10.5937/bjdm2602082E

BALKAN JOURNAL OF DENTAL MEDICINE

BaSS

ISSN 2335-0245

Effect of Different Irrigation Solutions on the Push-Out Bond Strength of Bioceramic and Resin-Based Root Canal Sealers: An in vitro Study

Büşra Efendioğlu,[1] Elif Bahar Çakıcı,[1] Hasan İlhan[2]

[1] Department of Endodontics, Faculty of Dentistry, Ordu University, Ordu, Turkey
[2] Biotechnology Institute, Department of Biotechnology, Ankara University, Ankara, Turkey

SUMMARY

Background: *The aim of this study was to compare the effect of continuous chelation using Dual Rinse hydroxyethylidene diphosphonate (HEDP) as a single irrigant with 17% ethylenediaminetetraacetic acid (EDTA) and 0.2% chitosan on the push-out bond strength of AH Plus and Bio-C root canal sealers to root dentin.* ***Materials and Methods:*** *One hundred twenty single-rooted mandibular premolars were standardized to 17 mm and randomly assigned to three irrigation groups and six sealer subgroups (EDTA–AH Plus/Bio-C; HEDP–AH Plus/Bio-C; chitosan–AH Plus/Bio-C). Root canals were prepared with a reciprocating NiTi system. Final irrigants were ultrasonically activated for 1 min. Obturation was performed with AH Plus or Bio-C using cold lateral compaction. After storage (37 °C, 100% humidity, 1 week), 1 ± 0.1 mm slices were obtained from apical, middle, and coronal thirds. Push-out testing was performed at 1 mm/min. Failure modes were examined under a stereomicroscope. Data were analyzed using parametric or nonparametric tests ($p < 0.05$).* ***Results:*** *AH Plus demonstrated significantly higher bond strength than Bio-C ($p = 0.041$). No significant difference was found among irrigation protocols when sealer type was disregarded ($p = 0.086$). Cohesive failure predominated (69.2%), and failure mode distribution differed significantly among groups ($p < 0.001$).* ***Conclusions:*** *Continuous chelation using Dual Rinse HEDP as a single irrigant demonstrated similar push-out bond strength compared with 17% EDTA and 0.2% chitosan. This approach may serve as an alternative to conventional multi-step protocols by potentially reducing clinical chair time and simplifying treatment steps.*

Keywords: Push-out bond strength; root canal sealer; HEDP; EDTA; chitosan

ORIGINAL PAPER (OP)
Balk J Dent Med, 2026;82-93

Introduction

Continuous chelation with HEDP may simplify irrigation protocols by eliminating the need for a separate chelating step and show similar bond strength to conventional multi-step irrigation regimens, reducing chair time and procedural complexity, thereby representing a clinically viable alternative in endodontic practice.

The long-term success of endodontic treatment depends not only on the effective cleaning of the root canal system but also on the ability of obturation materials to achieve adequate and stable adhesion to root dentin[1,2]. During mechanical preparation, the smear layer formed by the accumulation of organic and inorganic debris on the dentin surface occludes the dentinal tubules, thereby limiting the contact and penetration of filling materials into dentin[3]. Therefore, chelating agents are used during the chemomechanical preparation process to remove the smear layer. EDTA is one of the most commonly used chelating agents that enhances cleaning efficacy by dissolving the inorganic components of the smear layer[4]. However, its potential to cause dentin erosion and to reduce the effectiveness of sodium hypochlorite (NaOCl) has increased interest in alternative agents[5,6]. Recently, Dual Rinse® HEDP (Medcem GmbH, Weinfelden, Switzerland), also known as etidronic acid (1-hydroxyethylidene-1,1-diphosphonic acid), has been introduced to the market. Dual Rinse HEDP is a mild chelating agent that can be used in the same solution with NaOCl and provides "continuous chelation" during instrumentation, thereby simultaneously controlling the formation of the smear layer[7–9]. This approach simplifies the clinical protocol by eliminating the need for sequential irrigation and provides time efficiency for the clinician by shortening the procedure time through its application as a single solution. Furthermore, its chemical compatibility with NaOCl contributes to maintaining efficacy throughout the irrigation process. In addition to synthetic chelators, biologically derived agents are also being evaluated as alternative chelating agents; one of these is chitosan. Chitosan stands out as an alternative chelating agent due to its biocompatibility, biodegradable structure, and antibacterial properties; it has been reported to be effective in removing the smear layer through its capacity to bind calcium[10,11]. The chemical and morphological changes induced on the dentin surface by irrigation protocols can directly affect the bond strength of root canal sealers to dentin[12]. It has been reported that different irrigation strategies modify dentin surface characteristics in distinct ways and, accordingly, may result in variable bonding outcomes depending on the chemical composition of the sealer used[13]. The push-out bond strength test is frequently preferred for evaluating this adhesion and is considered a reliable method due to its ability to provide a stress distribution more closely resembling clinical conditions[14–17].

This study aimed to comparatively evaluate the bond strength of root canal sealers (AH Plus and Bio C) to root dentin following the use of different irrigation solutions (EDTA, Dual Rinse HEDP, and chitosan) in root canal treatment.

The hypotheses of the study were:

H_{01}: There is no difference between the push-out bond strengths of AH Plus and Bio-C root canal sealers to dentin in samples irrigated with 17% EDTA.

H_{02}: There is no difference between the push-out bond strengths of AH Plus and Bio-C root canal sealers to dentin in samples irrigated with Dual Rinse HEDP.

H_{03}: There is no difference between the push-out bond strengths of AH Plus and Bio-C root canal sealers to dentin in samples irrigated with 0.2% chitosan.

H_{04}: In samples obturated with AH Plus root canal sealer, different irrigation solutions (17% EDTA, Dual Rinse HEDP, and 0.2% chitosan) have no effect on push-out bond strength to dentin.

H_{05}: In samples obturated with Bio-C root canal sealer, different irrigation solutions (17% EDTA, Dual Rinse HEDP, and 0.2% chitosan) have no effect on push-out bond strength to dentin.

Materials and Methods

Ethical Approval

Ethical approval for this study was obtained from the Non-Interventional Scientific Research Ethics Committee of Ordu University, dated 09.05.2025 and numbered 2025/165.

