were compared, the CN subgroup consistently displayed higher microhardness values with CC throughout most regions and depths, except at the apical 50 µm depth, where differences were not significant. For EA and UAI agitation, CC generally maintained superior dentin hardness compared to SC, except at the coronal 100 µm level, where the difference was negligible. In the laser-activated subgroups, CC consistently resulted in increased microhardness relative to SC across all examined regions and depths **(Figure 5)**.

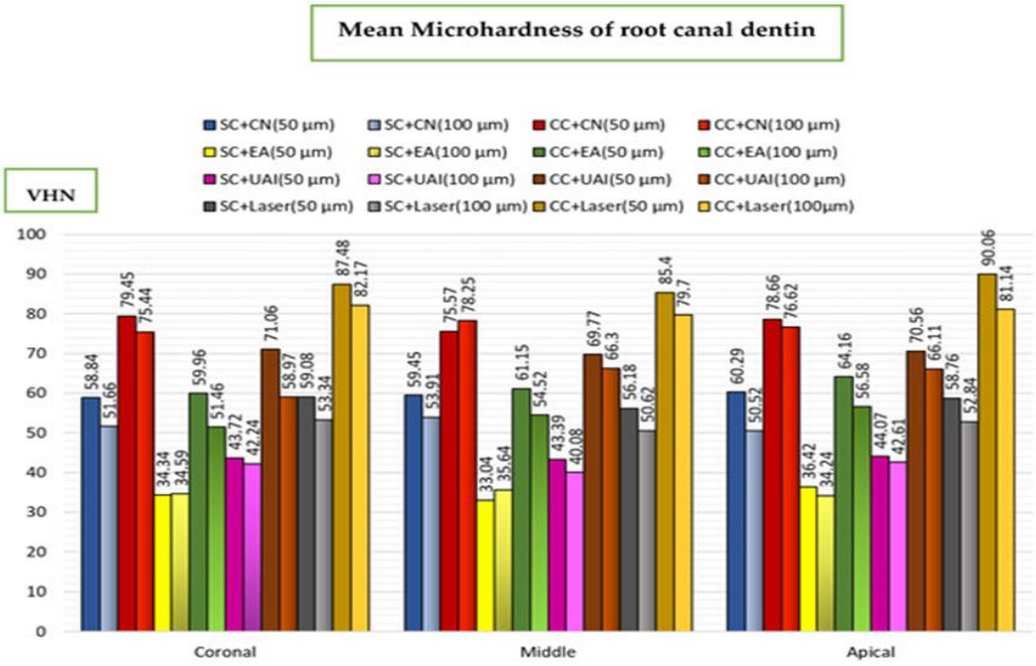


Figure 5. Bar chart illustrating the mean microhardness of root canal dentin after sequential SC and continuous chelation CC in coronal, middle, and apical sections at 50 and 100 µm from the canal surface using: CN (Conventional needle irrigation), EA(EndoActivator), UAI (Ultrasonic activated irrigation), Laser (Er:Cr:YSGG laser).

During root canal preparation, the formation of the smear layer on canal walls can hinder effective cleaning and compromise the three-dimensional seal of the obturation [23]. In this study, conventional needle irrigation showed no significant differences in smear layer removal between sequential chelation (SC) and continuous chelation (CC), supporting the null hypothesis and aligning with previous studies that observed similar outcomes without agitation [6]. However, some reports suggest that HEDP may be more efficient than EDTA in limiting smear layer formation and reducing hard tissue debris during instrumentation [24]. Conversely, under neutral pH conditions, EDTA has been found to outperform HEDP due to differences in chemical stability and ion chelation capacity [25].

Irrigant agitation plays a crucial role in enhancing canal cleanliness. In this investigation, significant variations were observed among the different agitation techniques **(Table 2)**. Both SC and CC with conventional needle irrigation exhibited extensive smear layer remnants throughout the canal length, consistent with earlier studies using other irrigants [22]. The limited efficacy of conventional needles is primarily due to restricted irrigant penetration and insufficient hydrodynamic forces to dislodge debris or biofilm from the canal walls [26].

