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# Effects of phytic acid and etidronic acid using continuous and sequential chelation on the removal of smear layer, dentin microhardness, and push-out bond strength of calcium silicate-based cement

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## Abstract

**Background** This study assessed the effects of sequential and continuous chelation using phytic acid and etidronic acid on smear layer removal, microhardness, and push-out bond strength (PBS) at radicular dentin.

**Methods** One hundred twenty single-rooted teeth were selected. Thirty teeth were split longitudinally, and initial microhardness was measured. The roots were then divided into six groups. In sequential chelation, 2.5% sodium hypochlorite (NaOCl) was used for 20 min, followed by 17% ethylenediaminetetraacetic acid (EDTA), 9% etidronic acid (HEDP), or 2.5% phytic acid (PA) for 2 min, while no chelator was applied in the control group. In continuous chelation, etidronic acid (DR HEDP) or phytic acid (DR PA) was mixed with NaOCl and applied for 20 min. Final microhardness values were measured, and the change was calculated as a percentage. Mid-root sections were obtained from the 60 teeth for the PBS test and divided into six groups. Irrigants were applied as in the microhardness test. Sections obturated with calcium silicate cement. PBS values were measured, and the types of failures were analyzed. Thirty teeth were analyzed using scanning electron microscopy (SEM). In sequential chelation, 2.5% NaOCl irrigation was performed during instrumentation, followed by 17% EDTA, 9% HEDP, or 2.5% PA for 2 min, while no chelator was applied in the control group. In continuous chelation, DR HEDP or DR PA was mixed with NaOCl and used during instrumentation. Images were taken, and smear layer scores were recorded. The PBS data were analyzed using one-way ANOVA, and the alterations in microhardness and remaining smear layer were analyzed using a two-way ANOVA test. The pairwise comparisons were analyzed using Tukey's HSD post hoc test. The failure patterns and smear scores were compared using Pearson's chi-squared test ($\alpha = 0.05$).

**Results** Results showed that DR HEDP ($4.02 \pm 0.68\%$) caused the least reduction in microhardness. DR HEDP ($10.26 \pm 1.74$ MPa) exhibited the highest bond strength, followed by PA ($7.97 \pm 0.92$ MPa) and DR PA ($7.74 \pm 1.16$ MPa).

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Failure patterns did not differ significantly. Lower percentages of the remaining smear layer area were observed in the DR PA (26.7 ± 18%), PA (24.2 ± 9.8%), and DR HEDP (37.1 ± 16.5%) groups compared to the others.

**Conclusions** The use of etidronic acid with the continuous chelation technique and phytic acid with the sequential chelation technique may be an alternative method to sequential EDTA irrigation.

**Keywords** Continuous chelation, Sequential chelation, Etidronic acid, Phytic acid, Microhardness, Push-out bond strength, Smear layer

## Background

The effectiveness of endodontic treatment relies on eliminating microorganisms from the root canal system through chemomechanical preparation, which combines mechanical instrumentation with chemical disinfection [1]. The complex anatomy of the root canal system, characterized by morphological variations, curvatures, and apical ramifications, presents a significant challenge to effective disinfection [2]. Without proper cleaning of the root canals, reinfection may occur due to the presence of residual microorganisms [3]. Consequently, irrigants play an important role in endodontic treatment.

The smear layer formed during mechanical instrumentation is an organic and inorganic layer that may contain dentin residues, pulp tissue residues, and bacteria [1, 2]. This layer acts as a barrier, impeding the direct access of irrigants to the dentin surface and tubules to prevent disinfection and alter the adhesion of root-filling materials to the canal walls [4]. Sodium hypochlorite (NaOCl) is commonly used as an irrigation solution, typically available in concentrations ranging from 1 to 5.25%, due to its effective tissue-dissolving and antimicrobial properties [1]. Since it cannot eliminate the inorganic components of the smear layer, it is recommended that a chelating agent be used in combination with NaOCl for the final irrigation of root canals [4].

Ethylenediamine tetraacetic acid (EDTA) is the most widely used chelator in endodontic treatment. It is employed alongside NaOCl to remove the smear layer, adequately disinfect the root canals, and dissolve organic tissue [5]. However, using EDTA during root canal treatment has several potential drawbacks. One is that the combination of EDTA and NaOCl can reduce the amount of free chlorine in the mixture. This reduction can affect both the antibacterial properties and the tissue-dissolving effectiveness of NaOCl [1, 4]. Therefore, it is advised not to mix these two solutions but rather to use them sequentially. Another disadvantage is that EDTA has a restricted ability to remove the smear layer in the apical third of root canals [4]. In addition, irrigation solutions directly interact with dentin bio-composite ingredients such as apatite nanocrystals or collagen fibers. In particular, chemical treatment can alter the dentin structure, which normally consists of approximately 70% mineral (primarily hydroxyapatite), 20% organic matrix (mostly type I collagen nanofibers), and 10% liquid [5, 6]. These changes due to the dissolution or oxidation will likely degrade the material's capacity to withstand mastication forces [6]. Exposure to NaOCl has been reported to decrease dentin microhardness due to its ability to dissolve organic tissue [5, 7]. EDTA is an effective chelating agent for inorganic di-covalent cations. It may detrimentally affect the calcium and phosphorus ratio when applied to dentin, rendering the tooth more prone to fracture [7]. Therefore, in recent years, alternative chelating agents that provide the most benefits with the least undesirable properties have been investigated to overcome the shortcomings of EDTA. Phytic acid (PA), also known as inositol hexaphosphate (IP6), is a naturally occurring organophosphorus compound primarily found in plant-based foods such as cereals, legumes, and oilseeds. It is also present in varying concentrations within mammalian cells. It functions as a chelator for several cationic ions, including calcium, magnesium, and iron [8]. This property aids in the removal of the smear layer, making it a recommended alternative irrigation solution to EDTA due to its lower toxicity and increased biocompatibility compared to EDTA [8, 9]. Etidronic acid (1-hydroxyethylidene-1, 1-bisphosphonate, HEDP, HEBP) is a systemically administered bisphosphonate used in the treatment of osteoporosis. It has been reported that it is an effective antimicrobial agent for removing the smear layer in root canals of teeth [10, 11]. There is no interaction between them that would reduce the effectiveness of sodium hypochlorite [4]. Therefore, HEDP and NaOCl can be mixed and used as a single solution, also called continuous chelation [4]. As a weak chelator, HEDP was stated to exert a less aggressive effect on dentine than EDTA [12].

Clinically, it is desirable to be able to complete endodontic treatment with a single irrigant. However, across-the-board in vitro studies are needed to evaluate the effectiveness of mixing irrigation agents. Because endodontic irrigation solutions can influence the mechanical properties of the radicular dentin, such as microhardness, by changing the chemical structure of the tissue [4, 5]. Changes in the dentin structure's calcium-phosphorus ratio result in dentin microhardness alteration. Thus, we can gain insight into indirect evidence of mineral loss or gain in the dental hard tissue by evaluating changes in dentin microhardness [5].

Biodentine (BD, Septodont, Saint Maur des Fosses, France) is a tricalcium silicate cement developed as a fast-setting bioactive dentin replacement material for pulp capping, apexification, and perforation repair [13]. BD is known to have a bonding ability with mineral tags in the dentin tubules. Considering the clinical applications, the bond strength of these materials with dentin plays a significant role in clinical practice. Chelators can also affect the push-out bond strength (PBS) between the radicular dentin and the materials used for obturation or repair [4, 11].

This in-vitro study investigated the effect of etidronic acid and phytic acid when used as continuous and sequential chelation on radicular dentin microhardness, smear layer removal, and the push-out bond of calcium silicate cement. Hypotheses of the study were that (1) there would be a difference in the effect of chelators on the microhardness change of radicular dentin; (2) there would be a difference in the effect of chelators on the push-out bond strength of calcium-silicate cement; (3) there would be a difference in the chelators' effect on smear layer removal.

## Methods

### Teeth selection

A total of 120 maxillary first incisor teeth, all exhibiting similar root lengths, were included in this study. These teeth were freshly extracted for periodontal reasons. The use of these teeth was conducted with the patient's consent and received approval from the Research Ethical Committee at Bulent Ecevit University (protocol: 2024/22).

The sample size was determined using G*Power (version 3.1.9.7, Kiel University, Kiel, Germany) along with a one-way analysis of variance (ANOVA) based on the results of a prior study [14]. The effect size (f) was 0.57, the type I error (α) was 0.05, and the statistical power (1-β) was 0.90. The total sample size required for this study was 60, with each group requiring a sample size of 10.

All teeth were examined using digital radiography from both buccal and proximal perspectives to confirm the presence of straight single root canals. Additionally, a dental operating microscope (EZ4W, Leica Microsystems, Milton Keynes, UK) was used at ×40 magnification to examine for any signs of root resorption, cracks, or fractures. Teeth with root fractures, resorption, calcification, root curvature, or a history of previous endodontic treatment were excluded. After removing soft tissues and calculus with a scaler, the teeth were stored in a 0.1% thymol solution at 4 °C until use.

