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# Effect of different chelating agents and their surface tension on the amount of apically extruded debris



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	canal preparation apical extrusion is an undesirable situa-
Debris extrusion; Maleic acid; Peracetic acid; Surface tensionthe effect of the presence of dif amount of apically extruded debri on the apical extrusion. Materials and methods: Ninety ex nal preparation, the teeth were m prepared with Reciproc instrumer liquid, 17% EDTA-gel, 7% maleic a Apically extruded debris was coll balance. The surface tension of so ring digital tensiometer. The statis cal extrusion and the Tukey for su surface tension was compared usi <i>Results:</i> The least amount of apic citric acid and EDTA-liquid ha tween apical extrusion and surface <i>Conclusion:</i> The presence of EDT preparation decreased the amount	e complications. The aim of the present study is to evaluate fferent chelators in root canals during preparation on the is and to investigate the effect of surface tension of irrigant stracted mandibular incisor teeth were included. Prior to ca- nounted to Eppendorf tubes. Root canals of the samples were hts in the presence of different chelating agents (17% EDTA- acid, 2.25% peracetic acid, 10% citric acid) and 5% NaOCL. Lected in Eppendorf tubes and weighted with an electronic olutions was calculated with the ring method using a du Noüy stical analysis was performed with Tamhane's T2 test for api- rface tension. The correlation between apical extrusion and ing Pearson's coefficient. cally extruded debris was with EDTA-gel, peracetic acid and each other. NaOCl had the highest surface tension whereas id the lowest. There was no significant correlation exists be-

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# Introduction

Effective root canal preparation is crucial for the success of root canal treatment. During this process, debris generated with endodontic instruments may extrude beyond apex along with microorganisms and result in complications including post-operative pain, inflammation and swelling.<sup>1,2</sup> In our day, several engine-driven rotary systems are present to facilitate root canal preparation and to minimize the procedural errors. Despite the recent advances in rotary instruments, numerous studies stated that all types of rotary instruments lead to apical extrusion of debris.<sup>1,3-5</sup> Reciproc (VDW, GmbH, Munich, Germany) is one of the contemporary single-file systems working with reciprocal motion. It is manufactured from a wire called Mwire which was subjected to a special heat treatment leading to increased strength. It is believed that using reciprocating single-file instruments extrude lesser amounts of debris.<sup>6</sup> However, Topcuoglu et al.<sup>5</sup> found that apically extruded debris during instrumentation with Reciproc files is more than other multi-file systems working with rotational motion. Similarly, Parirokh et al.<sup>7</sup> reported that 5.25% NaOCl cause more debris extrusion than 2.5% NaOCl and 2% chlorhexidine. Thus it can be assumed that additional precautions may be beneficial to reduce the amount of apically extruded debris. One of these precautions may be the use chelating agents during canal preparation which act as lubricant. Ethylenediaminetetraacetic acid (EDTA) is the most widely used chelating agent for this purpose. The other contemporary chemicals which can be considered as substitutes to EDTA are citric acid, peracetic acid and maleic acid.<sup>8-11</sup> However, these chemicals are evaluated in terms of their ability to remove smear layer. To the best of our knowledge their possible effects on debris extrusion was not evaluated. For this reason, the authors of the present study questioned whether the presence of these chelators in root canals during preparation may reduce the amount of apically extruded debris and surface tension of irrigants may effect the amount of apically extruded debris. The null hypotheses were that (i) the apically extruded debris would not be affected by investigated chelators and (ii) there would not be a correlation between surface tension and apically extruded debris.

## Materials and methods

The present study was approved by the Ethics Committee of Bolu Abant İzzet Baysal University, in accordance with the Declaration of Helsinki (17-KAEK-032).

A priori power analysis was performed to determine adequate number of samples to be included in the study. An effect size of 0.40 was added to a power b = 80% and

a=5% input into an F test family for analysis of variance, we needed ninety samples for six groups.

