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Efficacy of two different retreatment techniques in removing gutta-percha from root canals: A CBCT study

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Purpose: This study aimed to compare the efficacy of two rotary nickel-titanium file systems, ProTaper Universal (PTUR) and Remover (REM), in removing gutta-percha and sealer from root canals.

Methods: Forty-five extracted maxillary incisors were selected and prepared with the Revo-S file system. Root canals were filled with gutta-percha and a resin-based sealer using cold lateral condensation. The teeth were divided into three groups (n = 15) based on the file system used for gutta-percha removal: PTUR, REM, and hand files (H-files, as a control). The time taken for each system to remove the root canal filling was recorded. Three-dimensional images were obtained using dental volumetric tomography to measure the volume of the remaining filling material.

Results: Statistical analysis revealed a significant difference in the time required for retreatment and the percentage of residual gutta-percha among the PTUR, REM, and H-file groups ($P < 0.05$). The H-file group required the longest retreatment duration and left the highest percentage of residual material. While the REM group completed the retreatment faster than the PTUR group, this difference was not statistically significant.

Conclusion: The PTUR and REM systems were more efficient and faster than hand files in removing root canal fillings, although none achieved complete removal.

Keywords: Gutta-percha; root canal retreatment; rotary files.

Introduction

Endodontic therapy, commonly referred to as root canal treatment (RCT), is crucial for eradicating infections from the root canal system and safeguarding against future infections. The effectiveness of this treatment depends on several factors, such as complete removal of the infected pulp, meticulous cleaning and shaping of the canal, and accurate filling to protect against subsequent microbial incursions (1). Even with substantial improvements in endodontic procedures, treatment failures can still arise from insufficient disinfection, overlooked anatomical features, inadequate filling, or coronal leakage (2,3).

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When RCT fails, retreatment is often necessary to save the tooth. This process entails the removal of the existing root canal filling, along with subsequent re-cleaning, shaping, and -filling of the canals. Such procedures present multiple challenges, including the removal of established root canal fillings, the potential for perforation, and the imperative to preserve the tooth's structural integrity (4,5). The effectiveness of retreatment is highly contingent upon the thorough removal of the existing filling, which is essential for successful re-instrumentation and disinfection (4).

There have been several improvements to nickel-titanium (NiTi) endodontic file systems that make root canal cleaning and shaping more effective (6,7). Improved canal shape and increased safety during canal preparation have led to the development of novel file designs that incorporate features such as non-cutting tips, radial lands, variable tapers, altered rake angles, heat-treated materials, and modified pitch lengths. It has been suggested that motor-driven NiTi files be used to remove infill materials from canal walls. Several experiments have shown that these files are effective, clean well, and reliable (8).

Numerous research in the literature the efficacy of nickel-titanium rotary files for retreatment (9-12). These studies compare the efficacy of new-generation rotary canal files; however, none assessed all the recently released retreatment files collectively.

The ProTaper Universal Retreatment (PTUR) is a rotary file system designed by Dentsply, Maillefer, Ballaigues, Switzerland. It is specifically developed for the purpose of removing fillings from root canals. This system consists of 3 files, each with different lengths and tapers, specifically designed to sequentially eliminate fillings from various sections of the canal. The files are used in the following sequence: D1, which has a taper of 30/09 and a length of 16 mm, is utilized for removing filling from the coronal third. D2, with a taper of 25/08 and a length of 18 mm, is then applied to the middle third. Finally, D3, with a taper of 20/07 and a length of 22 mm, is specifically designed for the apical third (13).

In 2020, Coltène/Whaledent (Altstätten, Switzerland) introduced the Remover (REM) file system, an innovative file designed specifically for the extraction of root fillings. The REM is a single 30.07 file with a variable offset blade, a non-cutting tip, and a triple helix design. Constructed from NiTi, it undergoes a patented C-wire heat treatment, significantly enhancing its flexibility and shape memory, enabling the file to be prebent for improved maneuverability during procedures (14). To date, there are limited studies investigating its cyclic fatigue resistance, debris extrusion, and effectiveness in removing gutta-percha root fillings (14-16).