### Preparation of irrigation solutions

2.5% and 5% NaOCl (Sigma-Aldrich, St. Louis, MO, USA) were prepared by mixing pure chemicals with distilled water. The 17% EDTA solution was prepared by dissolving disodium EDTA (Sigma-Aldrich) in distilled water, with the aid of sodium hydroxide (Sigma-Aldrich), to facilitate dissolution. 2.5% and 5% PA solution was prepared by diluting a 50% concentration of phytic acid (inositol hexaphosphate, IP6, Sigma-Aldrich) with distilled water. Etidronic acid solutions were prepared by mixing the powder of one capsule (0.9 g) (Medcem GmbH, Weinfelden, Switzerland) with 10 mL of 2.5% NaOCl (DR HEDP; a dual-rinse solution of 9% etidronic acid with NaOCl used in continuous chelation) or 10 mL distilled water (HEDP, sequential chelation); the solutions were manually stirred for 2 min using a plastic spatula. All solutions were stored in dark containers and freshly prepared before experiments.

The materials used and study groups in this study are presented in Table 1.

### Microhardness test

Thirty teeth were used for microhardness testing. After preparing grooves along the long axis of the roots using a diamond disk mounted under water cooling, the roots were split longitudinally into two halves. Each half-root was embedded in acrylic resin blocks, with the dentin surface left exposed ($n = 10$). The samples were polished using #600, #1000, and #1200 grit SiC sandpapers for 20 s each with a polishing machine (Buehler, Lake Bluff, IL, USA) to achieve a smooth and flat surface.

The microhardness values were measured in each half-root using an automatic turret microhardness tester (HMV-G20S, Shimadzu Corp., Kyoto, Japan). The indentations were made with the square diamond pyramid shape indenter on each specimen using a 50-g load and a 10-s dwell time. All measurements were conducted at different thirds: coronal (11–13 mm from the apex), middle (6–8 mm from the apex), and apical (1–3 mm from the apex). At each third, three points were randomly selected and measured without overlapping. The values were obtained as the average of the results for the three measurements. After the initial measurements, the samples were divided into six groups based on the irrigation procedures. In the control, DR HEDP, and DR PA groups, the samples were immersed in 32 mL of solutions: 2.5% NaOCl, 9% HEDP + 2.5% NaOCl, and 2.5% PA + 2.5% NaOCl, respectively, for 20 min. They were then washed with distilled water for 1 min. The solutions were renewed for each sample. The EDTA, PA, and HEDP groups were immersed in 2.5% NaOCl and rinsed with distilled water as in the control group. Then, according to the groups, they were immersed in 17% EDTA, 2.5% PA, and 9% HEDP, each 4 mL, for 2 min and rinsed with

**Table 1** The materials used and the study groups in the study

| Irrigation Agents | Chelating Technique | Representative Group Name | Concentration and pH | Total Volume | Contact Time |
|---|---|---|---|---|---|
| Sodium hypochlorite | No chelator used | Control | 2.5% NaOCl (pH = 12) | NaOCl: 32 mL<br>DW: 5 mL | NaOCl: 20 min (including instrumentation time).<br>DW: 1 min |
| Sodium hypochlorite / Ethylenediaminetetraacetic acid | Sequential chelation | EDTA | 17% EDTA (pH = 7.4) | NaOCl: 32 mL<br>DW: 5 mL<br>EDTA: 4 mL<br>DW: 5 mL | NaOCl: 20 min (including instrumentation time).<br>DW: 1 min<br>EDTA: 2 min<br>DW: 1 min |
| Sodium hypochlorite / Etidronic acid | Sequential chelation | HEDP | 9% HEDP (pH = 2.4) | NaOCl: 32 mL<br>DW: 5 mL<br>HEDP: 4 mL<br>DW: 5 mL | NaOCl: 20 min (including instrumentation time).<br>DW: 1 min<br>HEDP: 2 min<br>DW: 1 min |
| Sodium hypochlorite / Phytic acid | Sequential chelation | PA | 2.5% PA (pH = 0.9) | NaOCl: 32 mL<br>DW: 5 mL<br>PA: 4 mL<br>DW: 5 mL | NaOCl: 20 min (including instrumentation time).<br>DW: 1 min<br>PA: 2 min<br>DW: 1 min |
| Etidronic acid and sodium hypochlorite mixture | Continuous chelation | DR HEDP | 9% DR HEDP (pH = 5.6) | HEDP + NaOCl: 32 mL<br>DW: 5 mL | HEDP + NaOCl: 20 min (including instrumentation time).<br>DW: 1 min |
| Phytic acid and sodium hypochlorite mixture | Continuous chelation | DR PA | 2.5% DR PA (pH = 7.8) | PA + NaOCl: 32 mL<br>DW: 5 mL | PA + NaOCl: 20 min (including instrumentation time).<br>DW: 1 min |

distilled water for 1 min. The solutions were renewed for each sample. Final microhardness tests of the samples were carried out as in the initial microhardness tests. The percentage change in hardness was calculated using the formula % = [(Initial microhardness—Final microhardness) / Initial microhardness] × 100. Representative Vickers indentation images at ×40 magnification of the groups at root thirds are presented in Fig. 1.

### Push-out test

Sixty teeth were embedded in auto-polymerized acrylic resin and coupled to a precision cutting machine (Micracut 201, Metkon, Bursa, Türkiye). Mid-root segments of 3 mm thickness were obtained by sectioning perpendicular to the long axis at 3 mm and 6 mm coronal to the apex. A single segment was taken from each root. The thickness of each slice was verified using a digital caliper (precision level +/−0.001 mm). Straight root segments with a single canal were uniformly instrumented up to a size #6 Peeso reamer (Dentsply, Tulsa, OK, USA) to create a uniform 1.7 mm, parallel-sided canal under water cooling. Root segments were randomly divided into six groups according to the irrigation procedure ($n = 10$). The samples were immersed in the solutions as described in the microhardness test. Root canals were obturated incrementally with a hand plugger (Queen Instruments, Hungary) using perforation repair material (Biodentine, Septodont, France) mixed per the manufacturer's instructions. Moist gauze was applied to the top and bottom of the sections to allow the material to set. The specimens were then maintained at 37 °C in distilled water for seven days before push-out testing.

The sections were positioned with the apical part of the root canal facing upward, centered at the bottom of the universal testing machine (PWS-E100, Shimadzu Co., Kyoto, Japan), and subjected to a push-out test (Fig. 2a). A 1.5 mm diameter tip was used for the push test. The testing machine was operated at a constant speed of 1.0 mm/min until maximum tension was achieved. The force (F) required for displacement was measured in Newtons (N), then converted into Megapascals (MPa) by dividing it by the area of adhesion of the luting material (SL) in $mm^2$, using the $\sigma = F/SL$ formula. SL was calculated using $SL = \pi Dg + (\pi D^2/4 - \pi d^2/4)$ formula: "D" is the average radius of the root-end cavity (mm), "d" is the average radius of the root canal (mm), and "g" is the relative height of the root-end cavity (mm).

After the PBS test, the root sections were observed for the failure patterns with a dental operating microscope at ×40 magnification. The failures were determined in percentages and classified as adhesive (the BD dislodged from the dentin, Fig. 2b), cohesive (fracture within the BD, Fig. 2c), and mix (the BD dislodged from the dentin and fracture within the filling material, Fig. 2d).