Ninety extracted intact human mandibular incisor teeth with mature apices were included. The presence of only one straight canal was confirmed with radiographs taken from mesio-distal and bucco-lingual directions. The teeth with canal curvature  $<\!10^\circ$  according to the method described by Schneider were included.<sup>12</sup> The teeth representing any fracture line, resorption or calcification were excluded. All samples were kept in 5% sodium hypochlorite (NaOCl, Whitedentmed, Erhan Kimya, İzmir, Turkey) for 2 h and any soft tissue remnants on the root surfaces were removed with scalers. The teeth were further examined under microscope (Zeiss Stemi 2000-C; Carl Zeiss Microlmaging, Göttingen, Germany) with a magnification of  $\times$  20 to discard the teeth with microcracks and craze lines. The teeth were kept in physiological saline at 4°C until they were used. The teeth were decoronated until 15 mm roots were obtained. Working length for each tooth was determined by progressing a size-10 K-file (Mani Inc. Tochigi, Japan) until it was visible at the apex and subtracting 1 mm from this length. All working lengths were recorded for canal preparation. All root canals were checked whether a size-20 K-file may passively reach to the working length and did not extrude the apical foramen. Teeth which do not meet this criteria were excluded. Thus, using a R40 Reciproc file (VDW), operated in a torque-controlled motor (Silver Reciproc; VDW) with "RECIPROC ALL" mode, was indicated according to the manufacturer's instructions. All irrigation and preparation procedures were performed by a single endodontist.

The experimental design for debris collection was based on the study by Myers and Montgomery.<sup>13</sup> Prior to canal preparation, weight of Eppendorf tubes (Labosel, İstanbul, Turkey) (1 for each tooth) was determined by using an electronic balance (Kern, Balingen, Germany) with an accuracy of  $10^{-4}$ g (g). Each tube was weighted 3 times (excluding their taps) and the average of 3 measurements was recorded as the weight of the tube. Then, the taps of the tubes were placed and a hole was created. The teeth were inserted throughout these holes. The opening between the teeth and the taps was sealed with cyanoacrylate (Pattex Super Glue; Türk Henkel, Inc., Istanbul, Turkey). A 24 gauge needle was inserted into the taps to equalize the pressure. Each tube was fitted into a bottle.

The teeth were divided into 6 equal groups according (n = 15) to a previously applied similar irrigation protocol.<sup>14</sup> The irrigation protocol were as follows.

#### Group 1

Prior to preparation, canals were rinsed with1 mL of 17% EDTA-liquid (Werax, Spot Dis Deposu AS, İzmir, Turkey). Immediately a R40 Reciproc instrument (VDW) was

progressed into the canal while the canal was filled with that chelating solution. After 3 pecking motion (nearly 9 s), canals were rinsed with 1 mL distilled water to remove EDTA. The flutes of the file were cleaned with sterile gauze and apical patency was checked with a size-10 K-file after three pecking motions. The canal again rinsed with 1 mL of EDTA-liquid and this biomechanical preparation cycle was repeated until the working length was reached. In each turn, it was paid attention to the presence of EDTA-liquid in root canals during instrumentation. Total volume of the EDTA-liquid and distilled water for each tooth was 10 mL and 10 mL respectively. At the end of preparation, each canal was rinsed with 5 mL distilled water.

### Group 2

All steps were similar to group 1 except; EDTA-gel (Werax) was applied into the canal by coating the gel around the active part of Reciproc files. After 3 pecking motion, canals were rinsed with 1 mL distilled water. Fresh EDTA-gel was coated around the file and instrumentation was continued by this way until the working length was reached. Total volume of both the EDTA-gel and distilled water for each tooth was 1 mL and 10 mL respectively. At the end of preparation, each canal was rinsed with 5 mL distilled water.

#### Group 3

All steps were similar to group 1 except 7% maleic acid solution (Sigma Aldrich, Saint Louis, MO, USA) was used instead of EDTA.

#### Group 4

All steps were similar to group 1 except 2.25% peracetic acid solution (Sigma Aldrich Chemie, Steinheim, Germany) was used instead of EDTA.

#### Group 5

All steps were similar to group 1 except 10% citric acid (Merck KGaA, Damstadt, Germany) instead of EDTA.

#### Group 6

In this group no chelating agent was used. Root canals were rinsed with 5% NaOCl throughout all preparation process with a total volume of 10 mL. Between each 3 pecking motion canals were rinsed with 1 mL distilled water. Total volume of distilled water used between each pecking motion was 10 mL. At the end of the preparation 5 mL of distilled water used as in other groups.

All irrigation procedures were performed with a 30gauge double side-vented needle (i-Tips, idental, Siauliai, Lithuania) which was placed 2 mm short of working length without tightening in the canal to provide standardization. To standardize flow rate of irrigation solutions each 2 mL irrigation was performed in 60 s. The contact time of each chelating agent during the root canal preparation did not exceed more than 40 s and the root canal was immediately irrigated with 1 mL distilled water to prevent the destructive effects of chelators on the mineralized dentin. Each file was used for the preparation of three roots.

