Abstract: The present study aimed to evaluate the degree of crack formation during canal preparation using reciprocating files. A total of 120 incisor teeth were divided into eight groups according to irrigation protocol (n = 15). The irrigation solution and chelating agents included distilled water, sodium hypochlorite (NaOCl), citric acid, maleic acid, peracetic acid, ethylenediaminetetraacetic acid (EDTA) gel, and EDTA liquid. The root canals were prepared using reciprocating instruments while one of the agents was present within the root canals. A control group was subjected to neither preparation nor irrigation. The roots were horizontally sectioned 3, 6, and 9 mm away from the apices. The numbers of cracks were recorded. Statistical analyses were performed using a chi-squared test. The chelating agents resulted in similar numbers of cracked sections. The use of one of the chelating agents during preparation using reciprocating instruments appears to be beneficial in reducing crack formation.

Keywords: chelating agents; citric acid; crack formation; EDTA; maleic acid; peracetic acid.

Introduction
Vertical root fracture (VRF) is a common sequel of root canal treatment, which can initiate from small cracks and/or craze lines present in the root dentin (1). These structural defects generally occur during canal preparation with rotary instruments (2). Stress generated on the root canal walls during preparation may cause crack formation, which may subsequently initiate VRF under continuing chewing forces (3). VRF is one of the main causes of post-endodontic extractions (4). Furthermore, bacteria can proliferate in crack lines and later establish biofilms on the root surface (5). Therefore, attempts to reduce the risk of dentinal crack formation are crucial for the long-term prognosis of root-filled teeth. In particular, more recent, time-saving, single-file systems with increased tapers may require the application of higher forces and result in a larger number of cracks than full-sequence file systems, which enhances the inquietudes of crack formation (6). Reciproc (VDW, GmbH, Munich, Germany) is one of these single-file systems; it includes three sizes of instrument (R25, R40, and R50), and one of these can be selected for use following consideration of the initial size of the root canals. These instruments are manufactured using M-wire technology, a special heat treatment, which provides the instrument with greater strength and flexibility. This system works using reciprocal motion; reciprocal motion is defined as a repeated clockwise rotation (release of the instrument) for 30° and counterclockwise rotation (cutting action) for 150°C.

Previous studies have evaluated the effects of motion style (6,7) and physical properties, including cross-section geometry and degree of the taper (1,8), on dentinal crack
formation. However, attempts to reduce the risk of crack formation have been limited. In previous studies (9-11), the effects of maintaining ethylenediaminetetraacetic acid (EDTA) within the root canals during preparation on crack formation were evaluated; these studies reported that the presence of EDTA during root canal preparation did not reduce dentinal crack formation. In recent years, other organic acids, including maleic acid (12), citric acid (13), and peracetic acid (14), have been shown to act as an inorganic tissue remover, similar to EDTA. However, their possible effects on dentinal crack formation have not been evaluated. Therefore, the present study aimed to determine whether these chelating agents can reduce dentinal crack formation. The null hypothesis is that the presence of maleic acid, citric acid, and peracetic acid does not affect the degree of dentinal crack formation in comparison with sodium hypochlorite (NaOCl).

**Materials and Methods**

**Sample preparation**

The present study was approved by the Ethics Committee of Gaziosmanpaşa University (17-KAEK-036) and included 120 extracted intact human mandibular incisor teeth with mature apices, free of any hard tissue defect. The presence of only one straight canal was confirmed with radiographs taken from the mesio-distal and buccolingual directions. All samples were stored in thymol solution until use. Any soft tissue remnants on the root surfaces were removed with scalers. The teeth were further examined under a microscope (Zeiss Stemi 2000-C; Carl Zeiss MicroImaging, Göttingen, Germany) with a magnification of 25× to discard any teeth with microcracks or craze lines on their outer surfaces. The teeth were decoronated until 15 mm of roots were obtained. The working length for each tooth was determined by radiographs taken from the mesio-distal and buccolingual directions. The total volumes of EDTA were recorded for each tooth were 10 mL and 10 mL, respectively. All working lengths were recorded for each tooth was determined by progressing a size-10 K-file (Mani Inc., Utsunomiya, Japan) until it was visible at the apex and subtracting 1 mm from this length. All working lengths were recorded for each sample preparation. All root canals were checked to confirm that a size-20 K-file was able to passively reach the working length but not extrude the apical foramen. No glide path was created, only a size-20 K-file was used to check the canal space. Teeth that did not meet this criterion were excluded. This was performed as R40 Reciproc instruments (VDW) were selected for canal preparation and the use of these files is indicated by the manufacturer if a size-20 hand file passively enters the canal and fits the working length. All roots were coated with a fine impression material (Impregum Soft; 3M ESPE, Saint Paul, MN, USA) to simulate the periodontal membrane and then embedded in acrylic blocks.