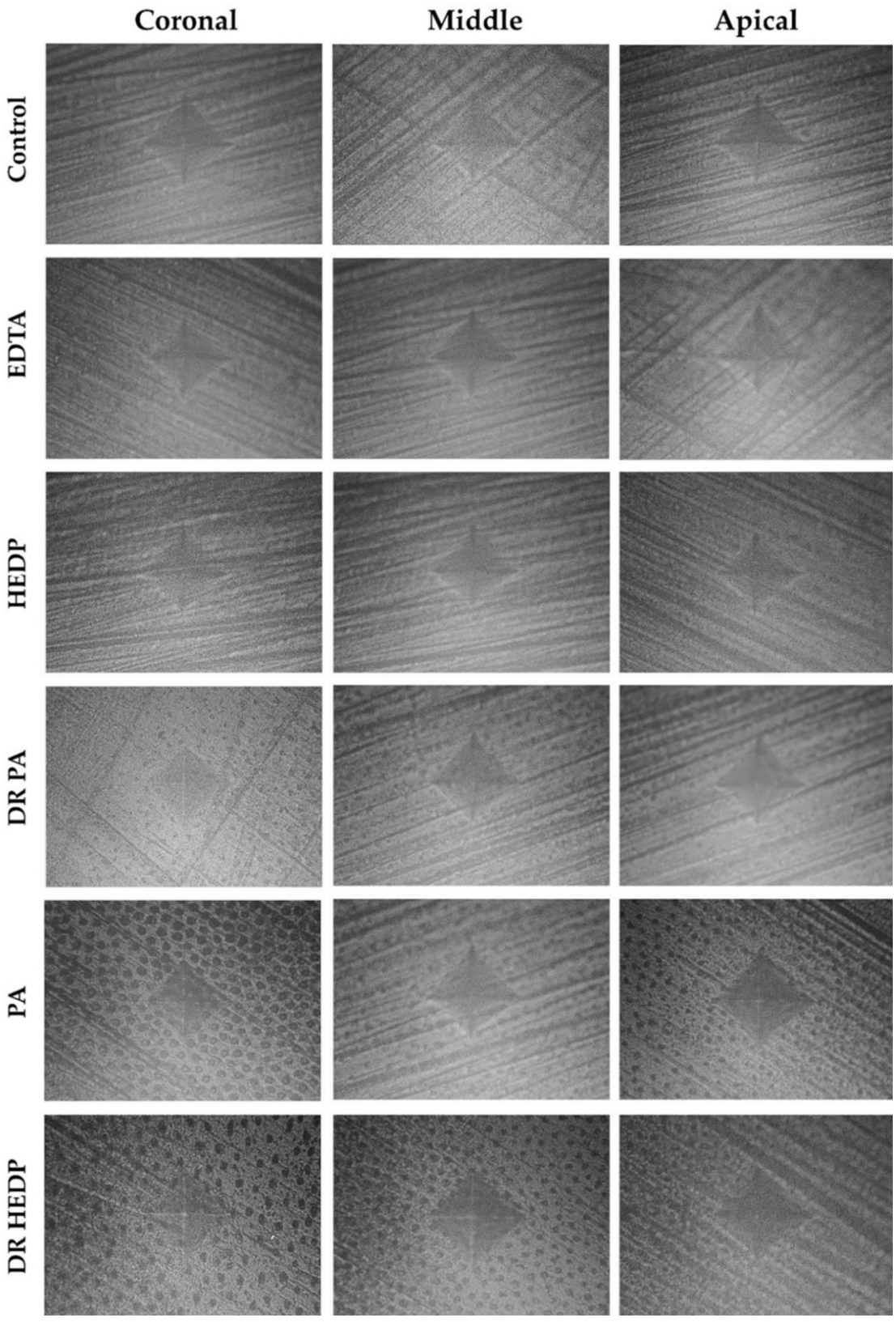


**Fig. 1** Representative Vickers indentation images at ×40 magnification of the groups at the root thirds

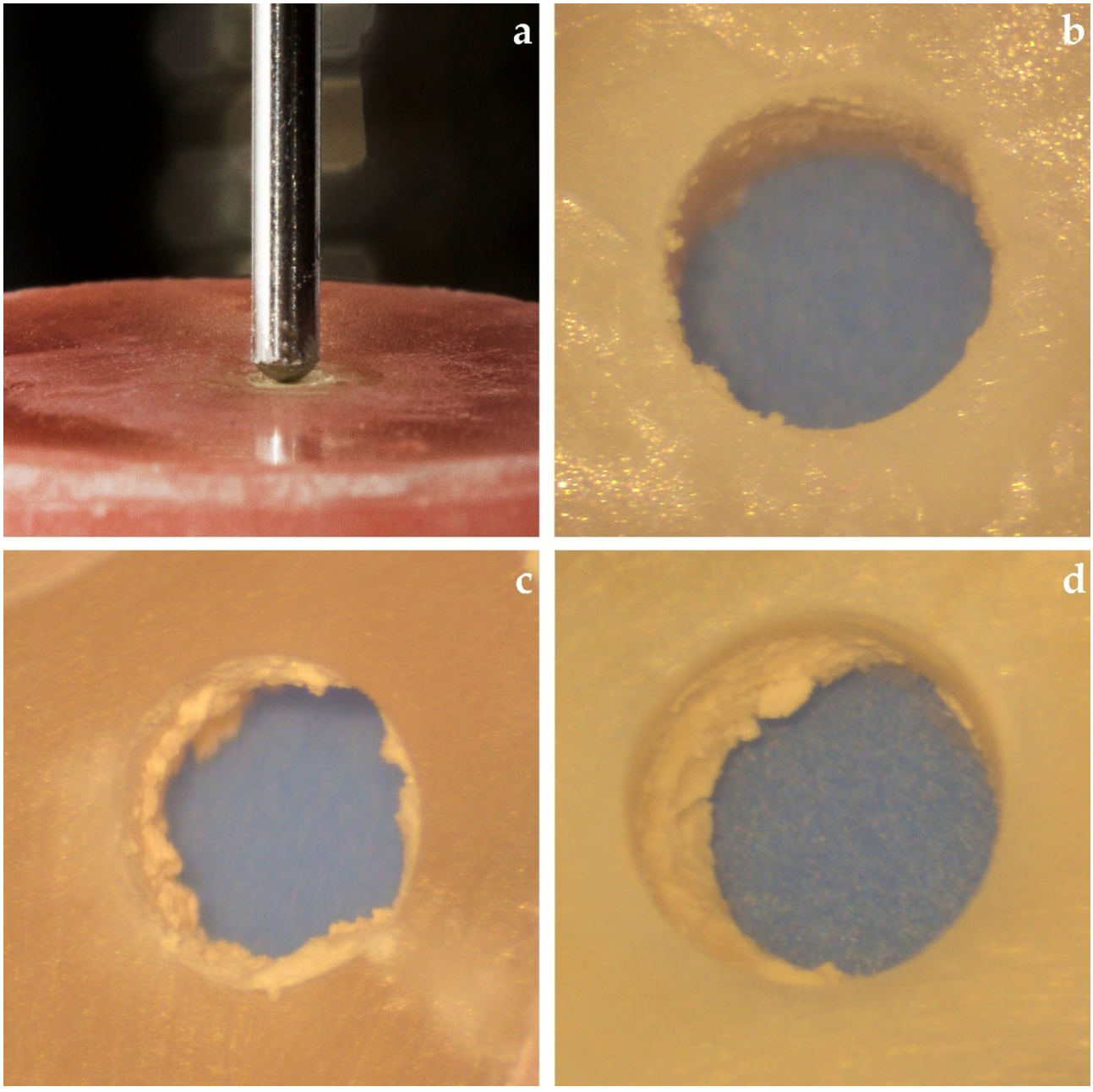


**Fig. 2** Representative images of **a**: push-out test, **b**: adhesive failure pattern, **c**: cohesive failure pattern, **d**: mix failure pattern

### Scanning electron microscopy (SEM) examination for smear removal

Thirty teeth were selected randomly to use in SEM observation. The coronal part of the teeth was sectioned at 15 mm from the apex to obtain standardized root canal lengths under water cooling (Isomet 5000, Buehler, Lake Bluff, IL, USA). Apical patency was verified using a #10 K file (Mani Inc., Tochigi-Ken, Japan). The specimens were randomly divided into six groups based on the irrigation procedure ($n = 5$). A single operator (EH) carried out the instrumentation and irrigation. Root canals were shaped using the ProTaper rotary system (Dentsply Sirona Endodontics, Tulsa, OK), following the manufacturer's instructions, up to size F4. Irrigation was conducted using a 27-G side-vented needle (Henry Schein, Melville, NY, USA), inserted 1 mm short of the working length. Following the final irrigation protocol, the root canals were dried using paper points (Dentsply Sirona Endodontics).

The irrigation regimen was as follows:

Control: Before starting the instrumentation, the root canals were irrigated with 2.5% NaOCl for 2 min. During

instrumentation, each file was used for 1 min, and after each file, the root canal was irrigated with 4 mL NaOCl for 2 min. Then, the root canals were flushed with 5 mL of distilled water for 1 min.

EDTA: The control group's described instrumentation and irrigation procedures were followed. The root canals were then irrigated with 4 mL of 17% EDTA for 2 min. A final rinse was performed with 5 mL of distilled water for 1 min.

HEDP: The control group's described instrumentation and irrigation procedures were followed. The root canals were then irrigated with 4 mL of 9% HEDP for 2 min. A final rinse was performed with 5 mL of distilled water for 1 min.

PA: The control group's described instrumentation and irrigation procedures were followed. The root canals were then irrigated with 4 mL of 2.5% PA for 2 min. A final rinse was performed with 5 mL of distilled water for 1 min.

DR HEDP: Before starting the instrumentation, the root canals were irrigated with 9% DR HEDP for 2 min. During instrumentation, each file was used for 1 min, and after each file, the root canal was irrigated with 4 mL 9% DR HEDP for 2 min. Then, the root canals were flushed with 5 mL of distilled water for 1 min.

DR PA: A 5% phytic acid solution is mixed with a 5% NaOCl solution in equal proportions to create a DR PA solution with a 2.5% phytic acid concentration and a 2.5% NaOCl concentration. Before starting the instrumentation, the root canals were irrigated with 2.5% DR PA for 2 min. During instrumentation, each file was used for 1 min, and after each file, the root canal was irrigated with 4 mL 2.5% DR PA for 2 min. Then, the root canals were flushed with 5 mL of distilled water for 1 min.

After completing the irrigation procedure, two parallel longitudinal grooves were created on the buccal and lingual surfaces of each root using a high-speed diamond bur (Horico Dental Hopf, Ringleb & Co. Gmbh & Cie, Berlin, Germany) with water cooling, ensuring no perforation of the root canal occurred. The F4 gutta-percha cone (Maillefer SA, Ecublens, Switzerland) was inserted into the canal, and a small cotton plug was used to seal the orifice. The gutta-percha cone served as an indicator for groove depth, preventing the bur from perforating the canals and avoiding contamination from debris produced during sectioning. Then, the root was longitudinally split into two parts using a chisel. The root sections were dehydrated for eight hours using varying concentrations of ethyl alcohol: 50%, 70%, 90%, and 100%, respectively. Followed by air-drying in a desiccator for 72 h. Then, the sections were examined with an SEM (QuantaTM 450 FEG, FEI, Oregon, USA) at 1.0 kV and ×2500 magnification ($n = 10$). All SEM images were acquired at three levels: coronal (11–13 mm from the apex), middle (6–8 mm), and apical (1–3 mm), by a technician blinded to the groups. The SEM images were then assessed to determine the effectiveness of the chelators on the removal of the smear layer at root thirds. Total area (Ta), debris, and smear layer areas (Sa) were calculated on all SEM images using Image J image analysis software. The percentage of the remaining smear layer compared to the total area was calculated using the formula 100 × Sa/Ta. The calculated values were then scored using a four-level scoring system: 1 = remaining smear layer less than 25% of the total area, 2 = remaining smear layer between 25% and 49% of the total area, 3 = remaining smear layer between 50% and 74% of the total area, and 4 = remaining smear layer greater than 75% of the total area [15]. Thus, errors that may occur in the calibration of observers have been reduced. Representative SEM images and scores of the groups at root thirds are presented in Fig. 3.