Cone-beam computed tomography (CBCT) is commonly used in endodontics, and has higher efficacy than conventional radiography for diagnosis of periapical pathologies and internal and external root resorption defects, evaluation of root canal morphology, and management of endodontic surgery (11,17). CBCT is also a precise imaging technique commonly utilized to evaluate remaining fillings in root canals after retreatment without causing damage to the tooth structure. This method helps in preserving the overall integrity of the treated tooth. CBCT generates extremely thin slices and provides accurate three-dimensional (3D) reconstructions with cubic voxels and consistent resolution (18).

This study aimed to compare the efficacy of 2 rotary nickel-titanium file systems [ProTaper Universal (PTUR); Dentsply Maillefer, Ballaigues, Switzerland & Remover (REM); Coltène/Whaledent, Altstätten, Switzerland] to remove the gutta-percha and sealer. The first null hypothesis states that there is no notable difference among these file systems in their ability to remove root canal filling. The second null hypothesis is that there is no difference in the time required for filling removal across all tested file systems.

Materials and Methods

Power analysis was calculated with G*Power version 3.1.9.4 software (Universitat Kiel, Kiel, Germany). Have been investigated effect size of 0.70 at the significance level of $\alpha = 0.05$, the required sample size was calculated to be 36. Based on this sample size, with an effect size of 0.70, three groups, and a significance level of 0.05, the statistical power was determined to be 0.957. However, to account for potential issues such as missing data, measurement errors, and outliers, it was decided to include more samples than the required sample size. Therefore, 45 samples were included in the study. At the end of the study, the power analysis was repeated with the final sample size of 45, an effect size of 0.70, and a significance level of $\alpha = 0.05$, resulting in a recalculated statistical power of 0.987.

The study utilized 45 freshly extracted maxillary incisors from humans, which were approved by the Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University (Ethical permission number: 18/09/2020 23-15). The study was conducted under the principles of the Declaration of Helsinki. The teeth that were selected for this study had completely mature roots, straight single canals canals and radiographs that showed patency without calcification. Canals were excluded unless they had an apical diameter larger than 15 mm. After cleaning them of any debris or soft tissue remnants, the teeth were placed in a physiological saline solution and stored at 4 °C until the experiment. To reach a standard root length of 18 mm, the crowns were removed using a diamond disc. The working length (WL) was calculated by subtracting 1 mm from the measurement taken after establishing access, which was used to push a size 10 K-file into the canal until it was visible at the apical foramen.

Following the manufacturer's instructions, the root canals were instrumented using the Revo-S NiTi file system (Micro Mega, Besançon, France). The procedure started with the SC1 (25/0.06) file, which was operated at 300 rpm with a torque of 2 Ncm. The final shape was completed by using SC2 (25/0.04) and SU (25/0.06) files up to the WL. A size 10 K-file was used for recapitulation, and 2 mL of 2.5% NaOCl was used for irrigation after after each file. The next step was a 1-min application of 5 mL of 17% EDTA, followed by a 5-mL rinse with distilled water. After that, the canals were dried using paper points and filled with gutta-percha (Meta-Biomed, Cheongju, Korea) and a resin-based sealer (AdSeal; Meta-Biomed) using the cold lateral condensation technique. Digital radiographs were taken from the buccolingual (BL) and mesiodistal (MD) directions to ensure the root filling was of high quality. The samples were kept at 37 °C with 100% humidity for 2-week after the access cavities were filled with a temporary filling (Cavit G; 3M Espe, Seefeld, Germany).

