



Original Article

# Effect of various calcium hydroxide removal protocols on the dislodgement resistance of biodentine in an experimental apexification model



Özgür İlke Ulusoy <sup>a\*</sup>, Keziban Olcay <sup>b</sup>, Mutahhar Ulusoy <sup>c</sup>

<sup>a</sup> Department of Endodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey

<sup>b</sup> Department of Endodontics, Faculty of Dentistry, Istanbul University-Cerrahpaşa, Istanbul, Turkey

<sup>c</sup> Department of Prosthodontics, Faculty of Dentistry, Near East University, Nicosia, Cyprus

Received 17 August 2020; Final revision received 19 October 2020

Available online 10 November 2020

## KEYWORDS

Biodentine;  
Calcium hydroxide;  
Etidronic acid;  
Peracetic acid;  
Immature roots

**Abstract** *Background/purpose:* Residual calcium hydroxide (CH) in the root canal dentine walls may influence the adhesion of tricalcium silicate-based materials. The aim of this study is to evaluate the effect of various CH removal protocols on the dislodgement resistance of biodentine from simulated immature root canals in an experimental apexification model.

*Materials and methods:* CH was applied to 120 simulated immature root canals. The samples were divided into 12 experimental groups (n = 10) according to the applied irrigation protocols used for the removal of CH: Group 1: Sodium hypochlorite (NaOCl), Conventional needle irrigation (CNI); Group 2: NaOCl, EndoActivator; Group 3: NaOCl, XP-endo Finisher; Group 4: NaOCl- Ethylenediaminetetraacetic acid (EDTA), CNI; Group 5: NaOCl-EDTA, EndoActivator; Group 6: NaOCl-EDTA, XP-Endo Finisher; Group 7: NaOCl+etidronic acid (HEBP), CNI; Group 8: NaOCl+HEBP, EndoActivator; Group 9: NaOCl+HEBP, XP-endo Finisher; Group 10: NaOCl-Peracetic acid (PAA), CNI; Group 11: NaOCl-PAA, EndoActivator; Group 12: NaOCl-PAA, XP-endo Finisher; Control Group: CH was not applied. Biodentine was placed at the apical thirds of 130 immature root canals. Vertical loading was applied to biodentine fillings inside the dentin discs. Maximum force to dislodge the material was statistically analyzed with ANOVA.

*Results:* The control, NaOCl+HEBP (CNI, EndoActivator, XP-endo Finisher) and NaOCl-PAA (EndoActivator, XP-endo Finisher) groups exhibited the lowest dislodgement resistance values (p < 0.001). When used CNI, irrigation with NaOCl+HEBP resulted in lower resistance to dislodgement of biodentine compared to NaOCl, and NaOCl-EDTA (p < 0.001).

*Conclusion:* Adhesion of apical barrier materials to root canal dentine can be influenced by the irrigation protocols used for CH removal.

\* Corresponding author. Department of Endodontics, Gazi University Faculty of Dentistry, First Street, Emek, Ankara, 06510, Turkey.  
E-mail address: [ilkeatasoy@yahoo.com](mailto:ilkeatasoy@yahoo.com) (Ö.İ. Ulusoy).

## Introduction

Apexification using tricalcium silicate-based materials as apical barrier is a widely accepted treatment option for the permanent immature teeth with necrotic pulp. Mineral trioxide aggregate (MTA), and more recently biodentine are the most commonly used tricalcium silicate-based cements for the purpose of inducing an apical calcified tissue barrier and prevention of root canal fillings' extrusion into the periapical area.<sup>1,2</sup> In the one-step apexification procedure using MTA or biodentine, disinfection of root canals with calcium hydroxide (CH) for approximately one week is generally recommended before placing apical plugs.<sup>3</sup>

Previous evidence has suggested that the CH remnants inside the root canal can adversely influence the penetration of root canal sealers into the dentinal tubules by acting as a physical barrier.<sup>4</sup> Therefore, dental practitioners frequently prefer to remove CH or other medicaments completely from the root canal system before placement of tricalcium silicate-based barriers. However, it is unclear in the literature whether the impact of CH residues on the adhesion of tricalcium silicate-based cements to the root canal dentine is positive or negative.