### Statistical analysis

IBM SPSS (V23, Chicago, IL, USA) software was used to analyze the data. The Shapiro–Wilk test was used to confirm if the data had a normal distribution according to the groups. One-way ANOVA was used to analyze the push-out bond strength, and the two-way ANOVA test was used to analyze the microhardness percentage change and the remaining smear layer area of the groups on root thirds. The pairwise comparisons were analyzed using Tukey's honest significant difference post-hoc test. The frequency percentages of the failure patterns and smear removal scores between the groups were compared using Pearson's chi-squared test. The differences were considered significant at $p < 0.05$.

## Results

Figure 4 presents the mean and standard deviation values for the change in microhardness among the groups in the root thirds. According to the results of the two-way ANOVA test used to evaluate the first hypothesis of the study, the chelators significantly affected the change in microhardness of radicular dentin ($p < 0.001$, $\mu p^2 = 0.672$). In contrast, the root thirds ($p = 0.963$, $\mu p^2 = 0.00$) and the interactions between the chelators and root thirds ($p = 1.00$, $\mu p^2 = 0.001$) did not significantly affect the change in microhardness. The Tukey HSD post hoc test indicated that the lowest percentage change in microhardness was observed in the DR HEDP (4.02 ± 0.68%) group ($p < 0.05$). Among the materials tested, the most remarkable change in microhardness was observed in the PA (10.12 ± 1.72%) group ($p < 0.05$). No statistical difference was found among the control (6.78 ± 1.17%), EDTA (7.73 ± 1.62%), HEDP (6.44 ± 0.96%), and DR PA (6.39 ± 1.27%) groups ($p > 0.05$).

Figure 5 presents the mean and standard deviation of push-out bond strength values for the groups tested in

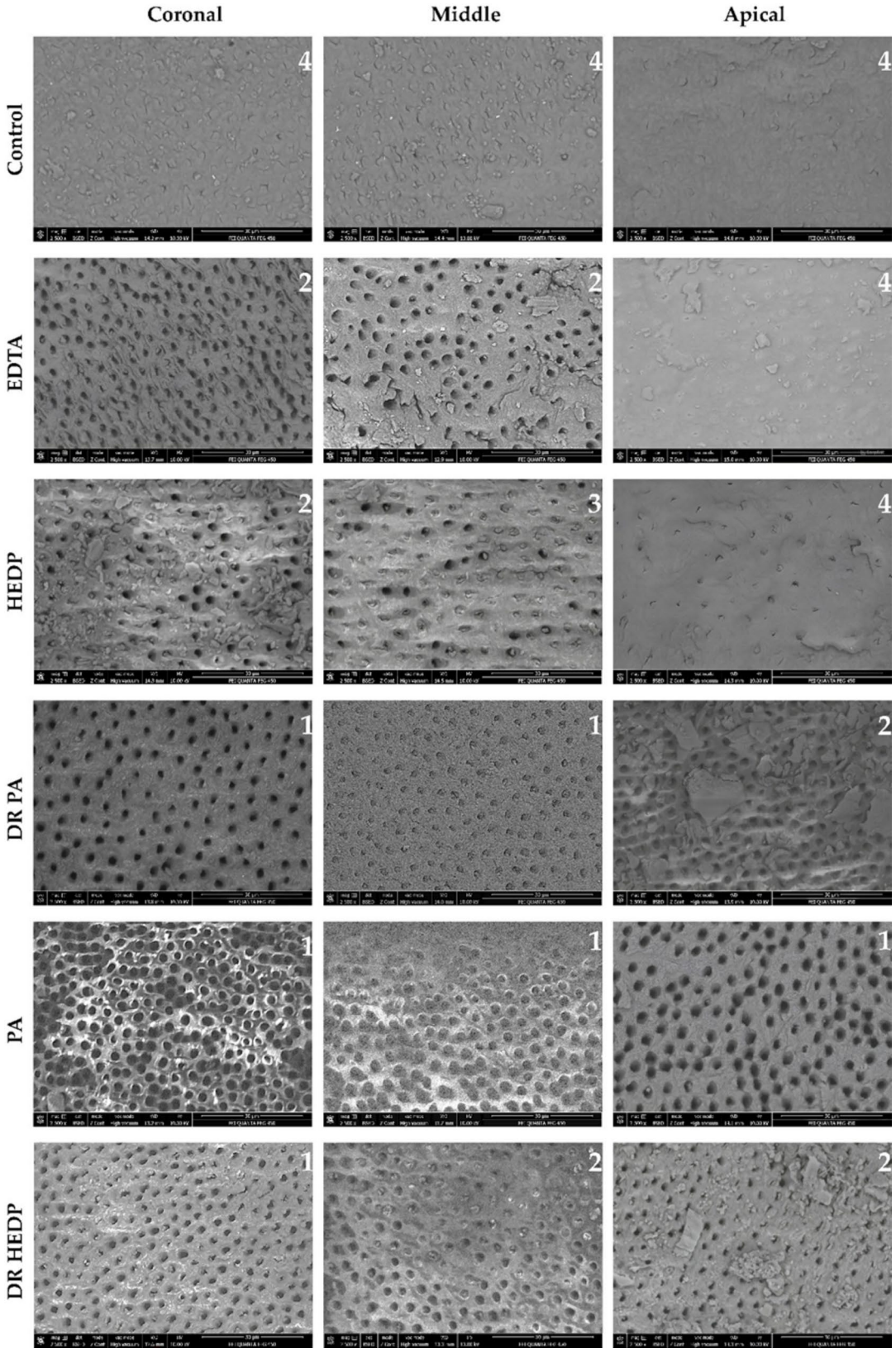


**Fig. 3** Representative SEM images and scores of groups at root thirds

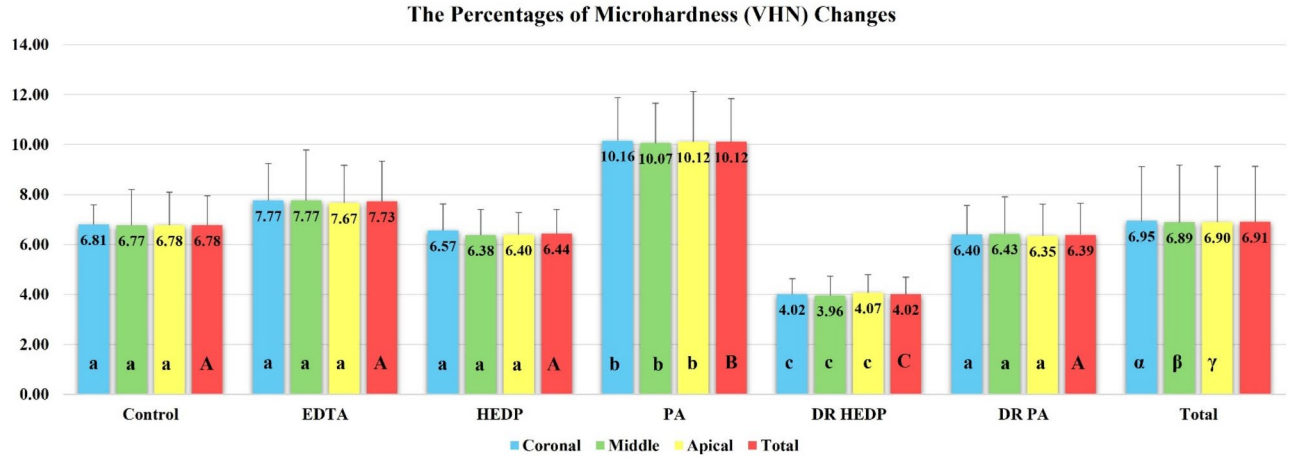


**Fig. 4** The mean and standard deviation values of the change in microhardness among the groups in the root thirds. (a-c): Different lowercase letters represent statistical differences among groups, (A-C): Different uppercase letters represent statistical differences among chelators, regardless of their root regions, (α-γ): Different symbols represent statistical differences among root thirds, regardless of the chelators used (Tukey HSD post hoc test, $p < 0.05$)