Sample Size Calculation

A power analysis was performed using G*Power 3.1.9.7 (Heinrich Heine University Düsseldorf, Germany). Based on a significance level of 5% ($\alpha = 0.05$) and a test power of 80% ($1–\beta = 0.80$), with an effect size of Cohen's f = 0.34, which was determined based on the available literature as reported by Veeramachaneni et al.[18], the minimum total sample size was calculated as 120 teeth for six experimental groups consisting of three main groups, each with two subgroups. Accordingly, 20 teeth were allocated to each group.

Selection and Preparation of Samples

In this study, 120 extracted human mandibular premolar teeth with completed apical development and single roots were used. Teeth extracted for periodontal or orthodontic reasons were included in the study. The teeth were obtained through the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Ordu University. The teeth used in the study were collected after providing the necessary information to the patients and obtaining their written informed consent. The teeth were stored in 10% formalin solution at room temperature until the experiment was conducted.

According to radiographic evaluation, teeth with root canals exhibiting a curvature greater than 5 degrees based on Schneider's classification, those with more than one canal, previously root canal–treated teeth, teeth with canal calcifications, external or internal root resorption, and teeth with restorations on the root surface were excluded from the study.

The teeth were trimmed to a standardized length of 17 mm using a diamond disc (Horico, Berlin, Germany).

The access cavities of the specimens, whose lengths were standardized by trimming, were prepared under water cooling using a high-speed handpiece. Initial canal patency was verified with a #10 stainless steel

K-file (Dentsply Maillefer, Ballaigues, Switzerland). The working length was established by subtracting 1 mm from the length at which the file tip was observed at the apical foramen. To simulate clinical conditions, the apices of all teeth were sealed with pink wax to prevent irrigant extrusion through the apex and to allow effective backflow of the irrigant, thereby creating a closed-end system.

Shaping of the Root Canals

Following pre-flaring of the root canals up to a size 20 K-file (Dentsply Maillefer, Ballaigues, Switzerland), each tooth specimen was prepared using the T-Endo Must 25/.06 and 40/.04 (Dentac, Istanbul, Turkey) NiTi reciprocating file system. Instrumentation was performed in reciprocation mode at 150° counterclockwise (CCW) and 30° clockwise (CW) angles, in accordance with the manufacturer's instructions, using the crown-down technique.

Experimental Design and Group Allocation

The teeth were first numbered and then randomly assigned, using the website (http://www.random.org), into three experimental groups according to the irrigation protocol and further subdivided into six subgroups based on the two different sealers used (AH Plus and Bio-C).

Group IA : Irrigation with NaOCl + EDTA, AH Plus root canal sealer
Group IB : Irrigation with NaOCl + EDTA, Bio-C root canal sealer
Group IIA : Irrigation with NaOCl/Dual Rinse HEDP, AH Plus root canal sealer
Group IIB : Irrigation with NaOCl/Dual Rinse HEDP, Bio-C root canal sealer
Group IIIA : Irrigation with 0.2% chitosan, AH Plus root canal sealer
Group IIIB : Irrigation with 0.2% chitosan, Bio-C root canal sealer

Groups IA, IB: During instrumentation, three pecking motions were performed with each file until the working length was reached. Subsequently, the canals were irrigated with 2 mL of 5.25% NaOCl (Imicryl, Konya, Turkey). Throughout this procedure, a 30-G side-vented irrigation needle (Fanta Dental, Shanghai, China) was used. The needle tip was positioned 2 mm short of the working length and moved in an in-and-out motion. While the syringe was moved in an apico-coronal direction, NaOCl was delivered passively and without pressure into the root canals. After completion of mechanical preparation, final irrigation was performed with 5 mL of 5.25% NaOCl (Imicryl, Konya, Turkey), followed by rinsing with 5 mL of distilled water. Subsequently, final irrigation was performed with 5 mL of 17% EDTA (Microvem, Istanbul, Turkey) for 1 minute.

Groups IIA, IIB: The root canals were prepared using the same instrumentation protocol as described for Group I; however, Dual Rinse HEDP (Medcem, Weinfelden, Switzerland) was used as the sole irrigating solution. The NaOCl/Dual Rinse HEDP solution was freshly prepared according to the manufacturer's instructions by dissolving 0.9 g of Dual Rinse HEDP powder (average content per capsule) in 10 mL of 5.25% NaOCl. The solutions were freshly prepared immediately before treatment. The root canals were irrigated with 2 mL of NaOCl/Dual Rinse HEDP at each instrument change using a 30-G side-vented irrigation needle (Fanta Dental, Shanghai, China), positioned 2 mm short of the working length. After completion of mechanical preparation, final irrigation was performed with 10 mL of NaOCl/Dual Rinse HEDP.

Groups IIIA, IIIB: The root canals were shaped according to the same protocol described for Group I. Following each instrument change, the canals were irrigated with 2 mL of 5.25% NaOCl (Imicryl, Konya, Turkey). Irrigation was performed using a 30-G side-vented needle (Fanta Dental, Shanghai, China). The needle tip was positioned 2 mm short of the working length and moved in an in-and-out motion within the canal. NaOCl solution was delivered passively into the root canals by moving the syringe in an apico-coronal direction. After completion of mechanical preparation, final irrigation was performed with 5 mL of 5.25% NaOCl (Imicryl, Konya, Turkey), followed by rinsing with 5 mL of distilled water. Subsequently, irrigation was performed with 5 mL of 0.2% chitosan solution for 3 minutes.

In all groups, activation of the irrigating solutions was performed using an ultrasonic tip and an ultrasonic device (D600 LED, DTE, Woodpecker, Guilin, China). The ultrasonic tip was positioned 1 mm short of the working length and operated without contacting the canal walls. For each irrigating solution, activation was carried out for a total duration of 1 minute, consisting of three 20-second activation cycles. The irrigating solution was refreshed after each cycle. To prevent the continued activity of the solutions and to standardize the duration of their effect, all root canals were irrigated with 5 mL of distilled water. The canals were then dried with paper points. After drying, in the A subgroups, AH Plus (Dentsply DeTrey, Konstanz, Germany) root canal sealer was mixed according to the manufacturer's instructions. Following sealer application to the canal walls, an R40 master cone was placed into the canal at the working length. The canals were obturated using the cold lateral condensation technique.