Several irrigation solutions and techniques have been recommended for removing the CH effectively from the root canal system.<sup>5,6</sup> Even though sequential use of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) along with mechanical instrumentation is one of the most widely used protocol to remove CH from root canals,<sup>7</sup> it has been deemed insufficient to achieve complete elimination of CH.<sup>8,9</sup> Therefore, different irrigation protocols have been researched regarding their capacity for CH removal. A 1-hydroxyethylidene-1, 1-bisphosphonate (HEBP-etidronic acid) has been introduced as an alternative chelator to EDTA as it shows no short-term interaction with NaOCl.<sup>10</sup> It was also demonstrated that use of HEBP efficiently removed CH dressings from root canal walls.<sup>6</sup> Peracetic acid (PAA), known as a strong disinfectant, has currently been used as another alternative to EDTA, and was suggested to form complexes with calcium when used in the root canal irrigation.<sup>11</sup>

Use of decalcifying agents without any activation during irrigation of root canals can not completely remove the intracanal medicaments including CH from root canal system. Therefore, utilization of various irrigation activation methods have been recommended to achieve effective CH removal.<sup>12–14</sup> EndoActivator (Dentsply Tulsa Dental Specialites, Tulsa, OK, USA) is a device which was primarily designed for the agitation of irrigation solutions in root canal treatment. The system releases sonic waves through three handpieces possessing different size and tapers. Alturaiki et al.<sup>15</sup> suggested that EndoActivator achieved better CH removal from the root canals compared to the EndoVac (negative pressure irrigation) and PiezoFlow (continuous ultrasonic irrigation), when used with NaOCl and EDTA. XP-endo Finisher (FKG, La Chaux-de-Fonds,

Switzerland) is a recently introduced instrument that was manufactured using a special titanium alloy (Martensite-Austinite-Electropolish-FleX), which provides high flexibility to the instrument.<sup>16</sup> This flexibility allows the file enhanced adaptation to the irregular-shaped root canals leading to a successful chemomechanical debridement. Marques-da-Silva et al.<sup>14</sup> stated that agitation of the final irrigants using XP-endo Finisher has removed CH dressings from simulated internal resorption cavities more successfully compared to the EndoActivator and XP-endo Shaper.

Calcium hydroxide removal abilities of the irrigation protocols may influence the dislodgement resistance of the apical barrier materials from the root canals of immature teeth. In addition, use of chelating agents before obturation of the root canals may also affect the adhesion of tricalcium silicate-based materials by destroying their structure and setting reactions.<sup>17,18</sup> It has previously been demonstrated that prior use of chelators significantly decreased the push-out bond strength of MTA and biodentine to the root-end cavities.<sup>19</sup> However, to the authors' knowledge no study has evaluated the CH removal protocols on the adhesion of biodentine to the root canals of immature teeth. Therefore, the aim of this study was to evaluate the effect of NaOCl, NaOCl-EDTA, NaOCl+HEBP, and NaOCl-PAA along with conventional needle irrigation (CNI), EndoActivator, and XP-endo Finisher on the dislodgement resistance of biodentine apical barriers from simulated immature root canals in an experimental apexification model.

## Materials and methods

The protocol of the current study was approved by an institutional non-interventional research ethical board (No: 10840098–604.01.01-E.12604).

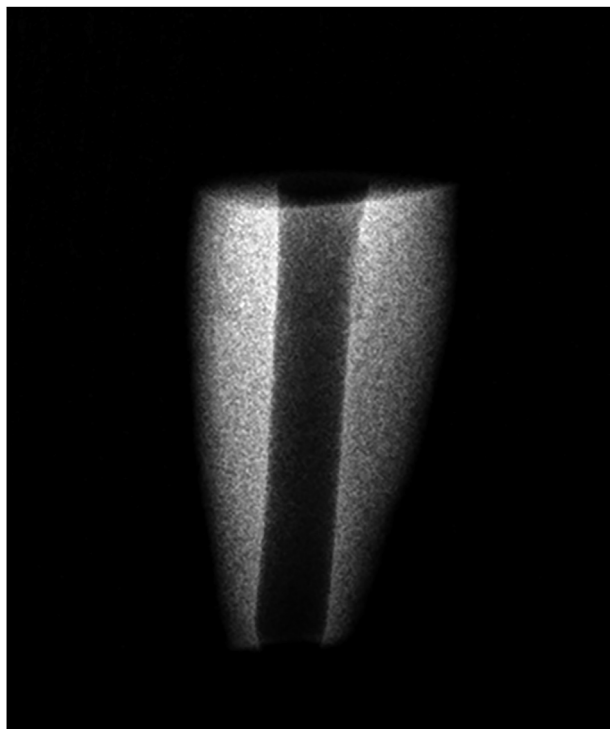
### Preparation of the simulated immature teeth