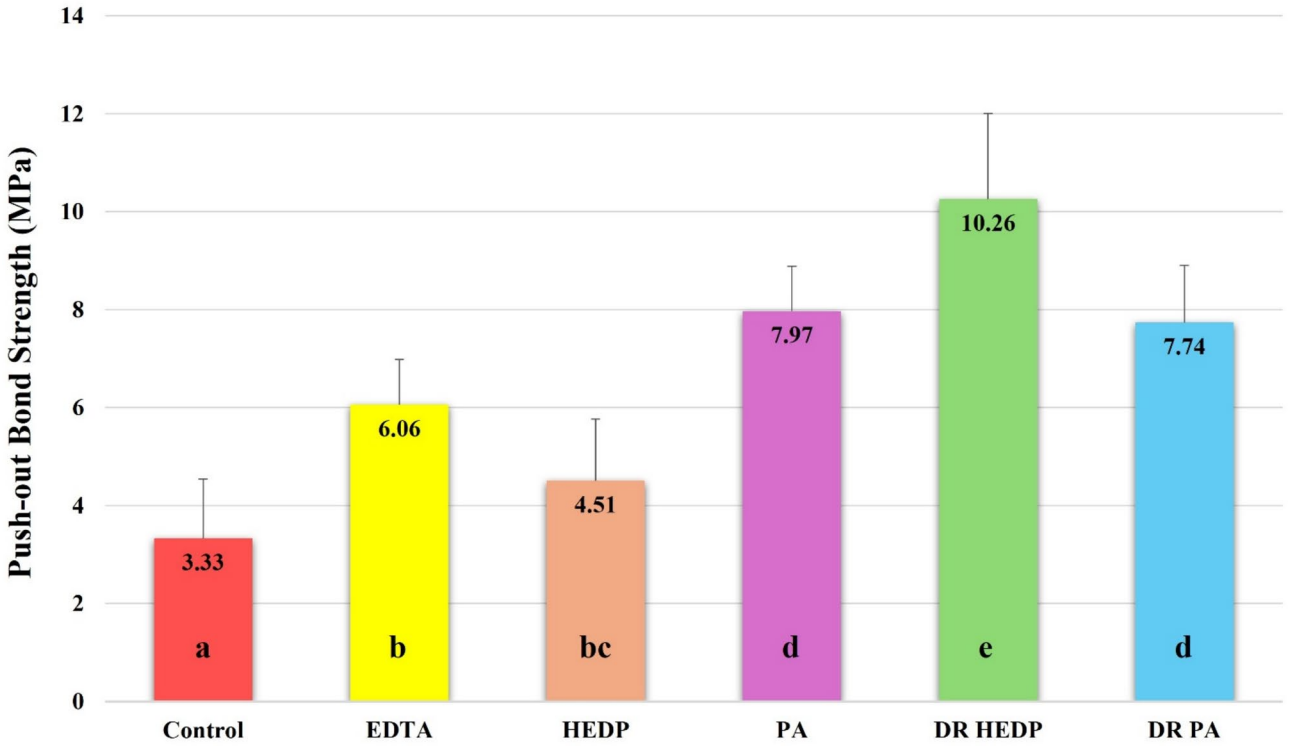


**Fig. 5** The mean and standard deviation push-out bond strength values of the groups. (a-d): Different lowercase letters indicate statistical differences between groups (Tukey HSD post hoc test, $p < 0.05$)

this study. According to the one-way ANOVA test used to evaluate the study's second hypothesis, there was a significant difference in PBS values among the groups ($p < 0.001$). According to Tukey's honest significant post hoc test, the highest fracture resistance values were observed in the DR HEDP group (10.26 ± 1.74 MPa), followed by the PA group (7.97 ± 0.92 MPa), DR PA group (7.74 ± 1.16 MPa), EDTA group (6.06 ± 0.92 MPa), HEDP group (4.51 ± 1.26 MPa), and the control group (3.33 ± 1.21 MPa) ($p < 0.001$). There was no statistical difference between the mean PBS values of the PA and DR PA groups ($p = 0.998$), EDTA and HEDP groups ($p = 0.073$), or HEDP and control groups ($p = 0.288$).

Figure 6 illustrates the distribution of failure patterns among the groups. According to the results of Pearson's chi-squared test, the failure patterns of the groups were not significantly different ($p > 0.05$). When examining intragroup comparisons, mix, and cohesive types

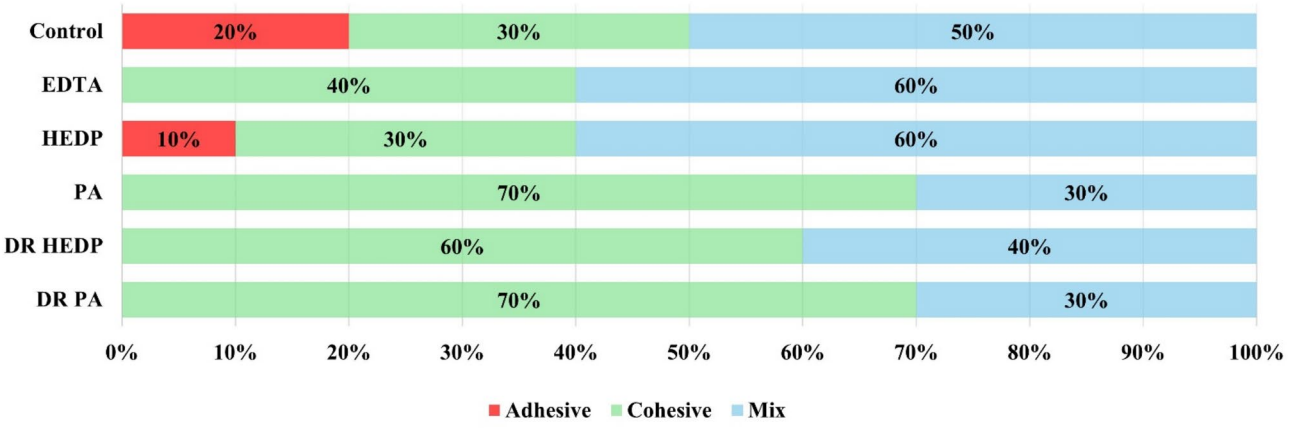


**Fig. 6** Distribution of percentage failure patterns among the groups (Pearson's chi-squared test, $p > 0.05$)

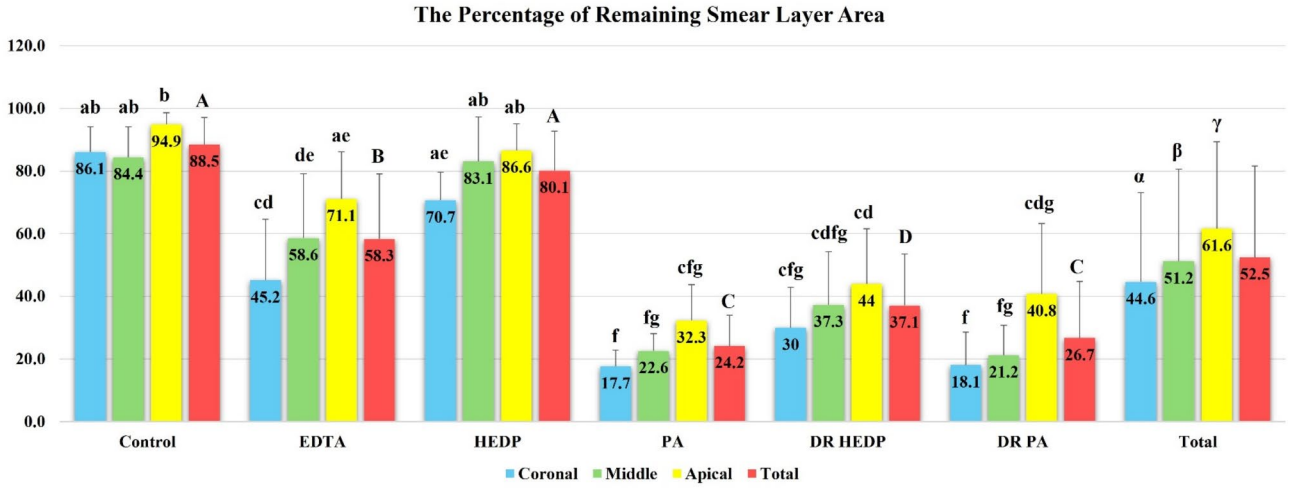


**Fig. 7** The mean and standard deviation values of the remaining smear layer area percentage in the groups. (a-g): Different lowercase letters represent statistical differences among groups, (A-D): Different uppercase letters represent statistical differences among chelators, regardless of their root regions, (α-γ): Different symbols represent statistical differences among root thirds, regardless of the chelators used (Tukey HSD post hoc test, $p < 0.05$)

emerged as the most common failure patterns among all groups, while the adhesive type was a significantly less frequent pattern. Adhesive-type failure was observed only in the control (20%) and HEDP (10%) groups.

Figure 7 presents the mean and standard deviation values of the remaining smear layer area percentages for the groups. Results from the two-way ANOVA test which was used to evaluate the third hypothesis of the study showed that both the root thirds ($p < 0.001$, $\mu_p^2 = 0.232$) and the chelators used ($p < 0.001$, $\mu_p^2 = 0.796$) had a significant effect on smear layer removal; however, the interaction between the root thirds and the chelators did not have a significant effect ($p = 0.494$, $\mu_p^2 = 0.055$). In all root thirds, the DR PA (26.7 ± 18%), PA (24.2 ± 9.8%), and DR HEDP (37.1 ± 16.5%) groups showed the highest efficacy in smear removal, whereas the control (88.5 ± 8.7%) and HEDP(80.1 ± 12.6%) groups showed significantly lower effectiveness.

Regarding smear scores, Pearson's chi-squared test showed that the PA, DR PA, and DR HEDP groups had significantly better scores than the other groups across all root thirds ($p < 0.05$). The scores of the control, EDTA, and HEDP groups were not statistically different in any of the root thirds ($p > 0.05$). The highest percentage of score 1 was observed in the PA (80%) and DR PA (60%) groups in the coronal root third, respectively (Fig. 8). In the middle third, while score 4 was not observed in the DR PA, PA, and DR HEDP groups, the most frequent scores in the HEDP, EDTA, and control groups were 3 and 4 (Fig. 9). The control group exhibited the highest rate of score 4 (100%) in the apical third, followed by the HEDP group at 80% in the apical third (Fig. 10).

