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Effect of Laser-Assisted Continuous Chelation Irrigation Protocols on Sealer Penetration and Bond Strength in Root Dentin: An Evaluation Using a Calcium-Specific Fluorophore and Push-Out Test

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Abstract

Background: Laser-assisted irrigation may enhance chemomechanical debridement and improve the sealing of root canals during endodontic treatment.

Objective: This study aimed to address the scientific deficit in understanding the laser-assisted sequential versus continuous chelation in root canal irrigation.

Methods: One hundred and twenty single-rooted teeth were prepared using Reciproc R40 instruments and randomly divided into six groups ($n = 20$) based on the irrigation protocol. In the sequential chelation groups, canals were irrigated with NaOCl during instrumentation, followed by 17% ethylenediaminetetraacetic acid using a needle, ultrasound, or erbium-doped yttrium aluminum garnet laser activation. In the continuous chelation groups, canals were irrigated with a mixture of 2% sodium hypochlorite and 9% hydroxyethylidene diphosphonic acid during and after instrumentation, followed by final activation using the same three techniques. Ten samples per group were used to evaluate sealer penetration with Fluo-3 dye, while the remaining samples underwent push-out bond strength testing. Statistical analyses were conducted using the Kruskal–Wallis and Friedman tests ($p < 0.05$).

Results: No significant differences in sealer penetration were found between sequential and continuous protocols in the apical third when needle or ultrasonic activation was used. However, laser activation combined with continuous chelation resulted in significantly higher sealer penetration and bond strength ($p < 0.001$). Sealer penetration followed the trend: coronal > middle > apical ($p < 0.05$) with all irrigation techniques.

Conclusions: Continuous chelation protocols, especially when combined with laser activation, were as effective or superior to conventional methods in improving sealer penetration and bond strength. Continuous chelation with laser-assisted irrigation may improve clinical efficiency and shorten treatment time in root canal procedures.

Keywords: lasers, Er-YAG, dentin, endodontics, laser confocal microscopy, fluorescence/fluorochromes

Introduction

Over the past decades, endodontic treatment philosophy has evolved, reflecting a shift from the traditional concept of "shape to obturate" to the more contemporary approach of "shape to irrigate."[1] It is well-known that the success of root canal treatment is largely dependent on effective chemomechanical preparation, which includes the removal of organic tissue remnants and smear layer.[2] Consequently, there has been significant growth in research aimed at developing devices that enhance the effectiveness of endodontic irrigants.[3]

Sodium hypochlorite (NaOCl) is the most commonly used irrigant in endodontic treatment, valued for its potent antimicrobial properties and its ability to dissolve organic tissue effectively.[4] However, as NaOCl is ineffective in removing inorganic components and the smear layer, its use is typically supplemented with a chelating agent, such as ethylenediaminetetraacetic

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acid (EDTA).[5] The use of ultrasonic activation has been proposed to enhance the effectiveness of irrigants by improving their dispersion and cleaning efficacy, particularly in the apical region and complex canal anatomy.[6] Laser-assisted irrigation has been proved to enhance smear layer removal and cleaning ability.[7,8] However, combining NaOCl with EDTA during instrumentation presents challenges, including compromised biomechanical properties of dentin[9–11] and reduced antimicrobial activity of NaOCl due to chlorine depletion.[5]

To address these limitations, an alternative approach, known as continuous chelation, has gained increasing attention in endodontic research in recent years.[12] This method employs a soft chelator, such as hydroxyethylidene diphosphonic acid (HEDP), which is compatible with NaOCl.[13] A combined NaOCl and HEDP solution allows simultaneous irrigation and chelation, reducing chair time and potentially improving clinical efficiency.[14] Dual Rinse HEDP, a new product by Medcem (Weinfelden, Switzerland), is provided as a capsule with 0.9*g* of HEDP to be mixed with 10 mL of a chosen NaOCl.[15]

Variations in dentin conditioning can influence the clinical performance of root canal sealers.[16] A final root canal irrigant not only affects the smear layer of the dentin surface but also the surface wettability.[17] There is a general lack of information available concerning the capacity of tubule penetration of hydraulic calcium silicate sealers when used together with continuous chelation strategy and a single cone filling technique. Potential differences of ultrasound and laser activation with continuous chelation have not been studied. Therefore, it is essential to investigate the penetration and the push-out bond strength of such materials following ultrasonic and laser activation of different irrigants to better understand their effectiveness in real-world scenarios.