Freshly extracted, sound human canine teeth with single roots were selected. All teeth were examined under stereomicroscope (Zeiss AxioZoom V16, Carl Zeiss, Jena, Germany) at x25 magnification for the absence of any caries and cracks. Absence of any root resorption areas, immature apex, structural deformations, and extra canal more than single was confirmed by radiographic examination. A total of 130 canine teeth meeting the inclusion criteria were used for the present study. A periodontal scaler was used to remove the residual tissues from the root surface of the sample teeth. The teeth were kept in 0.1% thymol solution at 4 °C until the experiment began. The crowns of teeth were then removed with the help of diamond discs (Komet, Gebr Brasseler GmbH & Co., Lemgo, Germany) under water cooling to obtain a standardized root length of 14 mm. After accessing to the root canals, the working length was determined to be 1 mm short of the apex using a #15 K-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) and

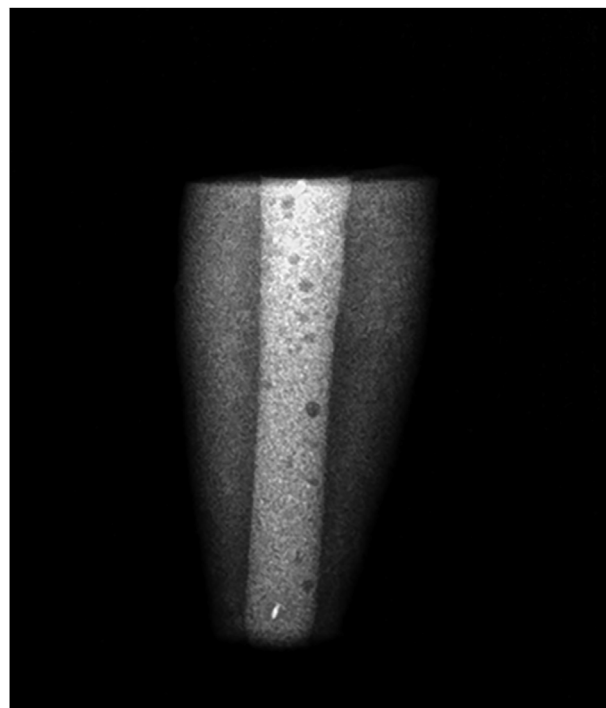
the root canals were instrumented with Protaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) to a master apical size of F5. To achieve a standardized internal root canal diameter and simulate immature teeth with open apex, peeso reamers (Mani, Inc. Tochigi, Japan) with sizes 1–6 were respectively used in working length (Fig. 1). Size 6 reamer was then extruded 1 mm beyond the apical foramen. Two ml 2.5% sodium hypochlorite (NaOCl) solution (Promida Co., Eskisehir, Turkey) was applied to the root canals using a plastic dental syringe and 27-gauge dental needle (Set Inject; Set Medical Instruments, Istanbul, Turkey) between each file during instrumentation. To simulate periodontal ligament and clinical conditions, the teeth were placed in a flower arrangement foam. The root canals were finally irrigated using 2 ml of 17% ethylenediaminetetraacetic acid (EDTA) (Cerkamed, Stalowa Wola, Poland) for 1 min and finally flushed using 5 ml distilled water. The root canals were dried with paper points. Calcium hydroxide powder (CH) (Vision, Istanbul, Turkey) that was mixed with saline was applied to the 120 root canals using a lentulo spiral. The quality of CH fillings was confirmed with radiographic evaluation for each sample (Fig. 2). The remaining 10 simulated immature root canals received no CH filling. The root canal orifices were restored with temporary restorative material (Coltosol F, Coltene Whaledent, Langenau, Germany) and the teeth were stored at 37 °C and 100% humidity for one week.

### Calcium hydroxide removal protocols

After one week incubation period, the teeth were randomly divided into 12 experimental groups (n = 10) according to



**Figure 1** Representative radiographic image of the simulated immature root model with open apex.



**Figure 2** Radiographic image showing homogeneous calcium hydroxide dressing inside the immature root canal.

the applied irrigation protocols used for the removal of CH, and one control group (n = 10) as follows:

#### Group 1 (NaOCl, Conventional needle irrigation)

The root canals were irrigated by 6 ml 2.5% NaOCl without any activation using a plastic dental syringe and 27-gauge dental needle (Set Inject) at a flow rate of 3 ml/min for 2 min. The needle tip was placed at a distance 1 mm short of the working length.