In the B subgroups, the injectable bioceramic sealer Bio-C (Angelus, Londrina, PR, Brazil) was delivered into the root canals using syringe tips according to the manufacturer's instructions. An R40 master cone was then placed to working length in the sealer-filled canal, and obturation was completed using the cold lateral condensation technique. Excess gutta-percha was removed with a heated hand instrument.

The canals were sealed with a temporary filling material, Cavit (Prevest DenPro Ltd., Jammu, India). The specimens were then stored in an incubator at 37 °C under 100% humidity in a dark environment for 7 days to allow complete setting of the root canal sealer.

Preparation of the Specimens for the Push-Out Test

The specimens removed from the incubator were embedded in cold-curing acrylic resin using polyvinyl chloride molds. To eliminate the potential influence of anatomical variations such as apical ramifications and lateral canals on the test outcomes, the apical 3 mm of the roots was first removed using a precision cutting device (Mecatome T180, Presi Metallography, Eybens, France). Subsequently, under water cooling, a precision cutting device (Mecatome T180, Presi Metallography, Eybens, France) equipped with a 0.5-mm-thick diamond disc (Presi, France) was used to obtain three horizontal sections from each specimen, each representing the coronal, middle, and apical thirds, with an approximate thickness of 1 ± 0.1 mm. Each section was marked on its apical surface to facilitate proper positioning during the push-out test. The apical and coronal diameters of the canal lumen in each dentin disc were measured using a digital caliper (Mitutoyo Corp., Kanogawa, Japan), which was also used to verify the thickness of each section.

Push-Out Test

The bond strength of the 360 prepared sections was tested using a universal testing machine (AGS-X, Shimadzu Corporation, Tokyo, Japan). The obtained sections were positioned on a apparatus with an underlying space to allow displacement of the root canal filling material during the push-out test. During the test, care was taken to ensure that the plunger tips contacted only the root canal filling material without touching the dentin. Due to the conical geometry of the root canal, the dentin discs were positioned with the apical surface facing upward to prevent binding of the filling material within the canal during testing, and the load was applied in an apical-to-coronal direction. A compressive load was applied at a constant crosshead speed of 1 mm/min until displacement of the root canal filling occurred. The load was continuously applied at 1 mm/min until debonding of the filling material. The maximum force recorded at the moment of separation was measured in Newtons (N) using TRAPEZIUM 2 software (Shimadzu Co., Kyoto, Japan). The obtained force values (N) were divided by the bonded surface area of the corresponding slices to calculate the bond strength in megapascals (MPa).

The following formula was used for the calculation:

Push-out bond strength (MPa) = Maximum force (N) / Root canal filling area (mm²)

Bonded area of the root canal filling (mm²) = $[\pi\ (R\ +\ r)]\ \times\ [(R\ -\ r)^2\ +\ h^2]^{\frac{1}{2}}$

Where:
π: 3.14
R: Coronal radius of the root canal filling
r: Apical radius of the root canal filling
h: Thickness of the slice

Analysis of Failure Modes After the Push-Out Test

Macroscopic evaluation of the specimens was performed using a Nikon SMZ25 stereomicroscope (Nikon Instruments Inc., Tokyo, Japan). Images were obtained at 1× and 2× magnifications using a macro lens attached to the device. High-resolution images were captured via the digital camera integrated into the microscope and transferred to a computer using the manufacturer's software for analysis.

After the push-out test, the specimen codes were concealed, and the samples were visually examined under a stereomicroscope by two independent observers to analyze the failure modes. Interobserver agreement was assessed using Cohen's kappa coefficient (κ). In cases of disagreement between the observers, a third observer was consulted to determine the final failure mode.

The evaluated failure modes were classified as follows:

Adhesive Failure: Separation occurring at the interface between the root canal sealer and dentin.

Cohesive Failure: Separation occurring within the root canal sealer itself or within the dentin.

Mixed Failure: A type of failure in which both adhesive and cohesive separation are observed simultaneously.

The experimental procedure steps of the study are presented in Figure 1.