This study aimed to assess the effects of using ultrasonic and laser activation on sequential versus continuous chelation protocols during root canal irrigation using fluorescent dye Fluo-3 as the investigative tool. By elucidating the comparative efficacy and impact of these protocols, the findings may provide evidence-based recommendations for optimizing irrigation strategies in clinical practice.

The null hypothesis tested in this study was: there is no difference between tested irrigation protocols in terms of sealer penetration into dentin tubules and push-out bond strength of the calcium silicate sealer.

Materials and Methods

Power analysis

A pilot study and power analysis were conducted to ensure sufficient statistical power for performing a test of the null hypothesis. The sample size for the experiments was calculated based on an effect size of 2, an alpha error probability of 0.05, and 80% power. The predicted sample size (n) was 6 samples per group. To account for any sample loss, a sample size of $n = 10$ for each group was selected.

Sample preparation

For this study, extracted human incisors ($n = 120$) were used from the Department of Oral Surgery, Dental School in Zagreb. This study was approved by the Research Ethics Committee of Dental School, University of Zagreb (approval number for the study: 05-PA-30-X-9/2022). Teeth were excluded if they had caries, incompletely formed apices, previous root canal treatment or more than one canal. The samples were cleaned of calculus and debris, stored in 1% chloramine and cut with a circular saw (Isomet; Buehler, Esslingen, Germany) under water cooling to standardize root length at 16 mm. The root length was determined using a digital caliper (Holex, Munich, Germany). Patency was verified by inserting a 10 K file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) into the canal space until the tip was visible at the apical foramen.

Root canal instrumentation and irrigation

The roots were randomly allocated into two equal groups: sequential and continuous chelating group (I and II); and randomly subdivided into needle irrigation (A), ultrasonic activation groups (B), and laser-assisted irrigation (C) ($n = 20$):

Group I A: NaOCl + EDTA needle irrigation.
Group I B: NaOCl + EDTA additional ultrasonic activation.
Group I C: NaOCl + EDTA additional laser activation.
Group II A: NaOCl/Dual Rinse HEDP needle irrigation.
Group II B: NaOCl/Dual Rinse HEDP additional ultrasonic activation.
Group II C: NaOCl/Dual Rinse HEDP additional laser activation.

Reciprocating single files R40 (25 mm length) supplied by VDW (München, Germany) with a speed of 300 rpm, 30° clockwise, and 150° counterclockwise rotation angles, using an automatic motor (VDW) were used for the mechanical instrumentation. The root canals of the sequential chelation groups were irrigated with 5 mL of 2% NaOCl solution during instrumentation using a plastic syringe with needle. Subsequently, 17% EDTA was administered (2.5 mL), either with a needle, with additional ultrasonic activation (Irrisafe Ultrasonic Tip 20/25 mm, 30 s, 35% power) or with additional activation with erbium-doped yttrium aluminum garnet (Er: YAG) laser (Skypulse, Fotona, Ljubljana, Slovenia) equipped with a H14 handpiece and conical fiber tip (SWEEPS 400/14, Fotona). The laser operated in SSP mode with a pulse duration of 30 μs, a repetition rate of 15 Hz, and a pulse energy of 20 mJ. Total time of laser activation was 30 s. All the irrigating solutions were introduced into the canal using a 30-gauge side-vented stainless-steel needle (Endo-top, Cerkamed, Stalowa Wola, Poland).