Following root canal preparation root canals dried with paper points, taps of the tubes (with the teeth and needles inserted to them) were removed. Each root surface was rinsed with 1 mL distilled water into its own Eppendorf tube. All tubes were kept in incubator at  $37 \degree C$  for 1 week to provide the evaporation of moisture and solutions in the tubes. Then, each pre-weighted tube was again weighted 3 times. The average of these 3 measurements was recorded as the last weight of the tube. The amount of apically extruded debris for each sample was calculated in g by subtracting the initial weight of the tube from the last weight.

The surface tensions of investigated liquid chelating agent, NaOCl and distilled water as a reference were measured at room temperature (22–23 °C) with du Noüy ring digital tensiometer (KSV Sigma 702, Finland). For each analysis, the du Noüy ring was cleaned using water, ethanol and acetone, and dried before each measurement. The tensiometer was calibrated and checked by measuring the surface tension of distilled water. To determine the surface tension of a liquid sample, the du Noüy ring was firstly immersed into the liquid. Then the ring was slowly lifted, during this period the liquid raised and form a meniscus. The force needed to detach the ring from the liquid surface was measured. The maximum force reading on the scale was recorded as a surface tension of the liquid. For each sample, ten measurements were performed and recorded.

One way analysis of variance were used to compare the continuous normal data among groups. For post-hoc comparisons between the pair-wise groups, the Tamhane's T2 test was used for apical extrusion and the Tukey for surface tension. Pearson's coefficient was used to compare the association between apical extrusion and surface tension. The level of significance was set to 0.05. Analyses were performed using SPSS 19 (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY, USA).

## Results

The mean amount of apically extruded debris for each group was represented in Table 1. EDTA-gel resulted in the

Table 1 Distribution of ap	Distribution of apical extrusion by groups.		
Groups	Mean $\pm$ Standard Deviation (g)		
G-1 EDTA-liq	$0.140 \pm 0.049^{a,b}$		
G-2 EDTA-gel	$\textbf{0.040} \pm \textbf{0.025^c}$		
G-3 MA	$0.111 \pm 0.030^{a,d}$		
G-4 PAA	$\textbf{0.067} \pm \textbf{0.020^c}$		
G-5 CA	$0.082 \pm 0.054^{b,c,d}$		
G-6 NaOCl	$0.170 \pm 0.066^{a}$		

Different superscript letters indicate a statistically significant difference.

EDTA; ethylenediaminetetraacetic acid, liq: liquid, MA; maleic acid, PAA; peracetic acid, CA; citric acid, NaOCl; sodium hypochlorite, g; gram.

least amount of debris extrusion while NaOCl lead to the most (P < 0.05). EDTA-gel and peracetic acid extruded significantly less debris than the other groups (P < 0.05) except citric acid. NaOCl caused significantly more debris extrusion than the other groups (P < 0.05) except EDTA-liquid and maleic acid.

The mean values of the surface tension of investigated irrigants are shown in Table 2. There were significant differences (P < 0.05) among all groups. Distilled water as a reference irrigant, showed the highest surface tension ( $70.36 \pm 1.15 \text{ mN/m}$ ) whereas that of the EDTA-liquid was found to be lowest ( $59.26 \pm 0.98 \text{ mN/m}$ ) (P < 0.05). The Tukey test showed that NaOCl ( $70.36 \pm 0.71 \text{ mN/m}$ ) showed significantly higher surface tension than other investigated irrigants (P < 0.05). EDTA-liquid and peracetic acid had a significantly lower surface tension than other irrigants (P < 0.05). Maleic acid ( $65.68 \pm 0.53 \text{ mN/m}$ ) had a statistically different surface tension from the other irrigants (P < 0.05).

When the relationship between apically extruded debris and surface tension of investigated irrigants was examined, the Pearson's correlation test showed no significant correlation between the two parameters (P > 0.05) (Table 3). Peracetic acid and NaOCl have a positive correlation between the results of surface tension and the amount of apical extruded debris, respectively r = +0.304 and r = +0.106. EDTA-liq, maleic acid and citric acid have a negative correlation between the results of surface tension and the amount of apical extruded debris, respectively r = -0.117, r = -0.404 and r = -0.208.