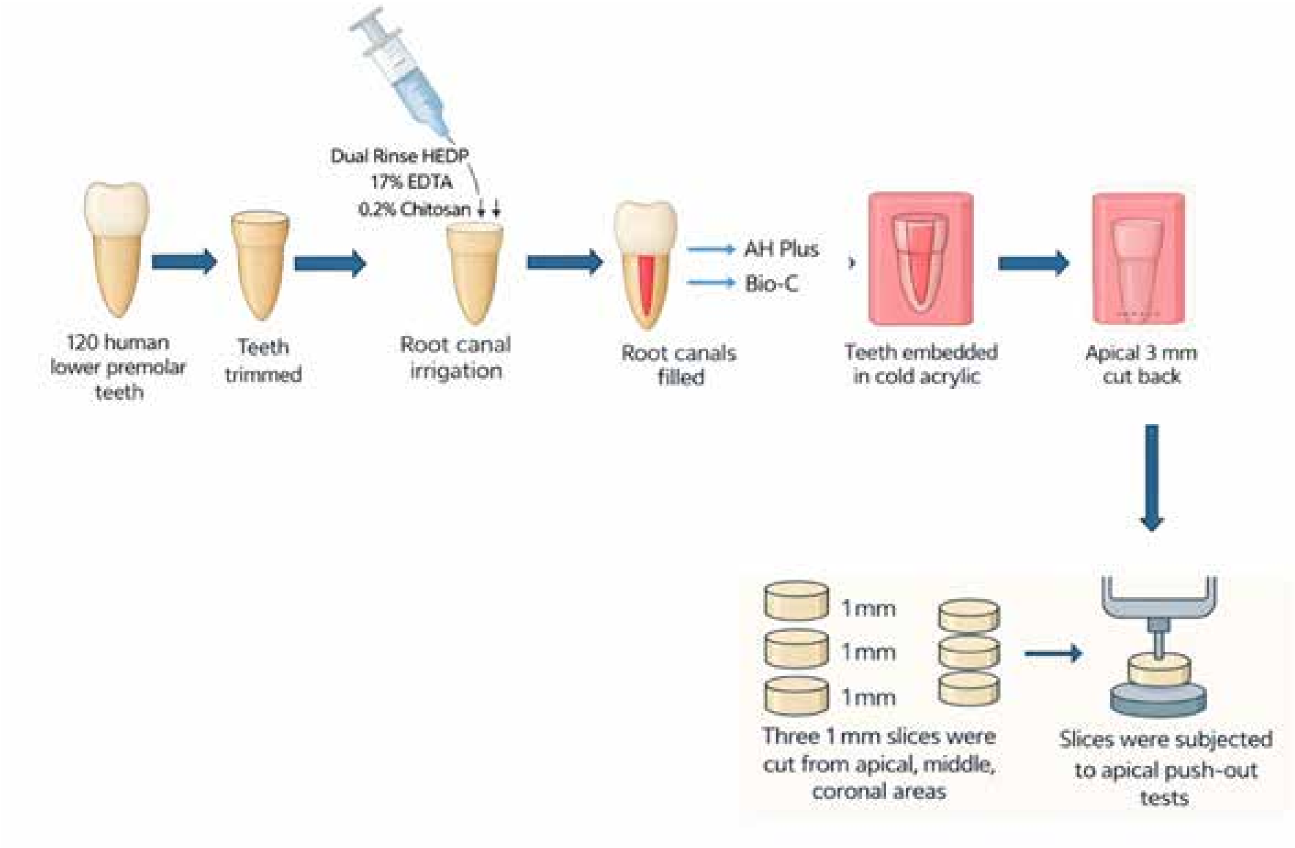

Figure 1. Experimental Procedure Steps of the Study

Blinding Procedure

The tooth samples were numbered by the principal investigator (E.B.Ç.), and access cavities were prepared by the operator (B.E.). The specimens were then randomized using the website Random.org into three experimental groups according to the irrigation protocol and further divided into six subgroups based on the root canal sealer to be used (AH Plus or Bio-C).

Following randomization, the teeth were delivered to the operator (B.E.), who was informed only of the irrigation protocol required for mechanical preparation to ensure procedural standardization. After instrumentation and irrigation, all specimens were returned to the principal investigator (E.B.Ç.).

Thereafter, the operator (B.E.) remained blinded to group allocation. According to the randomization results, the principal investigator (E.B.Ç.) assigned the sealer type and provided the specimens to the operator using coded identifiers only. Consequently, the operator performed root canal obturation without knowledge of the experimental groups, minimizing operator-related bias.

The push-out bond strength test was also performed under blinded conditions by the operator (B.E.). The test results, identified only by tooth codes, were transferred to the principal investigator (E.B.Ç.) and subsequently forwarded to an independent researcher for statistical analysis without revealing group allocation, thereby minimizing bias during data analysis.

Statistical Analysis

Push-out bond strength and fracture pattern data were analyzed using SPSS 31.0 software. Descriptive statistics were reported. The distribution characteristics of the data were assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated with Levene's test. For groups demonstrating normal distribution and homogeneity of variance, parametric analyses were performed using one-way ANOVA, and multiple comparisons were conducted with the Tukey HSD test. For data that did not demonstrate normal distribution, non-parametric tests were preferred, and the Kruskal–Wallis test and Mann–Whitney U test were applied. The distribution of fracture types among groups was evaluated using the chi-square test. When a statistically significant difference was detected, pairwise comparisons were performed with Bonferroni correction to determine which groups were responsible for the observed difference. For all statistical analyses, the level of significance was set at $p < 0.05$.

Results

Study Flow Diagram

A total of 148 human permanent mandibular premolar teeth were initially included in this study. Upon examination, 17 teeth were excluded for not meeting the inclusion criteria. During the mechanical preparation phase, 7 teeth were eliminated in total due to file fracture in 4 teeth and failure to reach the apical level in 3 teeth. At the sectioning stage, 2 teeth were excluded because equal-thickness slices could not be obtained as a result of disc fracture. During the push-out test, 2 teeth were excluded due to mechanical malfunction.

Consequently, the final number of teeth included in the study was determined to be 120 (Figure 2).

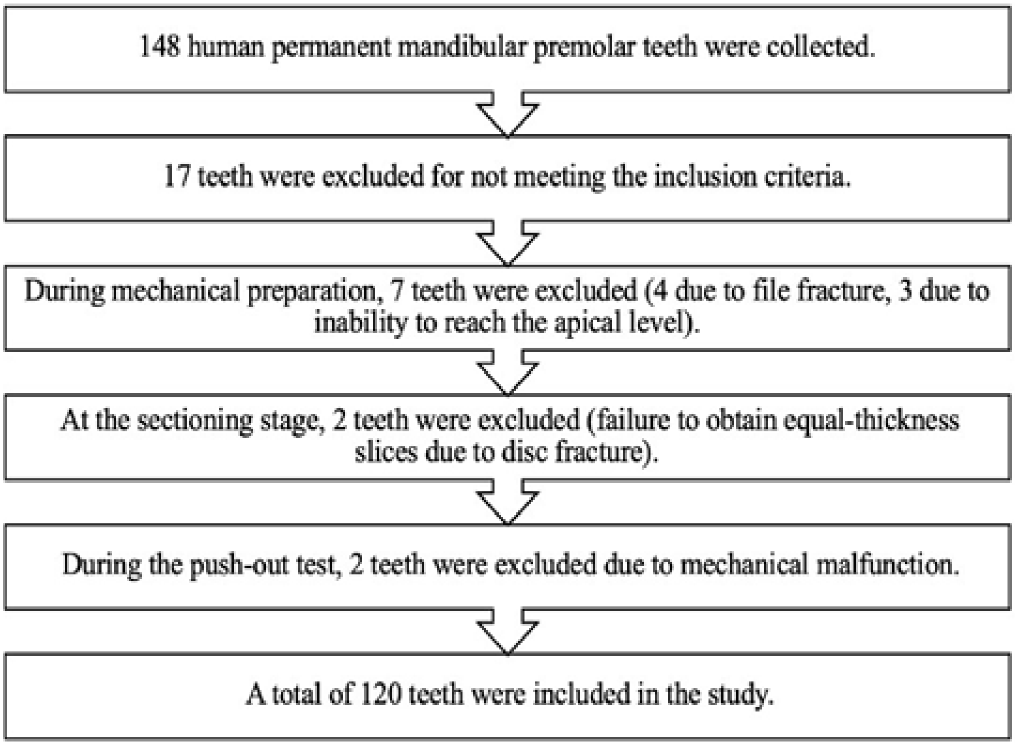

Figure 2. Study Flow Diagram

According to the statistical analysis, the Kruskal–Wallis test was used, and no statistically significant difference was found among the groups (p = 0.086) (Table 1).