In the continuous chelation groups, root canals were irrigated using a fresh mixture of Dual Rinse HEDP during and after instrumentation. One capsule (0.9*g*) of etidronate powder (Dual Rinse HEDP, Medcem, Weinfelden, Switzerland) was mixed with 10 mL of freshly prepared 2% NaOCl per application. Again, in one of these groups, the final irrigant (2.5 mL) was applied just with the irrigating tip, in the other group it was ultrasonically activated, and in the third group the laser was used for activation. All root canals were instrumented, irrigated and filled by the same person to reduce inter-operator variability. Ten samples from each group were tested for sealer penetration, and ten samples were used for push-out bond strength analysis.

Root canal obturation for sealer penetration assay

Obturation in all groups was performed with CeraSeal sealer mixed with fluorescent calcium indicator (Fluo-3; Thermo

Fisher Scientific, USA) and gutta-percha R40 (Reciproc) using a matched cone technique. For standardization, 1*g* of endodontic sealer was weighed on an analytic scale, and 0.002*g* of the Fluo-3 indicator was added and mixed manually. Teeth were temporarily closed with Cavit (3 M ESPE; St Paul, MN, USA) and placed within an ES 120 incubator (Nüve, Ankara, Turkey) set at a constant temperature of 37°C for 5 days to allow sealer to set.

Confocal microscopy

Samples were sectioned at 3, 6, and 9 mm from the root apex using a circular saw (Isomet) under water cooling. The disks (200-µm thick) were prepared and polished with Sof-Lex (3 M ESPE). Using confocal scanning laser microscope (TCS SP8 X FLIM, Leica, Wetzlar, Germany) at the absorption and emission wavelengths of Fluo-3 indicator (526 nm), samples were observed. For negative controls, teeth were filled only with sealer and gutta-percha cone, without using Fluo-3 florescent dye. After negative control was assessed, the samples were observed with 10x objective lens. Numeric values were obtained using ImageJ (National Institutes of Health, Bethesda, MD, USA). The maximum depth of sealer penetration (µm) was measured from the canal wall to the outer part of the recorded fluorescent signal. All measurements were evaluated by two examiners. The results of each examiner were recorded independently, and in case of diverging values, the mean value was used.

Push-out bond strength test

The obturation process in all groups was carried out using the CeraSeal sealer and the single cone technique, without the inclusion of a fluorescent calcium indicator. The samples were kept in an incubator at 37°C with 100% humidity for 1 week to allow the sealer to fully set. Tooth slices 2 mm thick were cut at intervals of 3, 6, and 9 mm coronal from the apex by using a water-cooled diamond saw (Isomet). The push-out test was performed using a universal testing machine (STEP Lab, Resana, Italy) with a crosshead speed of 1 mm/min. The samples were positioned on the testing machine with the apical part of the root canal facing upward. Plunger with tip diameters of 0.4, 0.6, and 0.8 mm were used for the apical, middle, and coronal sections. The plunger's diameter was selected to be smaller than that of the specimen, preventing any contact with the dentin walls during the test. A plunger was centrally positioned within the root canal space of each sample. The push-out strength was calculated in megapascals (MPa) by dividing the load in newtons (N) by the area of the bond interface:

$$\text{Bond area} = \pi\,(\mathrm{R} + \mathrm{r})\,\mathrm{h}$$

where $\pi = 3.14$, R = the radius of the sealer close to the crown, r = the radius of the sealer close to the apex, h = the height of the slice in mm.

Statistical analysis

Data were analyzed and graphically presented using GraphPad Prism software version 10.6.1 (GraphPad, La Jolla, CA, USA). The significance level was set at $p < 0.05$. Results are expressed as mean values ± standard deviation. Kruskal–Wallis test followed by multiple comparisons test were used to compare results among the groups on the apical, middle, and coronal levels of the canal. Friedman and multiple comparisons tests were used to compare results within the apical, middle, and coronal levels for each treatment group.