Initial CBCT Scanning

The specimens were placed on rubber base blocks and then used with the KaVo OP 3D Pro imaging technology from KaVo Dental GmbH in Biberach/Riss, Germany, to create a 3D model. Each block contained five samples. In order to get axial, frontal, and sagittal planes, protocols that were designed for a voxel resolution of 0.2 mm (5×5 cm FOV), 1.2 s, 3.2 mA, 90 kVp, and 34 mGycm^2 were used. All the collected CBCT images were exported as DICOM datasets. These data were then imported into 3D image semiautomatic segmenting and voxel-counting software, ITK-SNAP (Penn Image Computing and Science Laboratory, Philadelphia, USA), for the calculation of root filling volumes. The filling material was painted to distinguish it from the surrounding structure by selecting the red label (Fig. 1a, 1b). Then, the exact volume of the root filling material from the coronal to the apical third was calculated in mm^3 by ITK-SNAP. An oral and maxillofacial radiologist with five years of experience processed and reviewed each image three times. The Intra-Class Correlation Coefficient (ICC) was calculated as 0.997. Then the results were averaged to assess intra-observer reliability.

Retreatment Procedures

Based on the retreatment technique used, the specimens were divided into 3 groups: PTUR, REM, and H-files (control) (n = 15).

PTUR Group: In a crown-down technique, PTUR files (D1-D3) were used. To remove the filling from the coronal third, the D1 file was used. Afterwards, the D2 file was used for the middle third, with a size of 25 and a 0.08 taper. At last, the D3 file, with a size of 20 and a 0.07 taper, was advanced up to the WL. Following the instructions provided by the manufacturer, minimal apical pressure was applied. A constant speed of 500 rpm and a torque of 2 Ncm were used to run all files using an endodontic motor (XSmart Plus; Dentsply Maillefer).

REM Group: Using a constant speed of 400 rpm and 3 Ncm torque, a pilot hole of 2-3 mm was drilled into the gutta-percha using the NiTi file for coronal root canal flaring, One Flare (MicroMega), as instructed by the manufacturer. Afterwards, the REM file was used in a continuous rotational mode with a torque of 2.5 Ncm and a speed of 800 rpm. According to the manufacturer's recommendations, it was progressed into the filling in the coronal and middle thirds up to 3 mm from the WL using 2-3 mm back-and-forth movements without applying apical pressure. A One Curve 25.06 manufactured MicroMega in Besançon, France, was used to prepare the apical third to the WL. The rotation was constant at 350 rpm, and the torque was 2.5 Ncm.

H-file Group (control): To remove the gutta-percha from the coronal and middle thirds of the canals, retreatment was started using Gates-Glidden drills, sizes 3 (0.9 mm) and 2 (0.7 mm) (Dentsply Maillefer). Once the WL was reached, the canals were re-instrumented using a size 25 H-file (Dentsply Maillefer), utilizing a circumferential quarter-turn push-pull technique to remove any leftover gutta-percha and sealer.

During each retreatment procedure, the file was withdrawn from the canal after every 4 strokes to remove material trapped between the flutes using a sterile sponge. Retreatment was completed until the tool reached the WL and there were no remnants left in the canal. The canals were irrigated with 10 mL of distilled water using 27 G side-vented needles while the root canal filling was being removed. Any instance of tool breakage, deformation, or canal perforation was carefully recorded.

The retreatment was considered complete when a dental operating microscope (Global Dental Microscopes, St. Louis, MO, USA) established that there were no apparent remnants of gutta-percha or sealer on the file surfaces, within the root canal, or on the dentinal walls. All groups' retreatment procedures were timed using a chronometer. A single operator carried out all the retreatment procedures during the study to ensure consistency and minimize procedural variability.

CBCT Scanning after Retreatment

At the same position as the initial scan, a second CBCT examination was performed for each root after retreatment. A second assessment was conducted using the ITK-SNAP program, which was used for red labeling and staining as in the initial CBCT scanning procedure, to determine the volume of the residual filling material after retreatment. A five-year experienced oral and maxillofacial radiologist examined, processed, and assessed each image three times. The ICC was calculated as 0.993 between them. Then the results were averaged for intra-observer reliability. The formula to determine the proportion of the residual root canal filling was to divide the volume of the original filling by the volume of the remaining filling. A CBCT device (KaVo OP 3D DVT; KaVo Dental, Biberach, Germany) was used to scan each sample individually to evaluate the amount of filling that was left. With a field of view (FOV) of 5 × 5 cm, 1.2 s, 3.2 mA, 90 kVp, and 34 mGycm², the scanning settings comprised an isotropic voxel size of 0.2 mm. (Fig. 1c, 1d).