Table 1. Comparison of Bond Strength Values (MPa) Among Experimental Groups

Groups	**Bond Strength Value Mean ± SD (MPa)**	**Median (Min–Max) (MPa)**
IA 17% EDTA (AH Plus)	3.56 ± 2.63	3.19 (0.22–14.57)
IB 17% EDTA (Bio-C)	2.86 ± 2.48	1.93 (0.05–8.40)
IIA Dual Rinse HEDP (AH Plus)	3.47 ± 1.45	3.55 (0.32–6.22)
IIB Dual Rinse HEDP (Bio-C)	3.67 ± 2.59	3.59 (0.10–9.51)
IIIA 0.2% Chitosan (AH Plus)	3.69 ± 1.59	3.43 (0.16–6.76)
IIIB 0.2% Chitosan (Bio-C)	3.30 ± 2.69	2.68 (0.07–10.71)
Total (n = 360)	**3.42 ± 2.29**	**3.17 (0.05–14.57)**

**p = 0.086 (Kruskal–Wallis test)*

The Dual Rinse HEDP group exhibited the highest mean bond strength value compared to the other groups; however, the Kruskal–Wallis test revealed no statistically significant difference among the groups ($p = 0.086$) (Table 2).

Table 2. Comparison of Bond Strength Values (MPa) Among Irrigation Solutions, Independent of Sealer Type

Irrigation Solution	Mean ± SD (MPa)	Median (Min–Max) (MPa)
Dual Rinse HEDP (n = 120)	3.57 ± 2.09	3.57 (0.10–9.51)
17% EDTA (n = 120)	3.21 ± 2.57	2.65 (0.05–14.57)
0.2% Chitosan (n = 120)	3.50 ± 2.21	3.26 (0.07–10.71)
Total (n = 360)	**3.43 ± 2.29**	**3.16 (0.05–14.57)**

**p = 0.086 (Kruskal–Wallis test)*

According to the results of the Mann–Whitney U test, which compared the bond strength values of the root canal sealers, a statistically significant difference was found between the AH Plus and Bio-C groups ($U = 14186.00$, $Z = -2.04$, $p = 0.041$) (Table 3).

Table 3. Comparison of Bond Strength Values (MPa) Between Sealer Types, Independent of Irrigation Solution

Root Canal Sealer	n	Mean ± SD (MPa)	Median (Min–Max) (MPa)
AH Plus	180	3.57 ± 1.95	3.43 (0.15–14.57)
Bio-C	180	3.27 ± 2.59	2.64 (0.05–10.71)
Total	**360**	**3.42 ± 2.29**	**3.17 (0.05–14.57)**

**p = 0.041 (Mann–Whitney U test)*

In the evaluation according to sectional regions, the HEDP + AH Plus and Chitosan + AH Plus groups showed statistically significant differences in bond strength values depending on the section ($p < 0.05$). In these groups, the bond strength values in the coronal region were found to be significantly higher than those in the apical region. No significant differences were observed among the sectional regions in the other groups ($p > 0.05$) In the intergroup comparison according to root regions, no statistically significant difference was found in bond strength values among the coronal, middle, and apical regions ($p > 0.05$) (Table 4).

Table 4. Intra- and Intergroup Comparisons of Push-Out Bond Strength Across Root Canal Regions

Group	p Value (Intragroup)
EDTA + AH Plus	0.659
EDTA + Bio-C	0.613
HEDP + AH Plus	0.008*
HEDP + Bio-C	0.613
Chitosan + AH Plus	0.007*
Chitosan + Bio-C	0.922
Region	**p Value (Intergroup)**
Coronal	0.126
Middle	0.059
Apical	0.061

**Statistically significant (p < 0.05)*

Overall, the cohesive fracture type was predominantly observed. The proportion of cohesive fractures ranged approximately between 55% and 83% across the various combinations, reaching the highest levels particularly in the 0.2% Chitosan + AH Plus, Dual Rinse HEDP + AH Plus, and 17% EDTA + AH Plus groups. Adhesive fracture rates remained at the lowest levels in all groups. No adhesive fracture was observed in the Dual Rinse HEDP + AH Plus group, whereas in the other groups the rates varied approximately between 1% and 23%.

The highest proportion of mixed fracture type was observed in the Dual Rinse HEDP + Bio-C group, while the lowest rate was recorded in the 17% EDTA + AH Plus combination (Table 5).

Table 5. Distribution of Failure Modes Across Experimental Groups

Fracture Type	17% EDTA + AH Plus	17% EDTA + Bio-C	Dual Rinse HEDP + AH Plus	Dual Rinse HEDP + Bio-C	0.2% Chitosan + AH Plus	0.2% Chitosan + Bio-C
Adhesive	9 (15.0%)	14 (23.3%)	0 (0.0%)	7 (11.7%)	1 (1.7%)	10 (16.7%)
Cohesive	43 (71.7%)	33 (55.0%)	49 (81.7%)	36 (60.0%)	50 (83.3%)	38 (63.3%)
Mixed	8 (13.3%)	13 (21.7%)	11 (18.3%)	17 (28.3%)	9 (15.0%)	12 (20.0%)
Total (n)	60	60	60	60	60	60

Inter-observer agreement regarding fracture type evaluation was assessed using Cohen's Kappa coefficient, and the agreement was found to be at an excellent level ($\kappa = 0.971$; $p < 0.001$). The relationship between fracture type and irrigation solution/root canal sealer combinations was evaluated using the chi-square test. The analysis revealed a statistically significant difference among the groups ($\chi^2 = 31.803$, $df = 10$, $p < 0.001$).