Results

Sealer penetration

No autofluorescence was observed in the negative control samples that were filled without the addition of fluorescent dye (Fig. 1). In contrast, samples in which Fluo-3 was incorporated into the sealer and examined under fluorescent mode displayed green signal, indicating the presence of the labeled sealer (Fig. 2). All irrigation protocols facilitated sealer penetration into the dentinal tubules at the coronal, middle, and apical thirds of the root.

The maximum depth of sealer penetration among all six treatment groups across all root canal thirds is shown in Figure 3. At the apical level, the highest penetration was observed in the Dual Rinse HEDP group with laser activation (89.1 ± 14 µm), followed by the sequential NaOCl + EDTA group with laser activation (79.2 ± 12.8 µm). The lowest values were recorded in the Dual Rinse HEDP group with needle irrigation (34.1 ± 8.7 µm) and the sequential NaOCl + EDTA group with needle irrigation (38.3 ± 12 µm). When comparing overall results for the apical area, no statistically significant difference ($p > 0.05$) was found between the sequential and continuous irrigation protocols when the final irrigation was performed using either needle irrigation or ultrasonic activation. However, significantly greater sealer penetration was observed in the laser-activated groups compared with those using needle irrigation ($p < 0.001$) or ultrasonic activation ($p < 0.001$).

Comparing the overall results for middle area, no statistically significant difference ($p > 0.5$) existed between sequential and continuous irrigation protocols regarding the maximum sealer penetration depth if the final irrigation was performed only with needle irrigation or laser activation. However, penetrability results were higher in the groups with laser activation compared with needle irrigation ($p < 0.001$) and the ultrasound activation ($p < 0.001$). At the middle root level, significantly higher sealer penetration was found in samples irrigated with Dual Rinse HEDP combined with ultrasonic activation ($p <$ 0.001) in comparison with sequential irrigation with ultrasonic activation.

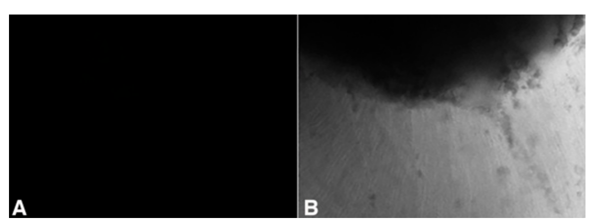

FIG. 1. Confocal image **(A)** and bright-field image **(B)** of the sample representing negative control, fluorescence was not detected. A root canal was filled only with the sealer and gutta-percha, without fluorescent dye. The investigated areas were in the dentin near the pulp space. The scale bar represents 50 µm.

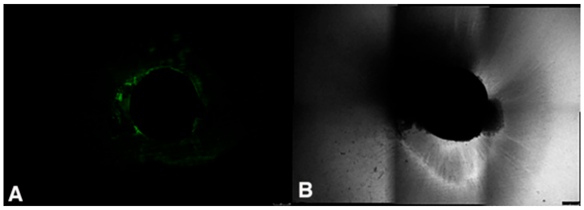

FIG. 2. Confocal image **(A)** and bright-field image **(B)** of the representative sample, green dots represent the sealer combined with Fluo-3. The scale bar represents 250 µm. The maximum depth of sealer penetration was measured from the canal wall to the point of maximum sealer penetration in the outer dentin.

Intragroup comparisons between the apical, middle, and coronal levels revealed significant differences in sealer penetration across all treatment groups ($p < 0.001$; Fig. 4). The coronal root sections exhibited the greatest maximum penetration depth, followed by the middle sections.

Push out bond strength

At the apical level, the highest bond strength was observed when continuous chelation with Dual Rinse HEDP was combined with laser activation, showing a statistically significant difference compared with the other groups ($p < 0.05$; Fig. 5). A similar trend was noted with ultrasonic activation, which significantly improved bond strength at both the apical and middle sections ($p < 0.05$). In contrast, no significant difference was found between sequential and continuous irrigation protocols at the coronal level ($p > 0.05$). The lowest bond strength across all root canal thirds was recorded with conventional needle irrigation alone.