#### Group 2 (NaOCl, EndoActivator)

The root canals were irrigated with 3 ml 2.5% NaOCl, which was activated using sonic energy (EndoActivator, Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) for 1 min at 10,000 cycles/min (medium power) with using a red tip (25/04). The needle tip was placed at 1 mm short from the open apex. The activation was stopped, and the root canals were again filled with freshly prepared 3 ml 2.5% NaOCl and activated using EndoActivator (Dentsply Tulsa) using the same protocol.

#### Group 3 (NaOCl, XP-endo Finisher)

The root canals were flushed with 3 ml of 2.5% NaOCl. Then the irrigant was activated using an XP-endo® Finisher file (FKG Dentaire, La-Chaux-de-Fonds, Switzerland). The XP-endo® Finisher was used with a torque-controlled endodontic motor (X-Smart Plus, Dentsply Maillefer, Ballaigues, Switzerland) at a speed of 800 rpm and a torque of 1 Ncm. Briefly, the file was cooled using a cold spray (Endo-Ice; Whaledent, Mahwah, NJ, USA) in its plastic tube. Then it was removed from its tube in rotation mode, inserted into the root canals filled with the irrigant and activated for 1 min. During activation, the XP-endo® Finisher was used

with slow, gentle and continuous in-and-out movements of about 7–8 mm amplitude to contact the full length of the canal. The activation was then stopped, and the root canals were irrigated with freshly prepared 3 ml 2.5% NaOCl, which was activated with XP-endo® Finisher for 1 min as described above.

#### **Group 4 (NaOCl-EDTA, Conventional needle irrigation)**

The samples were passively irrigated with 3 ml 2.5% NaOCl for 1 min at a flow rate of 3 ml/min using a 27-gauge dental needle (Set Inject). The root canals were then irrigated using 3 ml 17% EDTA (Wizard, Rehber Chemistry, Istanbul, Turkey) without any activation. The needle tip was placed at a distance 1–2 mm short of the working length during application of both solutions.

#### **Group 5 (NaOCl-EDTA, EndoActivator)**

3 mL 2.5% NaOCl was applied to the root canals, then activated by EndoActivator using the same protocol in the Group 2. Then the root canals were flushed with 3 ml 17% EDTA and EndoActivator (Dentsply Tulsa) procedure was repeated for 1 min.

#### **Group 6 (NaOCl-EDTA, XP-endo Finisher)**

The root canals were filled with 3 mL 2.5% NaOCl, which was activated with XP-endo® Finisher file (FKG) for 1 min as described in Group 3. Then, 3 ml 17% EDTA was introduced to the root canals and agitated with XP-endo Finisher for 1 min.

#### **Group 7 (NaOCl+HEBP, Conventional needle irrigation)**

Firstly, 18% (weight/volume) etidronic acid (HEBP) solution was obtained by mixing 60% aqueous HEBP solution (Sigma–Aldrich, St Louis, MO, USA) with ultrapure water. Three ml 18% HEBP and 3 ml 5% NaOCl solution were mixed to obtain a total volume of 6 ml single solution. As a result, the final concentrations of solutions in the mixture were 9% for HEBP and 2.5% for NaOCl. Six ml of this mixed solution was applied to the root canals without any activation using a 27-gauge dental needle (Set Inject) at a flow rate of 3 ml/min for 2 min as described in Group 1.

#### **Group 8 (NaOCl+HEBP, EndoActivator)**

Three ml mixed solution containing 9% HEBP and 2.5% NaOCl was prepared and applied to the root canals and activated with EndoActivator (Dentsply Tulsa) for 1 min using the same protocol in the Group 2. The activation was stopped and the root canals were filled with a freshly prepared mixture of 9% HEBP and 2.5% NaOCl. EndoActivator agitation was again performed for another 1 min.

#### **Group 9 (NaOCl+HEBP, XP-endo Finisher)**

The root canals were irrigated with 3 ml 9% HEBP and 2.5% NaOCl, which was activated using XP-endo® Finisher (FKG) for 1 min as defined above in the Group 3. Then, 3 ml of freshly prepared solution was applied to the root canals and activated using XP-endo® Finisher for additional 1 min.

#### **Group 10 (NaOCl-PAA, Conventional needle irrigation)**

The root canals were passively irrigated with 3 ml 2.5% NaOCl using a 27-gauge dental needle (Set Inject) for 1 min as described in the Group 1. 36–40% peracetic acid (PAA)

solution were obtained from a commercial source (Sigma–Aldrich, St Louis, MO, USA) and was diluted with distilled water to produce a 2% (w/v) PAA solution. Subsequently, 3 ml of 2% PAA solution was applied passively to the root canals via dental syringe for 1 min.