Statistical Analysis

Statistical analysis was performed on all data using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA). The normality of the initial volumes for the groups was examined with the Kolmogorov Smirnov test and it was seen that they exhibited a normal distribution. Then, whether there was a significant difference between the initial volumes was examined with the one-way ANOVA. Each group had their average retreatment time and mean percentage of residual gutta-percha determined. A one-way ANOVA was performed after ensuring data normality, post-hoc Dunnet T3 and Games Howell tests were used to detect statistically significant differences. A significance criterion of $P < 0.05$ was established.

Results

Table 1 shows the results of variance analysis and Table 2 shows the percentage of residual gutta-percha and the time (in sec) that elapsed during retreatment using PTUR, REM, and H-files. As a result of the statistical analysis performed for the initial volumes of the groups, there was no significant difference ($P > 0.05$). The total amount of time each group requiring retreatment varied significantly, with the H-file group having the longest duration ($P < 0.05$). The difference in residual gutta-percha among the groups was statistically significant ($P < 0.05$), with the highest amount of residual gutta-percha observed in the H-file group. There was no statistically significant difference between the PTUR and REM groups in terms of residual gutta-percha amount and retreatment time.

Table 1. Shows the result of the analysis of variance for the initial volume, the percentage of residual gutta-percha, and the time (in sec) that elapsed during retreatment.

	Sum of Squares	df	Mean Square	F	Sig.
Initial Volume (mm³)					
Between Groups	1102469.511	2	551234.756	0.166	0.48
Within Groups	139566753.067	42	3323017.930		
Total	140669222,578	44			
Residual filling (%)					
Between Groups	0.179	2	0.090	3.355	0.045*
Within Groups	1.123	42	0.027		
Time					
Between Groups	2123777.911	2	1061888.956	83.187	0.000*
Within Groups	536130.667	42	12765.016		

Table 2. Shows the percentage of residual gutta-percha, the time (in sec) that elapsed during retreatment using PTUR, REM, H-files, and diameter and taper of the last file used in the apex. Different letters indicate statistically significant difference within the same column according to post-hoc Dunnet T3 and Games Howell test ($P < 0.05$).

Groups	n	Residual filling (%) mean ± SE	Time (sec) mean ± SE	Diameter / taper
ProTaperUniversal Retreatment	15	$0.14 \pm 0,02^a$	205.66 ± 9.88^A	20 / 07
Micro Mega Remover	15	0.13 ± 0.03^a	178.26 ± 9.99^A	25 / 06
Hand File	15	0.24 ± 0.06^b	652.20 ± 48.53^B	25 / 02

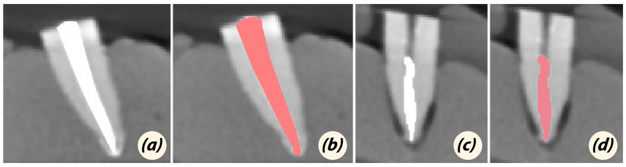

Fig. 1. CBCT images; **(a)** after root canal filling, **(b)** calculation of the volume of root canal filling, **(c)** after retreatment, **(d)** calculation of the volume of root canal filling after retreatment.

No file fractures occurred in REM group. In contrast, one D3 file fractured in the PTUR group, and two 25/02 H-files fractured in the H-file group. The affected samples were replaced with new ones. Additionally, no perforations were observed during the retreatment procedures in any of the groups.

Discussion

Since the efficacy of gutta-percha removal and time elapsed during the retreatment procedure significantly differed among the REM, PTUR, and H-file groups, two null hypotheses were rejected.

Endodontic failure is caused by persistent microbial infections in the root canal system and/or the surrounding area (19). As a result, when dealing with infections that persist or have just appeared, root canal retreatment is now favored over periradicular surgery (20). Thus, it is crucial to maximize the removal of gutta-percha and sealer during retreatment to expose any remaining necrotic tissue or bacteria that could serve as antigen sources (4).