Sonic agitation (EndoActivator) produced comparable results to passive ultrasonic irrigation (UAI), in line with previous research demonstrating similar smear layer removal using ultrasonic tips and polymer sonic tips in canals prepared to comparable apical diameters [27]. The reduced efficiency in the apical third may be explained by limited space for tip oscillation and irrigant movement, compounded by fewer and narrower dentinal tubules in this region [28–30]. Both sonic and ultrasonic activation were considerably more effective than conventional needle irrigation, consistent with prior findings showing superior debris and smear layer removal using mechanical agitation devices [31].

Laser-assisted irrigation using the Er,Cr:YSGG system demonstrated the most consistent and thorough smear layer removal across all canal thirds. The CC group activated with laser irrigation achieved the lowest smear layer scores, particularly in the apical third, likely due to cavitation and pressure wave effects that enhance irrigant penetration into dentinal tubules [32–34]. The high-energy photons generated by the laser disrupt the airlock effect in the apical region, allowing the solution to flow more efficiently and remove debris [35]. In addition, Er,Cr:YSGG lasers can thermally modify the inorganic dentin components, facilitating additional smear layer removal [36].

Regarding dentin microhardness, significant differences were observed among the groups at all canal levels and depths ($p < 0.05$), which contradicts the null hypothesis. Overall, CC irrigation with HEDP

preserved higher microhardness values compared to SC with EDTA, regardless of the agitation method. This finding is consistent with previous studies indicating that EDTA tends to extract more calcium from dentin, reducing its hardness, whereas HEDP is less aggressive and better maintains dentin's structural integrity [9, 37]. The preservation of microhardness with CC may thus have clinical advantages, helping maintain root canal wall strength during endodontic procedures.

In this study, dentin treated with EDTA exhibited a more pronounced reduction in surface microhardness compared to HEDP. This decline is primarily due to the substantial loss of mineral content and hydroxyapatite within the intertubular dentin, which weakens the structural integrity of human dentin [8]. In contrast, HEDP primarily exposes surface collagen fibers, which are subsequently degraded by sodium hypochlorite, resulting in a less pronounced reduction in hardness [38].

When considering the effect of agitation methods on chelator-induced demineralization, dentin subjected to conventional needle (CN) and laser activation retained higher microhardness values relative to sonic (EA) and ultrasonic (UAI) agitation, with the laser-treated specimens exhibiting the highest hardness. Previous research using Er:YAG lasers (2 W, 15 Hz) has also reported reductions in dentin microhardness when laser agitation is applied, consistent with our findings [39]. Other studies have shown that PIPS (photon-initiated photoacoustic streaming) did not further compromise dentin microhardness compared to static irrigation [40], suggesting that changes in microhardness are primarily governed by the chemical properties of the irrigants rather than the agitation technique itself. The extent of these effects depends on factors such as laser energy composition and the absorption characteristics of dentin [41]. In line with our observations, sonic and ultrasonic activation decreased microhardness, likely due to enhanced penetration of irrigants into dentinal tubules [42].

This study has inherent limitations due to its in vitro design, which restricts direct extrapolation to clinical practice. Experiments were conducted at room temperature rather than physiological conditions, which may influence the behavior of irrigants. Additionally, SEM imaging evaluates only localized areas of the canal walls and provides a two-dimensional view, limiting the assessment of smear layer thickness and distribution. A more comprehensive evaluation could be achieved using longitudinal imaging techniques such as micro-CT. Other potential sources of variability include differences in dentin properties among specimens, variations in irrigation delivery, and procedural inconsistencies during microhardness testing, which could influence results. Future in vivo studies are warranted to validate the clinical relevance of these irrigation strategies on smear layer removal and dentin ultrastructure.

Conclusion

The findings suggest that continuous chelation (CC) combined with laser activation is the most efficient method for smear layer removal. While CC and sequential chelation (SC) demonstrated similar efficacy in eliminating the smear layer from root canal walls, complete eradication was not achieved by any solution or agitation method. Importantly, CC had a substantially lower impact on dentin microhardness, particularly when paired with CN or laser activation, compared to SC. The preservation of higher dentin microhardness with CC supports its potential advantage in clinical endodontic procedures and may contribute to improved treatment outcomes.

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Ethics Statement: None

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