#### **Group 11 (NaOCl-PAA, EndoActivator)**

Three ml 2.5% NaOCl was introduced to the root canals and the solution was agitated with EndoActivator (Dentsply Tulsa) for 1 min as explained in the Group 2. The root canals were then irrigated using 3 ml 2% PAA solution that was activated with EndoActivator for 1 min.

#### **Group 12 (NaOCl-PAA, XP-endo Finisher)**

Three ml 2.5% NaOCl was applied to the root canals, and then activated using XP-endo Finisher (FKG) for 1 min using the same protocol in the Group 3. Following this procedure, the root canals were flushed with 3 ml 2% PAA solution, which was activated using XP-endo Finisher for another 1 min.

#### **Control group (n = 10)**

Any calcium hydroxide dressing was not applied to the simulated immature root samples in this group.

The total volume of the irrigation solutions was 6 ml in all experimental groups, the total irrigation time was 2 min in the conventional needle irrigation groups, and the total activation time was 2 min in the EndoActivator and XP-endo Finisher groups. To eliminate the negative effect of prolonged contact of root dentin with irrigation agents, all samples were finally flushed with 2 ml of distilled water for 30 s immediately after completion of respective irrigation protocol. Then the root canals were dried with paper points (DiaDent, Almere, Netherlands).

### **Preparation of biodentine apical barriers**

Biodentine (Septodont, Saint-Maur-des-Fosses, France) was prepared by trituration for 30s using a dental amalgamator according to the recommendations of the manufacturers. Prepared cement was placed in the apical 4–5 mm of the root canals using MTA carrier (Dentsply, Tulsa, OK, USA) to simulate apical barriers and compacted with Buchanan endodontic pluggers (Kerr Co., Orange, CA, USA).

### **Dislodgement resistance assessment**

The apical segments of the specimens were sectioned horizontally to obtain one slice with a thickness of  $2.0 \pm 0.1$  mm using a precision saw (Isomet, Buehler, NY, USA) at a slow speed under constant cooling. The thickness of each slice was confirmed using a digital caliper (Avenger Products Co., North Plains, OR, USA) with an accuracy of 0.001 mm in order to maintain the standardization of the slices and to discard the undesired effects caused from vibration of precision saw. The dislodgement resistance of biodentine was measured by application of vertical loads using cylindrical tips mounted on a universal testing machine (Lloyd Instruments Ltd, Fareham, UK). A gradually increasing force at a cross-head speed of 1 mm/min was applied to each specimen from the apical to the coronal

direction until bond failure occurred. The maximum force in Newtons to dislodge the material was divided by the surface area of biodentine filling (A) and thus converted to megapascals (MPa) for each specimen. Surface area of biodentine filling (A) was calculated using the formula:  $A \text{ (mm}^2\text{)} = \Pi (r_1+r_2) h$ , where  $\Pi = 3.14$ ,  $r_1$  = smaller radius of the filling material,  $r_2$  = larger radius of the filling material, and  $h$  = height of the filling material (2 mm).

Failure modes were analysed using a stereomicroscope and classified into one of the following categories: 'adhesive failure' between biodentine and dentinal wall interface, 'cohesive failure' within the biodentine or 'mixed failure' failure in both the biodentine and dentinal wall.

## Statistical analysis

The statistical analyses were carried out using Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean and standard deviation. All data were tested for normality prior to statistical analysis. For the comparison of control group and the experimental groups, one-way ANOVA test, followed by Dunnett's post-hoc test was used. Two-way ANOVA was performed to analyze the influence of the two factors (irrigation technique and irrigation solution) and their interactions on the dislodgement resistance. Post-hoc comparisons were evaluated using Bonferroni corrections. All statistical analyses were two-sided, and a P-value below 0.05 was considered to be statistically significant.

## Results

The mean values and standard deviations of the resistance to dislodgement in the experimental groups were shown in Table 1. One-way ANOVA revealed that the control samples without CH filling exhibited the lowest dislodgement resistance values ( $1.46 \pm 0.68$ ) ( $p < 0.001$ ). But this value was not statistically different from those of the samples irrigated with NaOCl+HEBP used with three irrigation techniques (CNI, EndoActivator, XP-endo Finisher), and NaOCl-PAA activated with both activation methods (EndoActivator, XP-endo Finisher). Two-way ANOVA revealed that, the dislodgement resistance of biodentine apical barrier was

significantly affected by the irrigation solution ( $p < 0.001$ ), but not influenced by the type of irrigation technique ( $p = 0.260$ ). There was no significant interaction between the irrigation solution and the irrigation technique ( $p = 0.580$ ). When combined with CNI, use of NaOCl+HEBP resulted in lower resistance to dislodgement of biodentine compared to NaOCl, and NaOCl-EDTA ( $p < 0.001$ ). The samples irrigated with NaOCl-EDTA that was agitated using EndoActivator showed higher dislodgement resistance compared to the ones irrigated with NaOCl+HEBP ( $p < 0.001$ ). In all the experimental groups, regardless of the irrigation solutions, there was no significant difference between the three irrigation techniques in terms of dislodgement resistance of biodentine ( $p > 0.001$ ).