Discussion

The results of this study suggest that continuous chelation protocols were as effective as, or more effective than, traditional sequential protocols in promoting sealer penetration and bond strength of calcium silicate-based cement. Therefore, null hypothesis was partially rejected.

This may be explained by the fact that both EDTA and HEDP are chelating agents that aid in smear layer removal, which allows sealer penetration into dentinal tubules.[18,19] Although HEDP produces shallower demineralization compared with EDTA,[20,21] this study indicates that the continuous chelation method does not reduce sealer penetration or sealer bond strength, particularly within the parameters of the tested irrigation and filling protocols. This can potentially be attributed to the fact that continuous chelation approach facilitates smear layer removal from the beginning to the final moment of irrigation. On the other hand, during sequential irrigation, smear layer removal occurs only at the final irrigation step.

Sealer penetration into dentinal tubules is a critical factor for achieving mechanical interlocking with root dentin, reducing microleakage, and preventing bacterial reinfection.[22,23] During instrumentation, endodontic files generate not only organic material but also a smear layer.[24] Therefore, eliminating the smear layer prior to root canal obturation is crucial, as it can retain bacteria that contribute to ongoing root canal infections.[25] Several factors can affect the depth of sealer penetration, such as dentin structure, the chelating ability of a solution, the effectiveness of smear layer removal, and the sealer's physical and chemical properties.[26] The preparation and conditioning of the canal walls are critical factors influencing the adhesion and overall performance of root canal sealers.[27] In this study, we focused on evaluating the maximum sealer penetration rather than the total area of sealer penetration. The latter metric is influenced by the dimensions of the tested samples, which could introduce variability and confounding factors. By emphasizing maximum sealer penetration, our approach aims to mitigate selection bias and reduce the impact of sample size heterogeneity, thereby providing a more standardized and reliable parameter for analysis.

Both for sequential and continuous irrigation, the results of the sealer penetration and bond strength exhibited significant differences between the values measured in the samples where