Following this result, pairwise comparisons were performed to determine which groups contributed to the observed difference. Pairwise chi-square comparisons were evaluated using Bonferroni correction, and it was found that the Dual Rinse HEDP + AH Plus group showed a statistically significant difference in fracture type distribution compared to the 17% EDTA + Bio-C and 0.2% Chitosan + Bio-C groups ($p < 0.0033$). No significant differences were detected among the other combinations.

Discussion

In the samples irrigated with 17% EDTA, no statistically significant difference was found between the push-out bond strength values of AH Plus and Bio-C root canal sealers to dentin; therefore, the H_{01} hypothesis was not rejected.

In the samples irrigated with Dual Rinse HEDP, no statistically significant difference was observed between the push-out bond strength values of AH Plus and Bio-C root canal sealers to dentin; therefore, the H_{02} hypothesis was not rejected.

In the samples irrigated with 0.2% chitosan, no statistically significant difference was detected between the push-out bond strength values of AH Plus and Bio-C root canal sealers to dentin; therefore, the H_{03} hypothesis was not rejected.

In the samples obturated with the AH Plus root canal sealer, different irrigation solutions (17% EDTA, Dual Rinse HEDP, and 0.2% chitosan) did not have a statistically significant effect on the push-out bond strength to dentin; therefore, the H_{04} hypothesis was not rejected.

In the samples obturated with the Bio-C root canal sealer, different irrigation solutions (17% EDTA, Dual Rinse HEDP, and 0.2% chitosan) did not have a statistically significant effect on the push-out bond strength to dentin; therefore, the H_{05} hypothesis was not rejected.

Researchers have reported that etidronic acid does not increase dentin surface roughness to the same extent as EDTA; however, by increasing surface energy, it enhances dentin wettability and improves micromechanical interlocking[19].

Accordingly, although not statistically significant, higher bond strength values have been reported in groups treated with etidronic acid compared to EDTA[20,21]. Similarly, in another study, higher, yet not statistically significant, bond strength values were reported in the group in which Dual Rinse HEDP was used compared with the EDTA group[22]. In parallel with these findings, the present study demonstrated that the continuous chelation approach (Dual Rinse HEDP) did not show a statistically significant difference in push-out bond strength values when compared with the conventional EDTA protocol. These results indicate that the single-solution continuous chelation protocol is as effective as the traditional NaOCl + EDTA regimen in terms of bond strength.

In a meta-analysis, AH Plus was reported to provide higher dislocation resistance than bioceramic sealers in all root regions. In accordance with these findings, the present study demonstrated that, independent of the irrigation solution, AH Plus exhibited significantly higher bond strength compared with Bio-C[23].

Since EDTA is a strong calcium chelator, it may negatively affect the hydration and mechanical properties of calcium silicate–based sealers by removing Ca^{2+} ions[24]. In line with this, the lowest bond strength value in the present study was observed in the group in which 17% EDTA was used as the final irrigant and Bio-C sealer was applied; however, this difference was not statistically significant.

Conflicting results have been reported in the literature regarding the effects of chitosan and EDTA on bond strength. Some studies have demonstrated that chitosan provides higher bond strength than EDTA in resin-based sealers[25,26], whereas other studies have reported that EDTA is superior or that no significant difference exists between the two solutions[27,28]. It has been reported that 0.2% chitosan resulted in higher bond strength values than EDTA when used with BioRoot RCS. Similarly, in the present study, 0.2% chitosan exhibited higher bond strength values than 17% EDTA in both sealer groups; however, this difference was not statistically significant[29].

In the present study, higher bond strength values were observed in the coronal region in the Dual Rinse HEDP – AH Plus and 0.2% Chitosan – AH Plus groups. This finding is consistent with the literature reporting that the reduced number and diameter of dentinal tubules in the apical region limit sealer penetration and micromechanical retention[30,31].

It has been demonstrated that bioceramic-based root canal sealers require approximately one week to complete their setting process[32]. Accordingly, in the present study, samples obturated with bioceramic sealers using the lateral condensation technique were stored at 37 °C and 100% humidity for 7 days to ensure complete setting of the sealers.

Studies have shown that an increase in temperature can affect the physical properties of bioceramic-based root canal sealers, particularly their flowability and setting time[33,34]. Therefore, in order to minimize technique- and operator-related variability in both sealer groups, the same obturation technique-cold lateral condensation-was preferred.

To minimize the frictional effect, the use of 1-mm-thick dentin discs has been considered more reliable[35,36]. In the present study, a total of three dentin slices, each 1 mm thick, were obtained from the apical, middle, and coronal regions of the roots.

It has been reported that selecting a plunger diameter corresponding to 70–90% of the canal diameter increases the accuracy of the push-out test[37]. Accordingly, based on the dentin slices measured in the pilot study, appropriate plunger diameters were determined for the apical, middle, and coronal regions.

A study has reported that cohesive failures in push-out tests occur within the material due to the strong adhesion of sealers to dentin and gutta-percha[38]. In the present study, cohesive fracture types were predominant in all groups. The high incidence of cohesive fractures, particularly in the AH Plus groups, may indicate the strong bonding ability of this sealer to dentin.

In the pairwise comparisons, only the Dual Rinse HEDP + AH Plus group was found to differ significantly from the 17% EDTA + Bio-C and 0.2% Chitosan + Bio-C groups in terms of fracture type distribution. This result suggests that

HEDP, due to its chelating effect and its advantage of simultaneous use with NaOCl, modifies the dentin structure in a more controlled manner and, particularly when combined with AH Plus, results in more consistent and predominantly cohesive fracture patterns.

A limitation of the present study is that the coronal, middle, and apical slices obtained from the same tooth were analyzed as individual specimens. Although this approach has been adopted in several previous push-out bond strength studies, the potential within-tooth correlation was not accounted for, which may have influenced the estimation of statistical significance. Future studies employing statistical models that account for clustered observations are therefore recommended.

Since this study was conducted under in vitro conditions, the obtained push-out bond strength results may not fully reflect the biological and mechanical factors present in the clinical environment. Furthermore, although the use of extracted single-rooted mandibular premolars enhanced standardization, the findings cannot be readily generalized to other tooth types with more complex root canal anatomies. Moreover, push-out bond strength testing alone cannot directly predict the clinical performance of root canal sealers. Therefore, further well-designed in vivo studies are required to validate these findings under clinical conditions.