The modes of failures of the specimens were listed in Table 2. The failures were predominantly cohesive in the samples irrigated with NaOCl, NaOCl-EDTA, and NaOCl+HEBP, whereas the majority of the observed failures were adhesive and mixed in the NaOCl-PAA treated samples.

## Discussion

Tricalcium silicate-based cements used as an apical barrier in the apexification of non-vital immature teeth should exhibit adequate adhesion to the root canal dentine, because they are exposed to several forces during operative procedures and mastication. However, use of intracanal medicaments and/or irrigation protocols for the removal of these medications can influence the dislodgement resistance of apical barrier materials.<sup>12</sup> It was demonstrated that prior use of CH has improved the dislodgement resistance of tricalcium silicate-based materials from the root canal dentine.<sup>12,20</sup> These results support the present findings, because the lowest dislodgement resistance values were derived from the samples which were not filled with CH before placement of biodentine apical plugs. Our results are also in agreement with those of Centenaro et al.,<sup>21</sup> who found higher dislocation resistance of biodentine from the root canals which were priorly medicated with CH, compared to the root canals that have no medication, although the difference was not statistically significant. This improvement in the adhesion can be

**Table 1** Means and standard deviations of the dislodgement resistance values of the samples in the experimental groups.

Irrigation techniques (IT)	Irrigation solutions (IS)								<i>P</i> -value of two-way ANOVA		
	NaOCl		NaOCl-EDTA		NaOCl+HEBP		NaOCl-PAA		IT	IS	IT x IS
	Means	(SD)	Means	(SD)	Means	(SD)	Means	(SD)			
Conventional needle irrigation	5.96	(1.5) <sup>Aa</sup>	4.97	(2.49) <sup>Aa</sup>	2.39	(1.49) <sup>Ab</sup>	4.52	(1.97) <sup>Aab</sup>	0.260	<0.001	0.580
EndoActivator	4.97	(2.47) <sup>Aab</sup>	5.19	(2.46) <sup>Aa</sup>	2.87	(0.60) <sup>Ab</sup>	3.41	(2.32) <sup>Aab</sup>			
Xp-endo Finisher	4.36	(1.92) <sup>Aa</sup>	4.65	(2.60) <sup>Aa</sup>	2.90	(1.08) <sup>Aa</sup>	3.09	(0.90) <sup>Aa</sup>			

\*SD, standard deviation; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; HEBP, etidronic acid; PAA, peracetic acid; *P*-value, statistical significance level.

The different upper case letters in each column indicate significant differences between the irrigation techniques. The different lower case letters in each row indicate significant differences between the irrigation solutions.

**Table 2** Types of adhesion failures observed in the experimental groups.

Irrigation techniques	Failure modes	Irrigation solutions			
		NaOCl n (%)	NaOCl-EDTA n (%)	NaOCl+HEBP n (%)	NaOCl-PAA n (%)
Conventional needle irrigation	A	4 (40)	1 (10)	2 (20)	5 (50)
	C	6 (60)	8 (80)	5 (50)	3 (30)
	M	0 (0)	1 (10)	3 (30)	2 (20)
EndoActivator	A	3 (30)	3 (30)	4 (40)	4 (40)
	C	6 (60)	6 (60)	6 (60)	5 (50)
	M	1 (10)	1 (10)	0 (0)	1 (10)
XP-endo Finisher	A	3 (30)	2 (20)	1 (10)	4 (40)
	C	5 (50)	6 (60)	7 (70)	4 (40)
	M	2 (20)	2 (20)	2 (20)	2 (20)

n, number; %, percentage; A, adhesive failure; C, cohesive failure; M, mixed failure; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; HEBP, etidronic acid; PAA, peracetic acid.

explained by possible chemical interaction of tricalcium silicate-based materials and CH residues in the root canal or the presence of water inside CH which may improve the setting reaction of tricalcium silicate-based materials.<sup>13,22</sup>