**Root canal preparation and irrigation**

Samples were divided into eight equal groups (n = 15) according to the irrigation protocol:

- **Group 1**: Prior to preparation, the canals were rinsed with 1 mL of 17% EDTA liquid (Werax, Spot Dis Deposu AS, İzmir, Turkey). A R40 Reciproc instrument (VDW) operated in a torque-controlled motor (Silver Reciproc; VDW) with “RECIPROC ALL” mode, was progressed into the canal while the canal was filled with EDTA liquid. Following three pecking motions, the canals were rinsed with 1 mL distilled water and rinsed again with 1 mL of EDTA liquid. The flutes of the file were cleaned with sterile gauze and the apical patency was checked with a size-10 K-file following three pecking motions. This cycle was repeated until the working length was reached. In each turn, the presence of EDTA liquid in the root canals during instrumentation was assessed. The total volumes of EDTA liquid and distilled water for each tooth were 10 mL and 10 mL, respectively. At the end of preparation, each canal was rinsed with an additional 5 mL of distilled water.

- **Group 2**: All steps were similar to group 1, with the exception that 17% EDTA gel (Werax) was applied into the canal by coating the gel around the active part of the Reciproc files. Following three pecking motions, the canals were rinsed with 1 mL distilled water. Fresh EDTA gel was coated around the file and instrumentation was continued in this manner until the working length was reached. The total volumes of EDTA gel and distilled water for each tooth were 10 mL and 10 mL, respectively. At the end of preparation, each canal was rinsed with 5 mL of distilled water.

- **Group 3**: All steps were similar to group 1, with the exception that 7% maleic acid solution (Sigma-Aldrich, Saint Louis, MO, USA) was used instead of EDTA.

- **Group 4**: All steps were similar to group 1, with the exception that 2.25% peracetic acid solution (Sigma-Aldrich Chemie, Steinheim, Germany) was used instead of EDTA.

- **Group 5**: All steps were similar to group 1, with the exception that 10% citric acid solution (Merck KGaA, Darmstadt, Germany) was used instead of EDTA.

- **Group 6**: In this group, no chelating agent was used. The root canals were rinsed only with 5% NaOCl throughout the preparation process with a total volume of 10 mL. Between all three pecking motions, the canals were rinsed with 1 mL distilled water. The 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.

- **Group 7**: All steps were similar to those of group 6,
with the exception that distilled water was used instead of NaOCl. The total volume of distilled water used between each pecking motion was 20 mL. At the end of the preparation, 5 mL of distilled water was used, as in the other groups.

All irrigation procedures were performed with a 30-gauge double side-vented needle (i-Tips, i dental, Siauliai, Lithuania) and it was placed 2 mm short of the working length without tightening in the canal to provide standardization. Each file was used to prepare three roots. All root canal preparations were completed by one endodontist.

Group 8: No preparation or irrigation was performed for this group (control group).

**Sectioning and image analyses**

Following canal preparation, each root was mounted on a resin block and horizontally sectioned 3, 6, and 9 mm away from the apices using a low-speed saw rotating at 300 rpm (Metkon Microcut 201 Precision Cutter, Metkon Instruments Ltd., Bursa, Turkey) with water cooling. All sections (n = 360) were examined under a microscope with a magnification of 25× and digital images were captured with a digital camera (AxioCam ERC5s, Germany) attached to the microscope (Zeiss Stemi 2000-C) (Fig. 1). The images were checked by two endodontists in a blinded manner. Surfaces containing at least one dentinal defect, such as a crack or craze line, were recorded as “1,” which indicated a cracked surface. Surfaces free from any defects were recorded as “0.” The number of sections with any defect and their ratio to all sections in each group were recorded. Statistical analysis was performed using the chi-squared test with IBM SPSS Statistics 19 (SPSS, Inc.; IBM Corp., Somers, NY, USA).

**Results**

The numbers of cracked surfaces are shown in Table 1. The ratios of cracked surfaces are shown in Figs. 2 and 3. In the overall evaluation of the study groups, the NaOCl group had the largest number of sections with cracks compared with the chelating agents (P < 0.05), but there was no significant difference between distilled water (P > 0.05). The differences among the chelating solutions were not statistically significant (P > 0.05). In the 3- and 6-mm sections, the NaOCl group had significantly more cracks than the chelating agents (P < 0.05), but did not show a significant difference with distilled water (P > 0.05). In the 9-mm sections, the distilled water group had more cracks than the other groups; however, the difference was not significant in the overall evaluation (P > 0.05).