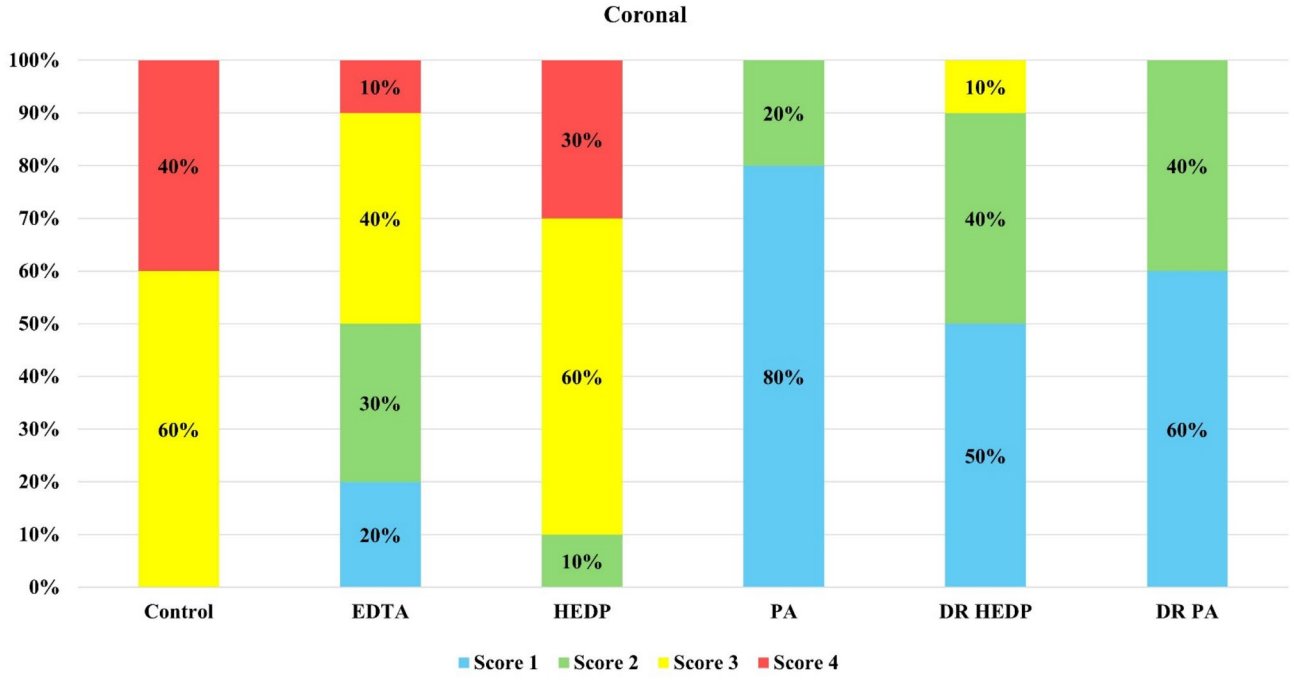


**Fig. 8** Distribution of the score percentages for the groups in the coronal root third (Pearson's chi-squared test, $p < 0.05$)

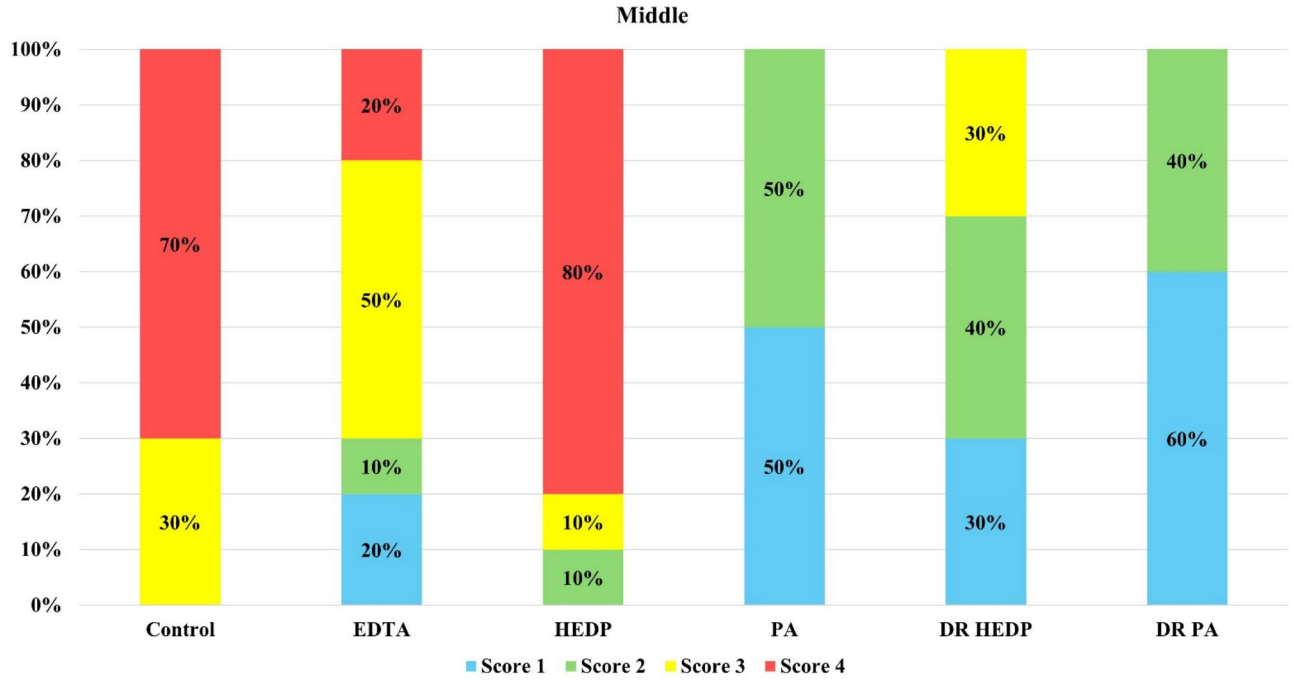


**Fig. 9** Distribution of the score percentages for the groups in the middle root third (Pearson's chi-squared test, $p < 0.05$)

## Discussion

The organic and inorganic structure and composition are critical for the dentin structure's mechanical integrity and biological function [4]. Ideally, irrigants should not have any negative effects on the physical and chemical structure of dentin [1, 2, 5, 7]. Therefore, it is substantial to assess the effects of irrigation agents used during root canal treatment on the mechanical properties of dentin. Although a slight softening of the root canal dentine can simplify mechanical instrumentation clinically, significant changes in hardness may lead to complications such as root canal transportation and reduced fracture

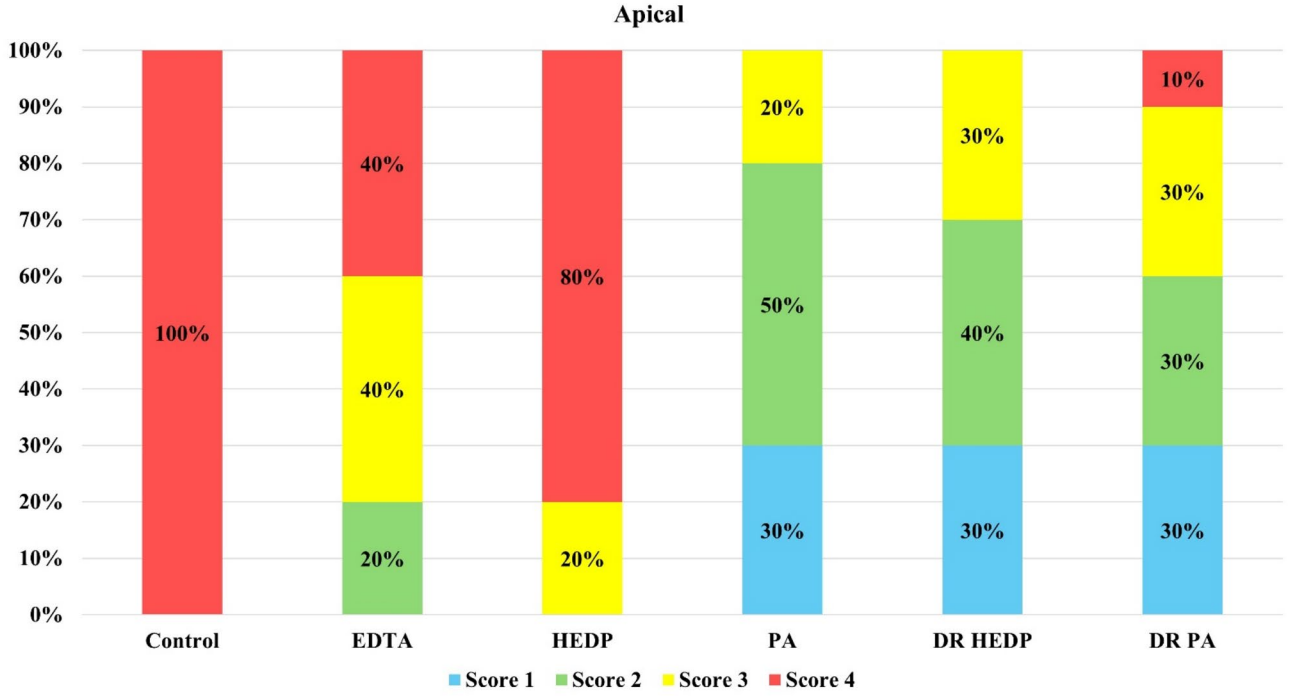


**Fig. 10** Distribution of the score percentages for the groups in the apical root third (Pearson's chi-squared test, $p < 0.05$)

resistance of the roots [16]. The characteristics of microhardness hold significant clinical importance as it is a widely utilized method for assessing the mechanical properties of surface dentin [17].