According to the present results, there seems to be a negative correlation between the removal efficiency of CH from the immature root canal walls and adhesion of biodentine apical plugs. The samples exposed to the NaOCl used with conventional needle irrigation showed the highest resistance to dislodgement of biodentine from root canal walls. Use of NaOCl without any activation has previously been demonstrated to be insufficient to remove CH from root canal walls.<sup>5</sup> Similarly, sequential use of NaOCl and EDTA resulted in stronger adhesion of biodentine compared to the use of NaOCl+HEBP when associated with three irrigation techniques, although this difference was not statistically significant in the XP-endo Finisher activation group. Limited demineralization capacity of EDTA in apical third of the root canals has been reported in previous studies, which could positively affect the adhesion of the apical barrier materials.<sup>23</sup>

The lowest dislocation resistance values for biodentine were obtained from the samples irrigated with NaOCl+HEBP when used with EndoActivator and CNI, despite these values were not statistically different from NaOCl-PAA irrigated samples. Superior chelating ability of HEBP over EDTA in removing smear layer from the apical third of root canals has previously been mentioned.<sup>23</sup> Although the chemical compositions of the smear layer and CH are different, irrigation of root canals with HEBP could also result in more effective removal of CH from immature root canals compared to EDTA, which in turn causes unsuccessful adhesion of biodentine as in the present study. A possible reason for the ability of HEBP in the elimination of CH can be its high acidity that compensates its weak chelating ability.<sup>6</sup> It was suggested that the high alkaline pH of CH increases the pH of the irrigation agents, thereby decreasing their chelating ability.<sup>6</sup>

In addition to the possible chemical interactions between tricalcium silicate-based materials and CH remnants, the micromechanical retention of the cements to the superficial CH residues can be another explanation for the stronger adhesion of biodentine in the samples irrigated with solutions with lower chelating ability and removal

potential of CH.<sup>22</sup> The lower adhesion values of biodentine in the NaOCl+HEBP treated samples can also be attributed to the possible alterations in the ultrastructure of root canal dentine. It was previously demonstrated that final irrigation of root canals with HEBP can lead to structural changes and hardness reduction in root canal dentine, which may adversely affect the adhesion of root canal sealers.<sup>24</sup>

The present results indicated that, regardless of the irrigation solutions, there was no statistically significant difference between the three irrigation techniques in terms of dislodgement resistance of biodentine. However, lower resistance to dislodgement was detected after use of XP-endo Finisher compared to the other techniques in the NaOCl, NaOCl-EDTA, and NaOCl-PAA groups. This can be explained by the superiority of XP-endo Finisher over EndoActivator and CNI on the removal of CH from irregular shaped root canals.<sup>14</sup> Therefore, use of XP-endo Finisher could have improved the limited CH removal abilities of NaOCl, EDTA and PAA, which could result in reduction in the adhesion of biodentine.

Based on the results of the present study, the type of the chelating agents rather than the irrigation technique seems to have an impact on the dislodgement resistance of biodentine apical barriers from simulated immature roots. This can be related to the abilities of irrigation solutions in the removal of CH as well as their effects on the ultrastructure of root canal dentine or tricalcium silicate-based cements. Further *in-vitro* and *in-vivo* research are needed to investigate the mechanism by which the irrigation protocols influence the adhesion of tricalcium silicate-based apical barrier materials to the root canals of immature teeth.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

## Acknowledgments

The authors thank to Prof. Dr. Bülent Celik for his help in the statistical analysis.

No funding used for this study.