![Fig. 1](image-url) Representative microscopic images of samples. Arrows point at dentinal defects. (a) Citric acid at 6 mm, (b) EDTA gel at 6 mm, (c) EDTA liquid at 9 mm, (d) NaOCl at 6 mm, (e) maleic acid at 6 mm without any dentinal defect, (f) maleic acid at 3 mm, (g) peracetic acid at 6 mm, and (h) distilled water at 3 mm. EDTA: ethylenediaminetetraacetic acid.

![Table 1](table-url) Distribution of the numbers of cracks by thickness and groups

| Group            | 3 mm | 6 mm | 9 mm | Total |
|------------------|------|------|------|-------|
| Control          | 15   | 0    | 15   | 45    |
| Peracetic acid   | 9    | 5    | 11   | 34    |
| Citric acid      | 10   | 5    | 12   | 35    |
| EDTA gel         | 9    | 6    | 11   | 34    |
| EDTA liquid      | 10   | 5    | 12   | 33    |
| Maleic acid      | 10   | 5    | 12   | 34    |
| NaOCl            | 1    | 14   | 2    | 12    |
| Distilled water  | 3    | 12   | 4    | 15    |
| Total            | 64   | 41   | 74   | 228   |

| P     | <0.001 | <0.001 | 0.053 | <0.001 |

0 indicates the number of surfaces free of cracks; 1 indicates the number surfaces with the presence of cracks. Different superscript letters represent the differences among the groups.

Different superscript letters indicate statistically significant values (P < 0.05). NaOCl: sodium hypochlorite; EDTA: ethylenediaminetetraacetic acid.
Discussion

Despite the fact that the use of single-file reciprocating instruments has increased in popularity in recent years, using one instrument with a high taper throughout the whole working length leads to increased stress generation on canal walls, particularly in the apical region, compared with full-sequence systems (6). The reason why using the Reciproc system is preferred in the present study is its widespread use, presumably relating to its increased strength and time-saving features. R40 was used for preparation as it was reported in a previous study (15) that size-35 apical enlargement is a threshold for the significant reduction of intra-canal bacteria regardless of the irrigant used. However, using R40 files with a 0.6 taper for the first 3 mm may lead to high stress generation in the apical root segment. This is evident in the present study, which showed a large number of cracked sections in the apical regions of all groups. Furthermore, the risk of root fracture increases with the quantity of tooth structure removed (16). Therefore, any attempt to reduce stress generation during preparation with single-file instruments may contribute to long-term success. The results of the present study revealed that the presence of one of the analyzed chelating agents during instrumentation reduced crack formation at 3-6 mm and in the total evaluation, compared with the NaOCl and distilled water groups. Therefore, the null hypothesis was rejected. The results of the present study suggest that maintaining the presence of one of the chelating solutions in root canals during instrumentation with Reciproc instruments is beneficial. Previous studies stated that using either rotary full-sequence or reciprocating single-file instruments with EDTA gel (10,11) or the presence of RC Prep (containing EDTA, Well-Prep, Vericom Co., Anyang, Republic of Korea) (9) in root canals did not reduce crack formation. In the present study, chelating agents resulted in a lower number of cracked sections than that following the use of NaOCl or distilled water. The discrepancy in the results may be correlated with the combined use of NaOCl and EDTA/RC Prep in previous studies, while no NaOCl was used in the present study (with the exception of group 6). A study by Ribeiro et al. reported that NaOCl degrades the organic matrix of root dentin, whereas EDTA binds to the calcium and phosphate ions of the hydroxyapatite structure, which leads to decreased yield strength and increased fracture tendency (17). Sim et al. reported that a high concentration of NaOCl reduces the physical strength of root dentin (18). This may be the reason why the NaOCl group exhibited the largest number of cracked surfaces in the present study. The combined use of NaOCl and EDTA in previous studies suggest that they may have synergistic effects in increasing crack occurrence. According to the present study, it can be advised that instrumentation with Reciproc single-file instruments should be performed in the presence of one of the chelating agents, and NaOCl irrigation could be minimized. Once the working length is reached, irrigation with NaOCl can be initiated or enhanced. Both liquid and gel forms of EDTA are capable of chelating root dentin, with the gel form generally preferred for the purpose of
lubrication. According to the present study, the results for both liquid and gel forms of EDTA showed the same degree of crack formation. This can be explained by the similar concentration and quantity of the two different forms.