Previous studies have stated that the density of dentinal tubules and the volume of intertubular dentin influence the hardness of root canal dentin. Consequently, teeth extracted from patients of different ages may show variations in dentinal tubule density, which could affect the mechanical properties of the radicular dentin [5–7]. Thus, in this study, it would be more meaningful to compare the percentage change in microhardness since the microhardness values of radicular dentin before the irrigation agents were applied may differ. According to the results of this study, a reduction in microhardness values compared to the initial measurements was observed in all groups. Thus, the first hypothesis of this study has been accepted. This result is consistent with previous studies [5, 18, 19].

This study examined the effects of continuous and sequential irrigation procedures, during which sodium hypochlorite was applied to all samples to simulate clinical treatment methods. In the continuous chelation groups, PA and HEDP were mixed with NaOCl. In the sequential irrigation groups, EDTA, PA, and HEDP were applied for 2 min after NaOCl irrigation. The effect of NaOCl on changing microhardness and mineral content has been reported to be time and concentration-dependent [5, 20]. There is no consensus about the ideal NaOCl concentration and application duration into the root canal [20], but the 2.5% NaOCl concentration is preferred because of the balance between efficacy and reduced cytotoxicity [21]. It has been reported that NaOCl contacts root canal dentin for 10–240 min during endodontic treatment [22]. It was reported that 0.5% NaOCl exhibited antimicrobial properties for 30 min, 1% NaOCl for 10 min, 2.5% NaOCl for 5 min, and 5.25% NaOCl for 2 min [21]. However, Iandolo et al. [23] reported that NaOCl showed its tissue-dissolving properties at a concentration of 4.26% in 22 min and 5.97% in 16 min. As a result, considering the approximate shaping time, requiring irrigation duration to disinfection, and tissue dissolution in a single root canal clinically, all samples in this study were exposed to NaOCl for 20 min. It has been reported that applying NaOCl for less than 3 min after chelators in the final irrigation does not change the microhardness value [17]. Therefore, additional short-term NaOCl irrigation was not performed after chelator application.

According to the results of this study, a significant decrease in microhardness was observed in the control group, which received only the application of NaOCl. Similarly, previous studies have reported that NaOCl irrigation reduces microhardness [5, 18–20]. Our findings align with previous research, which indicates that the maximum reduction in microhardness occurs during the initial irrigation with NaOCl rather than during medication application or final irrigation [19]. Sodium hypochlorite can influence dentin microhardness by altering mineral content and degrading the collagen matrix. It has

been reported that the released chlorine interacts with the calcium and phosphate in hydroxyapatite, leading to demineralization [4]. Additionally, chlorine can degrade the collagen fibers in the dentinal matrix, which further weakens the dentin and may result in the loss of structural integrity [4, 5].

In our study, it was observed that sequential chelator application after long-term sodium hypochlorite application did not significantly alter the microhardness compared to the control group without chelating. In addition, no significant difference was found between 2.5% PA, 9% HEDP, and 17% EDTA in 2 min irrigation. This suggests that chelators do not cause further detrimental effects on dentin, whose microhardness has already decreased with 20 min of NaOCl application. Previous studies have reported that 17% EDTA reduces dentin microhardness by values ranging from 6 to 15.5% [24, 25]. In our study, EDTA was used following NaOCl irrigation instead of being used alone to better simulate actual clinical irrigation procedures. While the microhardness in the EDTA group showed a slight decrease compared to the group that did not use a chelator, this difference was not statistically significant. Similar to our result, Viapiana et al. [26] reported that irrigation with 1% NaOCl for 20 min followed by 17% EDTA for 5 min resulted in similar microhardness values to the group that irrigated with NaOCl and distilled water. This result was consistent with the result of a recent review that reported that 17% EDTA, when used alone for 2 min, did not cause statistically significant changes to the dentine's structure or flexural strength despite a reduction in the apatite/collagen ratio [5].

There are a limited number of studies evaluating the effect of phytic acid on the microhardness of root dentin, and discordant results have been reported among these studies. El Banna et al. [24] stated that 2.5% PA did not significantly reduce the microhardness of radicular dentin when compared to the saline group. Nikhil et al. [27] reported that 1% PA reduced dentin microhardness, but this reduction was lower than that in the EDTA group. Muana et al. [28] reported that the microhardness value obtained with 1% PA was significantly lower than 17% EDTA and 10% citric acid. Eymirli et al. [19] reported that 1% PA reduced microhardness more than EDTA when used in final irrigation after triple antibiotic paste removal, and similar results were obtained with EDTA when used after calcium hydroxide removal. In our study, sequential or continuous irrigation with PA resulted in a similar decrease in microhardness to that of the EDTA or control groups. Direct comparisons with previous studies are not possible due to methodological differences. In our study, no significant difference was found in terms of microhardness change between sequential or continuous application of PA.

The interesting outcome of this study was that the decrease in microhardness in the DR HEDP group was less than in the groups in which NaOCl was applied alone for 20 min. This suggests that mixing etidronic acid with sodium hypochlorite and applying it using the continuous chelation technique reduces the negative effect of long-term sodium hypochlorite use on dentin microhardness. A recent study found that sodium hypochlorite solutions lost approximately 5–7% of their initial available chlorine after the addition of HEDP [29]. The authors reported that, despite this decrease, the tissue-dissolving properties of the solutions remained unaffected; however, both the viscosity and surface tension of the mixture increased. Due to the decreased available chlorine, the interaction of NaOCl with hydroxyapatite may decrease, resulting in reduced mineral loss. Additionally, the change in the physical and the chemical properties of the mixture may have resulted in less detrimental effects of dentin microhardness. Contrary to our study, Ulusoy et al. [30] found that mixing 9% HEDP with 2.5% NaOCl or saline increases the dentin nano-hardness more than EDTA or NaOCl used alone or sequentially. There are some methodological differences between the studies. In the mentioned study, a more sensitive evaluation method (nano hardness) was used, but the NaOCl application time was limited to 2 min to evaluate the final irrigation process. However, in clinical practice, NaOCl is applied for a longer period during instrumentation before final irrigation. We assume that this difference between the results is due to the time-dependent effect of NaOCl on dentin hardness, and the hardness decrease may not have occurred within 2 min. Supporting this assumption, no difference in nano hardness was found between NaOCl and distilled water in that study. In contrast, in the present study, prolonged exposure of the dentin surface to NaOCl affected microhardness more than all chelators used. Supporting the current findings, El Banna et al. [24] reported that applying 18% HEDP (prepared with saline) for 5 min did not significantly reduce the microhardness of radicular dentin compared to the saline group. In addition, recent studies reported that HEDP has less detrimental effects than EDTA [5, 12, 31].

Although the root thirds were structurally different, the decrease in microhardness of the root thirds was similar between within-group comparisons. This result suggests that when subjected to the same irrigation regimen, the root thirds exhibited similar behavior, demonstrating that direct contact between the irrigating solution and the dentin surface leads to similar alterations despite differences in structure across regions [17]. In this study, microhardness tests were performed by applying irrigation solutions to split roots embedded in acrylic molds. This open system model does not accurately reflect clinical situations. It is important to note that the root canal

functions primarily as a closed-end system during irrigation. A closed apical foramen creates significantly more complex flow patterns, posing considerable barriers to the penetration of irrigation solutions compared to open systems [32]. The chemical effects of irrigation are determined by concentration, volume, and time. Meanwhile, flow rate and agitation intensity primarily influence the mechanical effects [33]. According to our findings, the similar decrease in microhardness values in the root thirds might be due to the similarity of parameters such as irrigation volume, concentration, flow rate, etc., applied to all regions.

A tight seal between the root canal walls and the obturation materials is essential to prevent the penetration of microorganisms and their products into the root canal from the oral or periradicular field [3]. Calcium silicate-based cement used as an apical barrier, retrograde filling, and repair material should exhibit adequate adhesion to the root canal dentin because they are exposed to mechanical forces during operative procedures and mastication. The push-out test assesses the bond strength of root-end filling materials and is considered reliable despite potential variable influences. It measures shear bond strength, with fractures occurring parallel to the cement-dentin interface [34]. The PBS test is essential for evaluating the bond strength of calcium silicate-based cement in root-end cavities, especially when the seal's integrity is crucial for the achievement of endodontic treatments. This methodology aims to determine the effectiveness and durability of materials in clinical applications by evaluating their resistance to mechanical forces [14].

The results of this study showed that the push-out bond strength of calcium silicate-based cement was affected by the type of chelator and the method of application. Therefore, the second hypothesis of the study was also accepted. The lowest PBS values were obtained in this study when samples were irrigated with 2.5% NaOCl during instrumentation and rinsed with distilled water. Paulson et al. [35] found similar results to this study and assumed that this was due to the low smear layer removal property of NaOCl reducing the binding of BB to the root canal dentin. In this study, the highest PBS value was obtained in the DR HEDP group. This is consistent with a previous study that reported that the PBS values obtained when DR HEDP was used were higher than those obtained by the sequential application of NaOCl and EDTA [35]. Consistent with these results, Neelakantan et al. [36] stated that DR HEDP, unlike the sequential use of EDTA, does not adversely affect the hydration properties of calcium silicate-based cement. Also, Ulusoy et al. [37] reported that combining HEDP with NaOCl had less detrimental effects on cement's adhesion and microstructure of MTA cement than the sequential use of strong chelators like EDTA. In contrast with the results of this study, a recent study reported that DR HEDP, EDTA, and saline did not significantly affect the PBS of MTA Angelus and NeoPutty applied to retrograde cavities after endodontic treatment [38]. However, the calcium silicate-based cements used in that study were different from those used in this study. Ballal et al. [39] reported that the effects of irrigation solutions on the bond strength of different calcium silicate-based cements were variable, and the authors noted that different types of chelators did not change the push-out bond strength when MTA was used but did when BD was used [39].