## References

1. Rafter M. Apexification: a review. *Dent Traumatol* 2005;21: 1–8.
2. Songtrakul K, Azarpajouh T, Malek M, Sigurdsson A, Kahler B, Lin LM. Modified apexification procedure for immature permanent teeth with a necrotic pulp/apical periodontitis: a case series. *J Endod* 2020;46:116–23.
3. Elnaghy A, Elsaka S. Fracture resistance of simulated immature roots using biodentine and fiber post compared with different canal-filling materials under aging conditions. *Clin Oral Invest* 2020;24:1333–8.
4. Guiotti FA, Kuga MC, Duarte MA, Sant'Anna AJ, Faria G. Effect of calcium hydroxide dressing on push-out bond strength of endodontic sealers to root canal dentin. *Braz Oral Res* 2014;28:1–6.
5. Kuştarıcı A, Er K, Siso SH, et al. Efficacy of laser-activated irrigants in calcium hydroxide removal from the artificial grooves in root canals: an ex vivo study. *Photomed Laser Surg* 2016;34:205–10.
6. Chockattu SJ, Deepak BS, Goud KM. Comparison of efficiency of ethylenediaminetetraacetic acid, citric acid, and etidronate in the removal of calcium hydroxide intracanal medicament using scanning electron microscopic analysis: an *in-vitro* study. *J Conserv Dent* 2017;20:6–11.
7. Tatsuta CT, Morgan LA, Baumgartner JC, Adey JD. Effect of calcium hydroxide and four irrigation regimens on instrumented and uninstrumented canal wall topography. *J Endod* 1999;25:93–8.
8. Lambrianidis T, Kosti E, Boutsoukis C, Mazinis M. Removal efficacy of various calcium hydroxide/chlorhexidine medicaments from the root canal. *Int Endod J* 2006;39:55–61.
9. Taşdemir T, Celik D, Er K, Yildirim T, Ceyhanli KT, Yeşilyurt C. Efficacy of several techniques for the removal of calcium hydroxide medicament from root canals. *Int Endod J* 2011;44: 505–9.
10. Girard S, Paqué F, Badertscher M, Sener B, Zehnder M. Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1-bisphosphonate. *Int Endod J* 2005;38: 810–6.
11. De-Deus G, Souza EM, Marins JR, Reis C, Paciornik S, Zehnder M. Smear layer dissolution by peracetic acid of low concentration. *Int Endod J* 2011;44:485–90.
12. Amin SA, Seyam RS, El-Samman MA. The effect of prior calcium hydroxide intracanal placement on the bond strength of two calcium silicate-based and an epoxy resin-based endodontic sealer. *J Endod* 2012;38:696–9.
13. Gokturk H, Bayram E, Bayram HM, Aslan T, Ustun Y. Effect of double antibiotic and calcium hydroxide pastes on dislodgement resistance of an epoxy resin-based and two calcium silicate-based root canal sealers. *Clin Oral Invest* 2017; 21:1277–82.
14. Marques-da-Silva B, Alberton CS, Tomazinho FSF, et al. Effectiveness of five instruments when removing calcium hydroxide paste from simulated internal root resorption cavities in extracted maxillary central incisors. *Int Endod J* 2020;53: 366–75.
15. Alturaiki S, Lamphon H, Edrees H, Ahlquist M. Efficacy of 3 different irrigation systems on removal of calcium hydroxide from the root canal: a scanning electron microscopic study. *J Endod* 2015;41:97–101.
16. Leoni GB, Versiani MA, Silva-Sousa YT, Bruniera JF, Pécora JD, Sousa-Neto MD. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. *Int Endod J* 2017; 50:398–406.
17. Smith JB, Loushine RJ, Weller RN, et al. Metrologic evaluation of the surface of white MTA after the use of two endodontic irrigants. *J Endod* 2007;33:463–7.
18. Lottanti S, Gautschi H, Sener B, Zehnder M. Effects of ethylenediaminetetraacetic, etidronic and peracetic acid irrigation on human root dentine and smear layer. *Int Endod J* 2009;42: 335–43.
19. Ballal NV, Ulusoy Öİ, Chhapparwal S, Ginjupalli K. Effect of novel chelating agents on the push-out bond strength of calcium silicate cements to the simulated root-end cavities. *Microsc Res Tech* 2018;81:214–9.
20. Nagas E, Cehreli ZC, Uyanik MO, Vallittu PK, Lassila LV. Effect of several intracanal medicaments on the push-out bond strength of ProRoot MTA and biodentine. *Int Endod J* 2016;49: 184–8.
21. Centenaro CF, Santini MF, da Rosa RA, et al. Effect of calcium hydroxide on the bond strength of two bioactive cements and SEM evaluation of failure patterns. *Scanning* 2016;38:240–4.
22. Hosoya N, Kurayama H, Iino F, Arai T. Effects of calcium hydroxide on physical and sealing properties of canal sealers. *Int Endod J* 2004;37:178–84.
23. Ulusoy Öİ, Zeyrek S, Çelik B. Evaluation of smear layer removal and marginal adaptation of root canal sealer after final irrigation using ethylenediaminetetraacetic, peracetic, and etidronic acids with different concentrations. *Microsc Res Tech* 2017;80:687–92.
24. Silva e Souza PA, das Dores RS, Tartari T, Pinheiro TP, Tuji FM, Silva e Souza Jr MH. Effects of sodium hypochlorite associated with EDTA and etidronate on apical root transportation. *Int Endod J* 2014;47:20–5.