The distilled water group, which had no harmful effect on the microstructure of dentin, caused a statistically similar number of cracks when compared with the NaOCl group. This result shows that the chelators assessed reduced the formation of cracks by the positive effect on dentine during root canal preparation. Limited data are available in the literature on the effect of irrigation solutions on crack formation during root canal preparation. Adl et al. reported that saline caused fewer cracks than NaOCl, although without a significant difference (9). This result is in agreement with the results of the present study. However, Adl et al.’s study also reported that saline caused significantly fewer cracks than NaOCl, regardless of whether RC Prep was used (9). These differences may be attributed to variations in the preparation technique and irrigation protocol. Therefore, further investigations with different irrigants are required to investigate the effect of dentinal defect formation.

Unlike previous studies, the present study further evaluated the effect of the presence of different chelators, including peracetic acid, citric acid, and maleic acid, in root canals on crack formation during preparation. However, the results showed that none of these agents was superior in reducing crack formation. In a study by Hasheminia et al., the smear layer-removing capacity of 5% maleic acid and 17% EDTA was found to be similar (12). In other previous studies (13,14), the effects of peracetic acid and citric acid on smear layer removal were also similar. These similarities are associated with their similar mechanisms of action, which act on the calcium/phosphate ratio of root dentin. This is an important factor for the physical properties of dentin. This may explain why none of the solutions examined in the present study were superior in terms of dentinal crack formation. However, a study by Viola et al. reported that 1% peracetic acid was more cytoxic than 2.5% NaOCl (19). This was also true for 17% EDTA, whereas citric acid was less cytoxic (20). The relative biocompatibility of citric acid may be a matter of choice. Furthermore, the concentration of these chelating agents may affect the degree of change in the physical properties of root dentin. For this reason, the chelating agent included in the present study may serve different results unlike this study in terms of dentinal crack formation when used at different concentrations.

Another consideration in the action of chelating agents is their effect on instrumentation duration. Hulsmann et al. reported that chelating agents reduce the extent of smear formation by reducing the time required for instrumentation, and this is more important than the type of chelating agent (21). It is possible that the chelating agents used in the present study may have resulted in similar instrumentation intervals and thus led to similar levels of crack formation. However, the preparation time for the groups was not considered in the present study. The association between preparation time and crack formation may be investigated further.

A study by Anderson et al. found that RC prep reduced preparation stresses by 64% using GT instruments and by 27% using the ProFile system, compared to saline (22). In the present study, NaOCl and distilled water, which have no chelating effect, led to more cracks compared with the other groups. Therefore, further investigations including different instrumentation protocols (i.e., glide path preparation using 0.3 and/or 0.4 rotary instruments prior to Reciproc) with the chelating agents assessed in the present study may result in more valuable outcomes relating to this issue.

A study by Rivera and Walton reported that maxillary premolars, and mesial roots of mandibular molars and mandibular incisors with root canals narrower in the mesio-distal dimension compared with their bucco-lingual width were more prone to fractures (23). This was associated with their narrow and oval-shaped canal morphology, which may require more force during preparation. Ruckman et al. reported that these oval-shaped canals are relatively more common (24). For these reasons, mandibular incisor teeth were included in the present study. Mandibular incisors are more prone to be affected by forces generated during instrumentation due to their smaller dimensions and thin dentinal walls (25). However, the accurate simulation of clinical conditions is not possible. As stated by Burklein et al., even the storage medium of samples may affect their mechanical properties (6). The results of in vitro studies cannot be fully consistent with clinical conditions. Furthermore, the periodontal membrane, which dissipates occlusal forces is absent in in vitro conditions and this may affect the results. Therefore, the presence of any artificial structure to simulate the periodontal membrane is essential in studies. For this reason, in the present study, the roots of the specimens were coated with a thin layer of impression material to simulate the periodontal ligament. These polyether-based elastomeric materials are capable of simulating the periodontal membrane due to their viscoelastic properties being similar to those of periodontal membrane (25).
According to the results of the present study, the presence of one of the chelating agents assessed during instrumentation with Reciproc files appears to be beneficial in terms of crack formation. This is presumably related to the stress-reducing effect of these agents. However, whether these effects occur as a result of lubrication or changes in mineral content as a result of chelation remain to be elucidated. Further in vitro and in vivo studies are required to examine this.

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Conflict of interest
None declared.

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