Regardless of the use of solvents, hand file removal of gutta percha has historically been a time-consuming and laborious process, especially in cases with highly compacted root filling. Therefore, rotational NiTi tools help alleviate operator and patient discomfort during root canal retreatment (21). As a result, employing NiTi files can help alleviate fatigue for both the patient and the practitioner (22). Although many retreatment file systems have been developed for this purpose (6,7,13,22), there is no comprehensive study comparing all NiTi rotary file systems designed for retreatment. Our study compares the REM file system, about which there is limited information in the literature about its effectiveness and features, with PTUR and H-file, and it is thought that this will provide valuable data for future meta-analyses and enrich the literature.

Previous researches used different techniques to quantify the amount of gutta-percha and sealer, such as two-dimensional (2D) imaging, high-magnification tools like dental operating microscopes, sectioning the roots longitudinally, and making the teeth transparent for examining the remaining gutta-percha and sealer in the BL and MD directions by using a scoring system or measuring linearly to quantify the amount of gutta-percha and sealer (8,21,23). The accuracy of all these methods is questionable (24).

3D imaging techniques such as cone-beam CT have also been used to quantify the amount of remaining gutta (16,18). The use of CBCT in endodontic studies has made it possible to evaluate RCTs in 3 dimensions. Without physically altering or destroying the tooth, this non-invasive method permits extensive examination of morphological features (25). Root canal filling, root canal preparation quality, and tooth anatomy evaluations using this method have been extensively used (26-30). This study used CBCT to quantify the amount of filler material that remained inside the root canals following mechanical removal. For exact volume calculation in cubic millimeters, the CBCT software's "threshold" function allowed for precise delineation of the remaining filling.

Three critical characteristics of CBCT are voxel size, field of view, and slice thickness. The optimal voxel size is 0.2 mm, resulting in reduced scanning duration and diminished radiation exposure for the patient (31). A smaller voxel size enhances both noise and resolution. Images with reduced voxel sizes exhibit enhanced sharpness. For suspected root fractures, the voxel size employed should be less than 0.2 mm. The voxel size for assessing internal root resorption must be 0.16 mm. The voxel size employed for detecting the depth of proximal carious lesions is 0.125 mm. A voxel size of 0.2mm is more precise than one of 0.4mm. The voxel size significantly influences image quality and is directly related to scanning and image reconstruction duration, as well as the field of view, amperage, and voltage (32). This study employed a voxel size of 0.2 mm.

To further enhance the standardization of treatment across different file systems in this study, specific adjustments were made according to the unique recommendations of each manufacturer. For the REM file system, the manufacturer advises reaching the apical region with a file equivalent to their product specifications—specifically, a file with a diameter of 25 and a taper of 06, like the One Curve file (33). As a result, the apical preparation for the REM group was completed using a 25/06 file. Apical diameter of 20 and taper of 07 characterize the PTUR D3 file, which is designed for the apical third gutta-percha removal procedure. To align the manual technique more closely with these rotary systems, the H-file group's apical diameter was standardized to a 25/02 file. This adjustment was implemented to maintain consistency across different methodologies, despite the traditional variances in hand-filing techniques. It is well established that increasing the apical diameter facilitates the removal of a greater amount of gutta-percha (34). The increased amount of residual gutta-percha in the PTUR group compared to the REM group may be due to the apical diameter being enlarged only to size 20, even though there was no statistically significant difference within the PTUR group. The H-file group had the most residual gutta-percha, which is important to point out, and the reduced taper angle might be the reason for this. Confirmation of this claim and a complete understanding of the effect of file taper on gutta-percha removal require more extensive research.