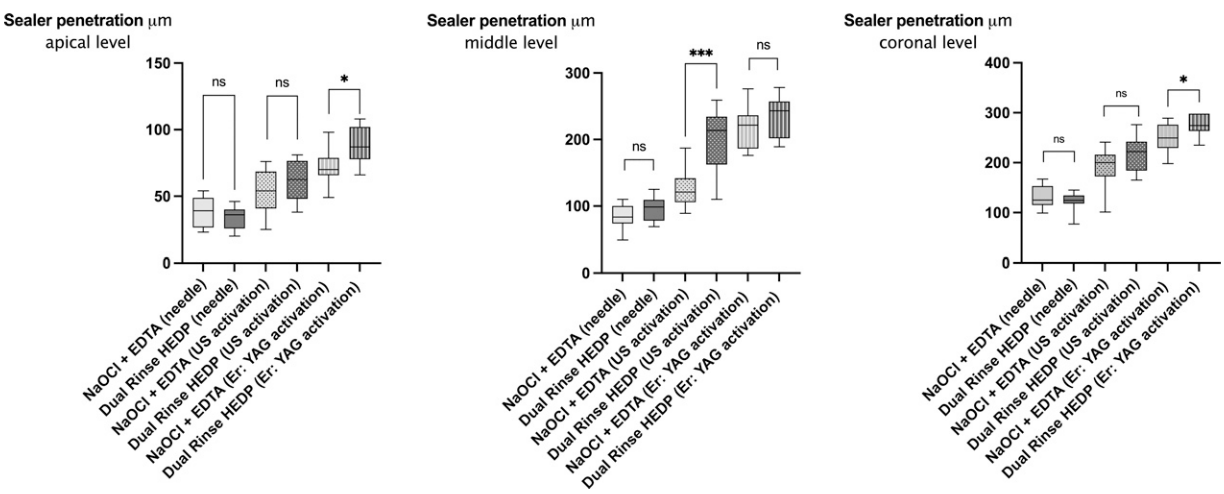

FIG. 3. Sealer penetration was observed at apical, middle, and coronal levels of each root. Bar chart presenting mean and standard deviation values for sealer penetration (µm) in different groups. US stands for ultrasonic activation. There was a significant increase (*$p < 0.05$) in sealer penetration at apical and coronal levels when continuous chelation was applied with laser activation, compared with sequential irrigation. The similar effect was observed at the middle level when ultrasound activation was applied (***$p < 0.001$).

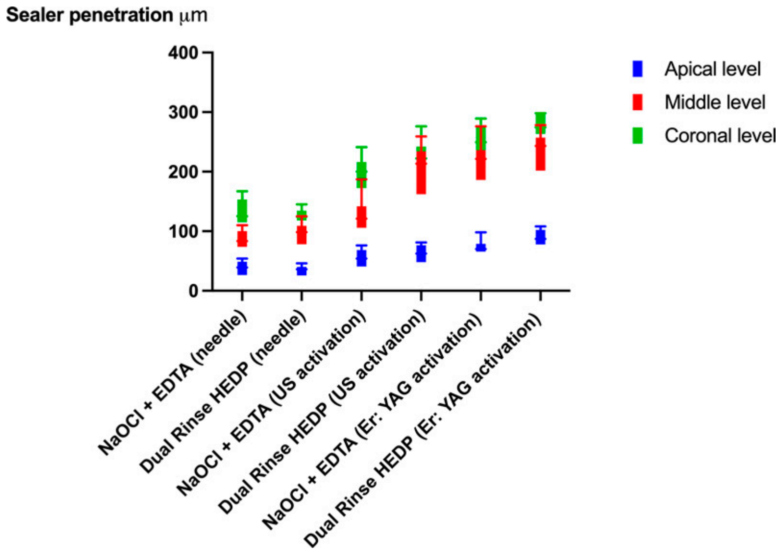

FIG. 4. The mean and standard deviation values of maximum sealer penetration were analyzed across different root levels. "US" denotes ultrasonic activation. At the apical level, sealer penetration was significantly lower ($p < 0.05$) compared with the middle and coronal levels across all irrigation techniques. No significant differences were observed between the middle and coronal levels in any group, except for the group treated with sequential irrigation (NaOCl + EDTA) enhanced by ultrasonic activation.

final irrigation was administrated only with a needle and the samples with ultrasonic and laser activation. These results are consistent with previous studies,[28,29] which have also highlighted the efficacy of ultrasonic activation in enhancing the penetration of sealers, particularly in the challenging apical region. The combination of continuous chelation using Dual Rinse HEDP and laser activation appeared to produce the most effective results within the present study. This suggests a synergistic effect between the chemical action of continuous chelation and the physical enhancement provided by laser activation. A laser is used to deliver energy into the root canal filled with an irrigant.[30] Er:YAG laser used in the current study induced cavitation via the formation and subsequent collapse of vapor bubbles, which generate strong shear forces and microstreaming within the canal.[31] Although the effectiveness of root canal irrigation with an Er:YAG laser has been highlighted in numerous previous studies, the majority of these investigations have focused on sequential irrigation protocols and studying final irrigation.[2–35] It has been found that the ultrasonic and laser activation of Dual Rinse HEDP enhanced the soft tissue dissolution in a similar manner as it affects the activation of NaOCl alone.[36] Surface tension and viscosity are features of the liquid that limit the irrigation capacity of the deeper areas of the root canal.[37] Dual Rinse HEDP shows higher surface tension and viscosity than that of NaOCl and EDTA,[15,38] which may affect the speed and lifetime of the primary cavitation bubble in laser activation.[39] As apical region is critical for preventing microbial reinfection and achieving a hermetic seal,[40] it is also the most challenging area to clean and disinfect during root canal treatment, and therefore it is important that continuous chelation improved sealer penetration and bond strength at the apical root level.