## Discussion

Apical extrusion of debris is an important issue since it is one of the major causes of postoperative apical periodontitis due to its microorganism content.<sup>6,15</sup> Previous studies evaluated either debris extrusion with different rotary instruments with different instrument designs and motion styles or the effect of needle type on apical extrusion.<sup>1,3,15,16</sup> Those studies generally focused on the amount of apically extruded debris during the use of different rotary instruments and after the use of files, distilled water or NaOCl were used for canal irrigation.<sup>1,3,4,16</sup> However, only one research was attempted to understand whether

Table 2 irrigants.	Mean surface tension values of investigated
Groups	Mean $\pm$ Standard Deviation (mN/m)
EDTA-liq	$59.26\pm0.98^{\mathtt{a}}$
MA	$65.68 \pm \mathbf{0.53^{b}}$
PAA	$59.86 \pm \mathbf{0.64^a}$
CA	$69.40 \pm \mathbf{0.35^c}$
NaOCl	$\textbf{70.36} \pm \textbf{0.71}^{d}$
DW	$\textbf{70.36} \pm \textbf{1.15}^{c,d}$

Different superscript letters indicate a statistically significant difference.

EDTA; ethylenediaminetetraacetic acid, liq: liquid, MA; maleic acid, PAA; peracetic acid, CA; citric acid, NaOCl; sodium hypochlorite, DW; distilled water, mN/m; millinewtons/meter.

Table 3	Pearson correlation between surface tension and
apical ext	rusion of investigated irrigants.

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Groups	r	Р
EDTA-liq	-0.117	0.747
MA	-0.404	0.247
PAA	+0.304	0.394
CA	-0.208	0.564
NaOCl	+0.106	0.772

EDTA; ethylenediaminetetraacetic acid, liq: liquid, MA; maleic acid, PAA; peracetic acid, CA; citric acid, NaOCl; sodium hypochlorite.

irrigation solutions may contribute to minimize the amount of apically extruded debris by Parirokh et al.<sup>7</sup> They investigated the effect of 2% chlorhexidine, 2.5% and 5.25% NaOCl on the amount of apical extrusion during root canal preparation. They reported that 5.25% NaOCl showed significantly greater amount of apically extruded debris. Their results showed that either the type of the irrigant or its concentration may affect the amount of apically extruded debris. To the best of our knowledge the presence of any chelating agent during canal preparation was not properly stated. Thus, the effect of chelating agents over the amount of extruded debris remained unclear.

According to our results, although the difference among EDTA-gel, peracetic acid and citric acid is not significant, the least amount of debris extrusion was observed with EDTA-gel. This may be presumably related to its gel form which helps to keep all dentinal debris removed during instrumentation in a muddy structure and removes throughout canal orifice attached to the instrument leading to minimal apical extrusion. Chelators were initially manufactured in liquid form. However, gel (or paste) forms have been more widely used with rotary files in order to provide lubrication-particularly in narrow canals and prevent instrument separations. All tested agents included in the present study except EDTA-gel, are in liquid (or solution) form. However, the results differentiated according to the solution kept in the canals during instrumentation. Peracetic acid resulted in lower amount of debris extrusion compared to maleic acid, EDTA-liquid and NaOCl. Thus, the null hypothesis (i) was rejected.

The lower surface tension of irrigation solution increases their wetting ability and allowing penetrating more into dentine. This was previously reported by Abou-Rass and Patonai<sup>17</sup> who stated that the less surface tension of irrigant the more the flow and penetration of them into root canals. In the present study EDTA-liquid and peracetic acid, which are not significantly different from each other, showed significantly lower surface tension than other irrigants. NaOCl, which no significant difference from distilled water, showed significantly higher surface tension than other irrigants. In contrast to these results, distilled water  $(72.13 \text{ mJ} \text{ m}^{-2})$  showed significantly different surface tension value than 5.25% NaOCl (48.90 mJ m<sup>-2</sup>).<sup>18</sup> Similarly, in another study, 17%EDTA, 5.25% NaOCl and distilled water showed significantly different surface tension value from each other (46.8, 49.0 and 72.1, mJ  $m^{-2}$ , respectively).<sup>19</sup> Tasman et al.<sup>20</sup> reported significantly different surface

tension values of 2.5% NaOCl, 5% NaOCl, distilled water and 17% EDTA (41, 43, 70 and 46 dyne/cm, respectively). These differences may be attributable to the variables in the irrigation solution's concentration and the used experimental design, and equipment.