In this study, using phytic acid as a dual rinse or a sequential chelator after sodium hypochlorite improved PBS results compared to applying EDTA or distilled water after sodium hypochlorite. Contrary to our study, a recent study has reported that 15 min of NaOCl followed by 3 min of saline, 17% EDTA, and 1% PA had no significant effect on the PBS of BD [40]. This might be due to methodological differences, such as the use of lower concentrations of phytic acid in the mentioned study, the different thicknesses (1 mm height) of the prepared slices, and the root section from which the samples were obtained. In this study, a single 3 mm-high dentin slice was prepared from each root, and 2.5% phytic acid was used. Increasing the thickness of the calcium silicate-based cement plug might enhance the resistance to displacement by improving the contact surface between the cement and dentin. The required minimum thickness for apical plugs to ensure a sufficient seal and resistance against displacement is 3 mm; therefore, slices measuring 3 mm in thickness were used in this study [41].

The number of studies examining the bond strength of calcium silicate-based cement after etidronic acid or phytic acid irrigation is limited. In a study in which both solutions were applied as a surface conditioner, it was reported that the application of 2.5% PA increased the shear bond strength of BD to coronal dentin, authors also reported similar SBS values were obtained when 9% DR HEDP 17% EDTA and saline used [42]. Although a direct comparison of the results of these studies cannot be made due to the different microstructural properties of root and coronal dentin, similarly, phytic acid application increased the PBS of BD more than EDTA in our study. Apart from other methodological differences, we think that the long-term application of DR HDP (20 min) might have yielded better results than EDTA in our study because there was no significant difference found between HEDP mixed with distilled water and applied for 2 min and EDTA according to our results. De-Deus et al. [12] reported that 300 s is required for optimal results if etidronic acid is used as a final irrigant. In line with the study results of De-Deus et al. [12], this study has also shown that etidronic acid is a weak chelating agent that

should not be used for final irrigation, and the application duration is essential.

Previous studies have suggested that decalcifying agents can damage the structure and setting reactions of tricalcium silicate-based cement, potentially affecting the particle-binding hydration phases and hydration mechanisms of MTA [14, 37, 40]. However, BD has a smaller particle size and higher tubule penetration capacity than MTA. BD liquid, which contains calcium chloride, shortens the material's setting time and increases its mechanical properties [13]. Previous research has shown that BD has higher POBS than MTA, and the formation of a tag-like structure in the dentin tubules [40]. Therefore, we assume that removing the smear layer with chelating agents and exposing the tubules is important for improving the PBS of BD.

When push-out bond failure types were evaluated, mixed and cohesive failures were observed at similar rates, consistent with a previous study [40]. Adhesive-type fractures were rarely observed (at three specimens), consistent with studies reporting that BD bonds well to dentin via mineral tag formation [35, 40]. Different chelators or irrigation procedures applied did not affect the push-out bond failure type of BD [40].

In endodontic treatment, removal of the smear layer is recommended to disinfect the dentinal tubules and increase the bonding of endodontic sealers to the root canal walls [11]. The results of this study indicated that the effectiveness of chelators in removing the smear layer varied. Consequently, the third hypothesis of the study was accepted. According to the results of this study, the highest smear removal was observed in the phytic acid groups and DR HEDP group in all root thirds. Phytic acid is a highly negatively charged molecule with a high affinity for calcium and a high chelation ability [28]. Consistent with our findings, a recent literature review reported that PA at concentrations between 0.5% and 1% provided similar or better smear removal than 17% EDTA [43]. In a previous study, Puvvada et al. [44] reported that phytic acid has better smear layer removal efficiency when applied by mixing with NaOCl. However, unlike the results of Puvvada et al. [44], in this study, mixing phytic acid with NaOCl did not contribute to the smear removal ability. According to the results of our study, no difference was found between the smear removal efficiencies of the DR PA and DR HEDP groups. In contrast to these results, Sunanda et al. [45] reported that DR PA showed better chelating ability than DR HEDP. The inconsistency between the results of these studies and ours is due to differences in methodologies. Unlike in our study, the mentioned studies used only saline during instrumentation and employed chelators as final irrigants. These dual rinse chelators used during instrumentation in this study were applied to the root canals for a longer period of time. Therefore, the long-term application of etidronic acid, which is known to be a weak chelator, may have provided a better smear removal. On the other hand, in our study, etidronic acid used for 2 min showed similar scores to those of the control group and was not found effective in smear removal. Similar to our findings, it has been reported that etidronic acid prepared with distilled water, despite being sonically activated, removed less smear than EDTA and DR HEDP, and similar scores were obtained with the group without chelator application [46]. However, in the mentioned study, the effectiveness of DR HEDP and sequential EDTA was similar in terms of smear removal and sealer penetration. In contrast, our study found that EDTA was less effective at removing the smear layer. In our study, since 6 shaping files were used instead of 4 shaping files, a larger amount of irrigation solution was used, and the contact of the solutions with the root surfaces probably lasted longer. Studies reported that when DR HEDP is used during instrumentation, better values are obtained in removing debris accumulation from isthmuses or the penetration of root canal sealer than when sequential EDTA is used [10, 11, 47–49]. Considering the findings of this study and previous studies, it is a better method to apply etidronic acid long-term during instrumentation with a continuous chelation technique rather than applying it with a sequential chelation technique.

As in most previous studies, in our study, the smear removal percentage of irrigants decreased from coronal to apical. This could be attributed to the low tubular density in the apical region of the root canal. Intragroup evaluations, smear removal efficiency in the apical region was found to be lower than in the coronal region in the EDTA group and the DR PA group. This result is consistent with studies reporting that EDTA is unable to remove the smear layer in the apical region [4, 11, 50]. In the other groups, no difference was observed in the effectiveness of the smear layer removal at the root thirds.

This study had some limitations. There is no consensus on exactly how the final irrigation procedure should be performed. In this study, as in a recent publication, no irrigation with NaOCl was performed after the chelator application [46]. After rinsing the applied chelators in different procedures with distilled water, the effect on the bonding of calcium silicate-based cement and microhardness was investigated. However, we think it would be helpful to evaluate this situation separately in future studies.

The phytic acid and sodium hypochlorite mixture was included in the study because it was previously reported that using this mixture increased smear removal [44, 45]. Nassar et al. reported that when phytic acid was used at a concentration of 1%, the effervescent effect was low, and the chlorine in the solution was not depleted

[8]. However, when 2.5% phytic acid was used, exothermic reaction, bubbles, and gas formation were observed above expectations. Since the effects of the solutions on the dentin surface were evaluated in this study, the gas formed was not evaluated. It is estimated that this reaction involves a possible acid-base reaction and releases chlorine gas. This solution was prepared experimentally, but caution is needed regarding gas formation.

Another limitation of the present study was the technique used to treat the push-out bond strength test specimens with irrigants. The dentin sections were immersed entirely in irrigants, which does not directly simulate actual clinical conditions. Clinically, in apexification or endodontic treatment procedures, these solutions only come into contact with the inner surface of the root canal. To overcome this limitation, nail polish was applied to the axial portion of the dentin discs before irrigation was applied. Preparing samples from 3 mm sections rather than the entire root may seem distant from the clinical scenario, but this method simplifies the preparation of standard samples. Especially in teeth with oval-shaped canals, when the roots are instrumented and obturated, it is difficult to determine whether the prepared cavity covers the entire root canal wall. When the section is taken before shaping and obturation, it is easier to detect variations in root canal morphology in cross-sections by examining the root segment. Additionally, cutting the roots after obturation may compromise the bonding interface between calcium silicate cement and radicular dentin.

Finally, there were limitations in evaluating the remaining smear layer. SEM is frequently used in studies such as smear removal and morphological evaluation of the dentin surface, but it has limitations regarding reproducibility and accuracy [40, 44–47, 50]. Furthermore, it cannot provide an image of the entire canal because it only allows for assessing limited areas of the canal lumen. However, using study methods by a trained technician and scoring with an objective process, such as calculating the remaining smear layer area, helped minimize discrepancies related to the standardization and reproducibility of the methods, such as those related to the area selection of the samples, in this study.

## Conclusion

Within the limitations of this in vitro study, the following conclusions can be drawn from the results:

1. Etidronic acid mixed with sodium hypochlorite and used as a continuous chelator during root canal instrumentation had the least detrimental effect on root dentin, while it had a positive effect on the smear removal and bonding of calcium silicate-based cement.
2. Short-term use of etidronic acid as a sequential chelator is not sufficiently effective.
3. It has been observed that mixing phytic acid with sodium hypochlorite and using it during instrumentation procedures does not provide any additional benefit in the methods evaluated in this study.
4. Clinically, the use of etidronic acid with the continuous chelation technique and phytic acid with the sequential chelation technique may be an alternative method to sequential EDTA irrigation.

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**Author contributions**
The methodology was prepared by E.H. and A.H.; the investigation was conducted by E.H.; the experiments were carried out by E.H. and A.H.; the original draft preparation was done by E.H.; the writing, including review and editing, was handled by E.H. and A.H.; visualization was completed by A.H.; supervision was provided by E.H. All authors read and approved the final manuscript.

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**Data availability**
All data generated or analysed during this study are included in this published article.

## Declarations

**Ethics approval and consent to participate**
The study was conducted in accordance with the Declaration of Helsinki and approved by the Non-Interventional Clinic Research Ethics Committee of Zonguldak Bülent Ecevit University (2024/22, 11.12.2024). The consent that was obtained from all of the participants was informed.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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