Previous studies demonstrated the decreasing degree of sealer penetration from the coronal to apical third of the root canal,[41,42] which could be due to a lower number of dentinal tubules in apical region[43] and tubular sclerosis.[44] This was also confirmed within the current study.

Endodontic irrigation solutions can affect the mechanical properties of radicular dentin, such as its microhardness.[45] Since HEDP solution dissolves less calcium ions from the

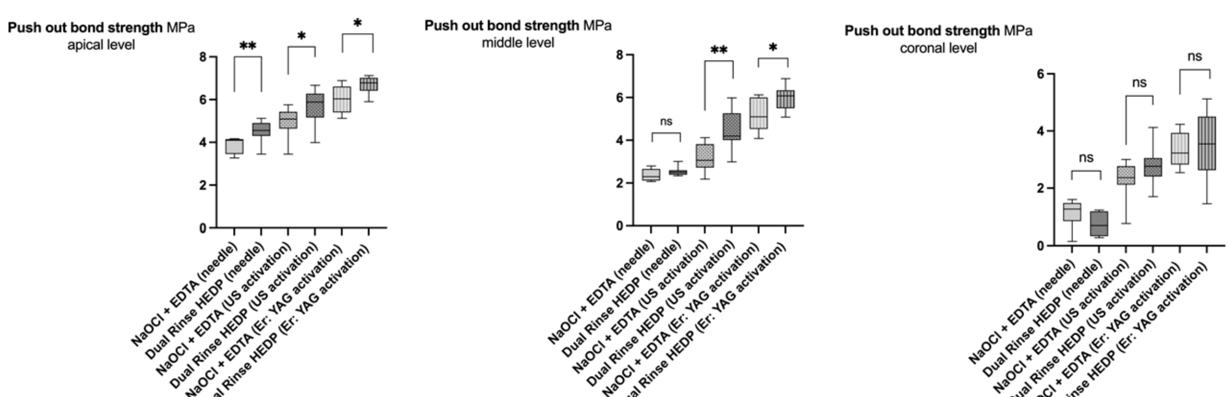

FIG. 5. Push-out bond strength was measured for samples from apical, middle, and coronal part of the root. Bar chart presenting mean and standard deviation values for push-out bond strength (MPa) in different groups. US stands for ultrasonic activation. Continuous chelation improved sealer penetration at the apical level of the root (**$p < 0.01$) when needle irrigation was used. The similar trends at apical and middle levels were observed when ultrasound and laser were applied.

hard tissue comparing with EDTA,[20] HEDP/Dual Rinse provides a balance between smear layer removal and preservation of the dentin surface, which is particularly beneficial for calcium silicate-based sealers. Bioceramic sealers set through a moisture-activated hydration reaction, producing a calcium silicate hydrate matrix and calcium hydroxide.[33] Therefore, irrigation of root canal enhances sealer penetration, chemical interaction with dentin, and long-term sealing ability.[46]

In this study, a fluorescent dye, Fluo-3 was used to explore whether different irrigation protocols with additional ultrasonic and laser activation; sequential chelation with EDTA versus continuous chelation with NaOCl and HEDP. To the best of the authors' knowledge, these systems have not been previously comparatively evaluated using this method. Traditionally, sealer penetration has been evaluated using Rhodamine B as a fluorescent dye with confocal laser scanning microscopy.[47–49] However, rhodamine B dissolves in water at room temperature, and its small particle size allows it to penetrate effectively into dentin tubules.[50] Thus, Rhodamine B's hydrophilic properties and ability to dissolve in water can result in overestimation of sealer penetration depth, particularly with calcium silicate-based sealers.[51,52] To address this limitation, Fluo-3, a calcium-specific fluorophore, was employed in the present study. This specific fluorophore binds to calcium ions within the calcium silicate-based sealer, enabling a more accurate assessment of actual sealer infiltration into dentinal tubules.[50,53] This fluorophore provides a novel and sensitive approach to evaluating calcium silicate-based sealer penetration into dentin tubules by enabling real-time imaging of calcium ion dynamics.[52] Unlike conventional dyes used for confocal microscopy studies about sealer penetration in previous studies,[47,48] Fluo-3 facilitates the direct visualization of calcium, offering critical insights into the biochemical interactions between sealer and dentin.[51]