It has been suggested that irrigation solutions with low surface tension can wet the dentin walls better and allow the irrigation solution to penetrate deeper.<sup>19,21,22</sup> However, it was shown that the reduced surface tension of different endodontic chelator solutions did not improve their calcium removal capacity from the root canal walls.<sup>23</sup> When the correlation between apically extruded debris and surface tension of used irrigants was investigated, no significant relation was observed. Therefore the null hypothesis (ii) was accepted. The results of this study were not compared properly due to there has been no similar previous studies comparing the surface tension of endodontic irrigants and its effect on apical extrusion. Therefore, further investigations are needed to clarify the effect of surface tension on apical debris extrusion.

Another possible explanation for the results of the present study may be the statements of Hülsmann et al.<sup>22</sup> who reported that root canal preparation with chelators shortens the contact time of instruments with canal walls and thereby decreases the amount of debris removed from root dentine. The different chelating solutions in the present study might have provided different lubrication conditions leading to different amounts of debris formation. Thus, the different amounts of apically extruded debris in the present study may be mainly related to the amount of intra-canal debris formation resulting from the difference in preparation time. A previous study showed single-file systems were associated with a reduced preparation time than multi file systems.<sup>15</sup> In the present study, a single file instrument working with reciprocal motion was used during the root canal preparation. So that the amount of intracanal debris formation may be affected by irrigation solutions, preparation time and teeth micro-structure more than file system. A limitation of this study was that, it did not use a different rotary file with a different motion principles as a control and the preparation time was not recorded for each irrigation solution.

Reciproc is one of the contemporary single file instruments working with reciprocal motion-a modification of balanced force technique. In the present study, canals were prepared with Reciproc instruments for its currency. On the other hand, studies over this subject included mandibular premolar teeth.<sup>1,3-5,15</sup> According to the study of Li et al.<sup>24</sup> compaction of debris results in hardness in flushing the debris out and causes more extrusion. This situation is more prominent in mandibular incisor canals owing narrower morphology. For this reason different from the previous studies, we preferred to use mandibular incisor teeth with narrower canals compared to mandibular premolars to gain more compaction and hardness and to better compare the effect of chelator on apical extrusion. This may explain why the most mean extrusion weight is 0.170 g for the present study while the previous studies with Reciproc reported averages of 0.00121 g and 0.00167 g.<sup>5,15</sup> There are conflicting results in the literature that the width of apical opening may increase or decrease the amount of apically extruded debris.<sup>25,26</sup> Therefore in the present study only the teeth with apical width correspond to a size-20 K-file were included.

It was previously reported that apical debris extrusion was not observed when the irrigation solution was not used during the root canal preparation. This situation was explained by the fact that the debris creates a plug in the apical part of the root canal and preventing the extrusion.<sup>7</sup> Apical patency was maintained with a size-10 K-file after three pecking motions to prevent apical plug formation.

The tip design of needle comprehensively influences the apical extrusion of the debris.<sup>16</sup> An open end needle allows the irrigation solutions to move through the apical foramen rather than root canal walls. Thus creating high pressure in the apical region of the canal can lead to irrigation solutions and debris extrusion.<sup>16,27</sup> Silva et al.<sup>16</sup> report double side-vented needles creates less debris extrusion than open and side-vented needles in mild and moderate curved root canal. For this reason, double side-vented needles were used for irrigation procedures in the present study.

The present experimental model was also used in previous studies.<sup>1,3,15,16</sup> However, as reported by Karatas et al.,<sup>3</sup> it lacks the ability of simulating the back pressure provided by physiological periodontal tissues which limits the extrusion of debris. Thus, the present findings do not exactly render clinical results. Furthermore, development of apical periodontitis after root canal preparation is related with not only the amount of apically extruded debris, but also the immunological response of the subject which may vary. Mechanical and chemical irritations during preparation may also initiate apical periodontitis.<sup>6</sup> Thus, it can be stated that ex-vivo conditions are not capable of fully reflect the intra-oral events. However, the results of the present study reveals that apically extruded debris which is a major cause of post treatment flare-up can be decreased by selecting the correct chelator as intra-canal lubricant during canal preparation with rotary instruments. Due to the lack of a similar previous study, we are unable to perform a proper comparison and evaluation. Further studies may be beneficial to better clarify this issue.

Within the limitations of the present study, all examined agents extruded debris beyond the apical foramen. The presence of EDTA-gel, peracetic acid and citric acid in root canals during preparation with single-file reciprocating system seems advantageous to reduce the amount of apically extruded debris. There was no significant correlation exists between the surface tension of investigated irrigants and their amount of apical extrusion. Further investigations are required over this issue.

## **Declaration of Competing Interest**

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

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