Conclusion:

Within the limitations of this in vitro study, no statistically significant differences were observed among the irrigation solutions tested. Continuous chelation using Dual Rinse HEDP as a single irrigant demonstrated bond strength values comparable to those obtained with conventional 17% EDTA and 0.2% chitosan. These findings suggest that Dual Rinse HEDP may be a promising alternative to conventional multi-step irrigation protocols; however, further in vivo and clinical studies are required to confirm its clinical applicability.

References

1. Ferracane JL. Developing a more complete understanding of stresses produced in dental composites during polymerization. Dental Materials [homepage on the Internet] 2005 [cited 2026 Mar 15];21(1):36–42. Available from: https://www.sciencedirect.com/science/article/abs/pii/S010956410400171X?via%3Dihub
2. Reyes-Carmona JF, Felippe MS, Felippe WT. The Biomineralization Ability of Mineral Trioxide Aggregate and Portland Cement on Dentin Enhances the Push-out Strength. J Endod [homepage on the Internet] 2010 [cited 2026 Mar 15];36(2):286–291. Available from: https://www.jendodon.com/action/showFullText?pii=S0099239909008838
3. Silva P V., Guedes DFC, Nakadi F V., Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. *Int Endod J* 2013;46(4):332–338.
4. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int Endod J [homepage on the Internet] 2002 [cited 2025 Dec 1];35(11):934–939. Available from: https://pubmed.ncbi.nlm.nih.gov/12453023/
5. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. *Int Endod J* 2003;36(6):411–415.
6. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod [homepage on the Internet] 2005 [cited 2025 Dec 1];31(11):817–820. Available from: https://pubmed.ncbi.nlm.nih.gov/16249726/
7. Zehnder M. Etidronate (Dual Rinse HEDP) for root canal irrigation in clinical use. 2019;
8. Tartari T, Guimarães BM, Amoras LS, Duarte MAH, Silva e Souza PAR, Bramante CM. Etidronate causes minimal changes in the ability of sodium hypochlorite to dissolve organic matter. Int Endod J [homepage on the Internet] 2015 [cited 2025 Dec 2];48(4):399–404. Available from: https://pubmed.ncbi.nlm.nih.gov/24893624/
9. Rath PP, Yiu CKY, Matinlinna JP, Kishen A, Neelakantan P. The effects of sequential and continuous chelation on dentin. *Dental Materials* 2020;36(12):1655–1665.
10. Vilanova W V., Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YTC. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J [homepage on the Internet] 2012 [cited 2025 Nov 25];45(1):42–48. Available from: https://pubmed.ncbi.nlm.nih.gov/21899566/
11. Morin-Crini N, Lichtfouse E, Torri G, Crini G. Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry.

Environmental Chemistry Letters 2019 17:4 [homepage on the Internet] 2019 [cited 2025 Dec 1];17(4):1667–1692. Available from: https://link.springer.com/article/10.1007/s10311-019-00904-x
12. Shokouhinejad N, Sharifian MR, Jafari M, Sabeti MA. Push-out bond strength of Resilon/Epiphany self-etch and gutta-percha/AH26 after different irrigation protocols. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology [homepage on the Internet] 2010 [cited 2025 Dec 2];110(5). Available from: https://pubmed.ncbi.nlm.nih.gov/20875756/
13. Rahman Hashem AA, Ghoneim AG, Lutfy RA, Fouda MY. The Effect of Different Irrigating Solutions on Bond Strength of Two Root Canal-filling Systems. J Endod [homepage on the Internet] 2009 [cited 2025 Nov 26];35(4):537–540. Available from: https://pubmed.ncbi.nlm.nih.gov/19345800/
14. Goracci C, Tavares AU, Fabianelli A, et al. The adhesion between fiber posts and root canal walls: Comparison between microtensile and push-out bond strength measurements. Eur J Oral Sci [homepage on the Internet] 2004 [cited 2026 Mar 15];112(4):353–361. Available from: /doi/pdf/10.1111/j.1600-0722.2004.00146.x
15. Drummond JL, Sakaguchi RL, Racean ? D C, Wozny ' J, Steinbergl AD, Wiley J. Testing mode and surface treatment effects on dentin bonding. [cited 2026 Mar 15]; Available from: /doi/pdf/10.1002/%28SICI%291097-4636%28199612%2932%3A4%3C533%3A%3AAID-JBM6%3E3.0.CO%3B2-S
16. Pawar AM, Kfir A, Metzger Z, et al. Can Type of Instrumentation and Activation of the Final Irrigant Improve the Obturation Quality in Oval Root Canals? A Push-Out Bond Strength Study. Biology (Basel) [homepage on the Internet] 2022 [cited 2026 Mar 15];11(1). Available from: https://pubmed.ncbi.nlm.nih.gov/35053055/
17. Cecchin D, Souza M, Carlini-Júnior B, Barbizam JVB. Bond strength of Resilon/Epiphany compared with Gutta-percha and sealers Sealer 26 and Endo Fill. Aust Endod J [homepage on the Internet] 2012 [cited 2026 Mar 15];38(1):21–25. Available from: https://pubmed.ncbi.nlm.nih.gov/22432822/
18. Veeramachaneni C, Aravelli S, Dundigalla S. Comparative evaluation of push-out bond strength of bioceramic and epoxy sealers after using various final irrigants: An in vitro study. J Conserv Dent [homepage on the Internet] 2022 [cited 2025 Nov 25];25(2):145–150. Available from: https://pubmed.ncbi.nlm.nih.gov/35720830/
19. Tartari T, Wichnieski C, Bachmann L, et al. Effect of the combination of several irrigants on dentine surface properties, adsorption of chlorhexidine and adhesion of microorganisms to dentine. Int Endod J [homepage on the Internet] 2018 [cited 2026 Mar 15];51(12):1420–1433. Available from: https://pubmed.ncbi.nlm.nih.gov/29862516/
20. Neelakantan P, Varughese AA, Sharma S, Subbarao C V., Zehnder M, De-Deus G. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal sealer to root dentine. Int Endod J [homepage on the Internet] 2012 [cited 2026 Mar 15];45(12):1097–1102. Available from: /doi/pdf/10.1111/j.1365-2591.2012.02073.x
21. Tartari T, Wichnieski C, Silva RM, Letra A, Duarte MAH, Bramante CM. Final irrigation protocols can be used to promote stable long-term bond strength of AH Plus to dentin. Journal of Applied Oral Science [homepage on the Internet] 2023 [cited 2026 Mar 15];31:e20230005. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10287094/
22. Sfeir G, Bukiet F, Hage W, Hachem R El, Zogheib C. Impact of Final Irrigation Protocol on the Push-Out Bond Strength of Two Types of Endodontic Sealers. Materials (Basel) [homepage on the Internet] 2023 [cited 2025 Dec 2];16(5). Available from: https://pubmed.ncbi.nlm.nih.gov/36902876/
23. Silva EJNL, Canabarro A, Andrade MRTC, et al. Dislodgment Resistance of Bioceramic and Epoxy Sealers: A Systematic Review and Meta-analysis. Journal of Evidence-Based Dental Practice [homepage on the Internet] 2019 [cited 2025 Dec 10];19(3):221–235. Available from: https://pubmed.ncbi.nlm.nih.gov/31732099/
24. Lee YL, Lin FH, Wang WH, Ritchie HH, Lan WH, Lin CP. Effects of EDTA on the hydration mechanism of mineral trioxide aggregate. J Dent Res [homepage on the Internet] 2007 [cited 2025 Nov 25];86(6):534–538. Available from: https://pubmed.ncbi.nlm.nih.gov/17525353/
25. Antunes PVS, Flamini LES, Chaves JFM, Silva RG, Cruz Filho AM da. Comparative effects of final canal irrigation with chitosan and EDTA. J Appl Oral Sci [homepage on the Internet] 2019 [cited 2025 Nov 25];28. Available from: https://pubmed.ncbi.nlm.nih.gov/31800870/
26. Ratih DN, Sari NI, Santosa P, Kaswati NMN. Time-Dependent Effect of Chitosan Nanoparticles as Final Irrigation on the Apical Sealing Ability and Push-Out Bond Strength of Root Canal Obturation. *Int J Dent* 2020;2020.
27. Hashem sarah. Effectiveness of Chitosan as Final Irrigating Solution on Bond Strength of Resin Based Sealer to Radicular Dentin. *Al-Azhar Dental Journal for Girls* 2019;0(0):0–0.
28. Choudhury S, Shivanand S, Patil AC, Patil SA, Doddwad PK, Patil C. Evaluation of Push-out Bond Strength of a Resin Sealer to Dentin after a Final Flush of Three Irrigants. J Contemp Dent Pract [homepage on the Internet] 2020 [cited 2025 Nov 25];21(9):982–985. Available from: https://pubmed.ncbi.nlm.nih.gov/33568582/
29. Agarwal S, Raghu R, Shetty A, Gautham P, Souparnika D. An in vitro comparative evaluation of the effect of three endodontic chelating agents (17% ethylenediamine tetraacetic acid, 1% peracetic acid,