This study analyzed the effects of continuous and sequential irrigation, using NaOCl across all samples to simulate standard clinical practice, as it is considered the gold standard in root canal disinfection. In the continuous chelating groups, NaOCl was mixed with HEDP. It was shown that NaOCl weakened the root dentine by damaging the inner tubule surfaces near the canal, and higher concentrations led to more significant deterioration.[54] For this reason, a concentration of 2.5% NaOCl was used in all the experiments. A recent study suggested that continuous irrigation with NaOCl combined with Dual Rinse HEDP resulted in a smaller reduction in dentine microhardness compared with using NaOCl alone or sequential irrigation with EDTA following NaOCl.[42]

While ultrasonic and laser activation primarily enhance canal cleaning and irrigation efficacy, they may also contribute to improved time efficiency during root canal treatment by facilitating solution dispersion.[55] Balancing thoroughness and efficiency in time management is essential to achieve optimal clinical outcomes and ensure patient satisfaction.[56] From a clinical perspective, completing endodontic treatment using a single irrigant is considered advantageous.

Our results align with previous research demonstrating similar outcomes when a bioceramic sealer was combined with an alternative obturation technique, such as warm vertical compaction.[42] On the contrary, Ozel et al.[2] as well as Gawdat et al.[57] showed contradictions in the results with the current study which may be due to the different fluorescent dye used for root canal obturation. The single cone technique used in this study is a recommended approach for root canal obturation with bioceramic sealers.[58]

This study is limited by its nature as a purely laboratory-based investigation. Nonetheless, efforts were made to simulate conditions that could arise in clinical practice. This research was limited to single-rooted teeth with a single canal in order to ensure standardization and reproducibility of experiments. However, it is essential to acknowledge that multi-canal teeth more accurately represent the complexity of clinical cases, highlighting the need to complement single-rooted tooth research with studies on multi-rooted systems.

The teeth used in the present study were extracted for reasons unrelated to this research, and as a result, the age of the patients was not recorded. It is important to note that dentin undergoes age-related changes, including sclerosis, reduced tubule density, and decreased permeability, which can negatively affect root canal sealer penetration.[59] Additionally, age-related mineralization may render dentin more resistant to chelating agents, such as EDTA or HEDP, potentially reducing the effectiveness of irrigation protocols and further limiting sealer infiltration into the dentinal tubules.[60] Another limitation of the current study lies in the fact that tooth sectioning occurred post-instrumentation and obturation, possibly compromising the adhesion between calcium silicate-based cement and the radicular dentin, as well as sealer penetration. Hazar and Hazar investigated push out bond strength on the 3 mm thick dentin sections that were cut prior the irrigation and obturation;[45] however, this approach involves immersing the samples in the tested solutions and performing root canal obturation with a hand plugger, which does not accurately replicate real clinical conditions.

Conclusions

In conclusion, while the choice between sequential and continuous chelation protocols does not significantly affect sealer penetration depth or push-out bond strength, ultrasonic and laser activation enhance penetration, particularly in the apical region. Among the protocols tested, the combination of continuous chelation and laser-assisted irrigation demonstrated the most effective results in terms of both sealer penetration and bond strength. Further research on multi-rooted teeth is needed to validate these findings in clinically relevant conditions.

Author Disclosure Statement

No competing financial interests exist.

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