0.2% chitosan) on the push out bond strength of gutta percha with a new bioceramic sealer (BioRoot RCS). J Conserv Dent [homepage on the Internet] 2019 [cited 2025 Nov 25];22(5):475–478. Available from: https://pubmed.ncbi.nlm.nih.gov/33082665/

30. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and the smear layer. *Int Endod J* 2009;42(4):335–343.
31. Paqué F, Luder HU, Sener B, Zehnder M. Tubular sclerosis rather than the smear layer impedes dye penetration into the dentine of endodontically instrumented root canals. Int Endod J [homepage on the Internet] 2006 [cited 2025 Dec 10];39(1):18–25. Available from: https://pubmed.ncbi.nlm.nih.gov/16409324/
32. Leal F, Simão RA, Fidel SR, Fidel RAS, Prado M do. Effect of final irrigation protocols on push-out bond strength of an epoxy resin root canal sealer to dentin. Australian Endodontic Journal [homepage on the Internet] 2015 [cited 2025 Dec 8];41(3):135–139. Available from: /doi/pdf/10.1111/aej.12114
33. Chen B, Haapasalo M, Mobuchon C, Li X, Ma J, Shen Y. Cytotoxicity and the Effect of Temperature on Physical Properties and Chemical Composition of a New Calcium Silicate–based Root Canal Sealer. *J Endod* 2020;46(4):531–538.
34. Qu W, Bai W, Liang YH, Gao XJ. Influence of Warm Vertical Compaction Technique on Physical Properties of Root Canal Sealers. *J Endod* 2016;42(12):1829–1833.
35. Goracci C, Grandini S, Bossù M, Bertelli E, Ferrari M. Laboratory assessment of the retentive potential of adhesive posts: A review. J Dent [homepage on the Internet] 2007 [cited 2025 Dec 10];35(11):827–835. Available from: https://pubmed.ncbi.nlm.nih.gov/17766026/
36. Skidmore LJ, Berzins DW, Bahcall JK. An in vitro comparison of the intraradicular dentin bond strength of Resilon and gutta-percha. J Endod [homepage on the Internet] 2006 [cited 2025 Dec 10];32(10):963–966. Available from: https://pubmed.ncbi.nlm.nih.gov/16982274/
37. Pane ES, Palamara JEA, Messer HH. Critical evaluation of the push-out test for root canal filling materials. *J Endod* 2013;39(5):669–673.
38. Donnermeyer D, Dornseifer P, Schäfer E, Dammaschke T. The push-out bond strength of calcium silicate-based endodontic sealers. Head Face Med [homepage on the Internet] 2018 [cited 2025 Dec 10];14(1):13. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6102912/

Received on May 2, 2026
Revised on July 12, 2026
Accepted on July 14, 2026

Conflict of Interest: The authors declare that there is no conflict of interest.
Financial Disclosure Statement: This research did not receive any specific grant or financial support from funding agencies in the public, commercial, or not-for-profit sectors.
Human Right Statement: N/A
Animal Rights Statement: N/A

Correspondence

Büşra Efendioğlu
Department of Endodontics, Faculty of Dentistry,
Ordu University, 52200 Ordu, Turkey
E-mail: busraef61@gmail.com
Phone: +90506 3397354