While some research has shown the reverse to be true, many studies have demonstrated that the use of any solvent to dissolve gutta-percha in canals provides faster results than either hand files or rotary NiTi devices (35-37). Hülsmann & Stotz have noted that H-files are more rapidly removed from the root canal by solvents, and rotary files have also been shown to be significantly faster when used with solvents (35). However, they also reported that root canal cleaning has proven to be less satisfactory. Researchers have suggested that this may be due to the softening process, where softened gutta-percha is 'rubbed' against the root canal walls. Researchers found that chemically softened gutta-percha could be easily pushed into difficult-to-reach canal structures such as isthmuses, cul-de-sacs, lateral canals, and irregularities. Because of this, removing filling becomes more of a challenge and takes more time (38). Hülsmann & Bluhm found no significant difference in the time required for retreatment using rotary files or H-files, with or without the use of eucalyptol for gutta-percha removal (39). Furthermore, to avoid any interaction with periradicular tissues, researchers have emphasized the significance of not using solvents in the apical portion of the root canal (40). The use of chloroform as a solvent for gutta-percha remains a topic of debate, as it is known to be locally toxic when it comes into contact with periradicular tissues (40). Furthermore, chloroform has been demonstrated to possess hepatotoxic and nephrotoxic properties, and it is classified as a carcinogenic substance (41). In our study, no solvent was used due to the controversy over the use of solvents and to ensure standardization.

Heat-treated retreatment files have been found to have improved fracture resistance (14) and their use is recommended in the treatment of curved root canals due to their increased flexibility (42). Ünal et al. (43) reported occurrences of file fractures and procedural errors such as perforations during retreatment of curved root canals, attributing these issues to increases in the taper angle of the files (44). Although the current study was conducted on straight canals, with only one instance of a D3 file fracture observed, it highlights the need to investigate the effectiveness and fracture incidence of heat-treated retreatment files in curved root canals as well. Additional studies are needed to assess the performance of these files in curved root canals, thereby expanding upon the findings of this research.

In this study, all retreatment methods left some residual filling within the canals. This outcome is consistent with previous research, where similar results were reported using a range of retreatment files, techniques, and solvents (21,39,45). Schirrmeister et al. (24) evaluated the performance of PTUR and H-files in removing gutta-percha from curved root canals and found no notable differences in the cleanliness of the root canal walls between the 2 techniques. However, PTUR files showed a higher rate of file fractures compared to manual files, suggesting possible limitations for their use in curved canals during retreatment. Shrivastava et al. (45) assessed the performance of PTUR, R-Endo, and H-files in removing gutta-percha from root canals, determining that H-files were the most effective in cleaning, followed by R-Endo files, whereas PTUR left the largest amount of residual gutta-percha. Their study found no significant differences between PTUR and R-Endo files, although both were considerably less effective than H-files. In contrast, our study revealed that PTUR and REM files performed similarly in gutta-percha removal, with H-files being significantly less efficient and requiring the longest time to complete the procedure. This discrepancy could be due to variations in methodology or differences in operator experience and technique. The effectiveness of H-files may heavily rely on the operator's skill and familiarity with manual instrumentation, indicating that operator experience could have a substantial impact on the observed outcomes.

Chudasama et al. (44) performed a comparative evaluation of PTUR, 2 rotary retreatment systems, and H-files using a stereomicroscopic method. Their findings indicated that PTUR was the most effective in gutta-percha removal, leaving the least residual filling, whereas conventional H-files with Gates-Glidden drills were the least effective. Jagtap et al. (7) compared the performance of H-files, PTUR, R-Endo, and Gutta-Percha Remover (GPR) Mani retreatment files for eliminating root canal filling, utilizing stereomicroscopy and AutoCAD software to analyze the residual filling. Their results demonstrated that PTUR files were the most efficient. Their results indicated that PTUR files were the most effective, leaving the least residual material, while manual H-files were the least effective. These findings are consistent with our study, which also demonstrated that NiTi rotary systems (PTUR and REM) were more efficient than manual methods in gutta-percha removal.

In order to investigate the technique that was most effective in removing gutta-percha from curved root canals, Özyürek & Özsezer-Demiryürek examined ProTaper Next (PTN), PTUR, and H-files (46). They observed that both PTN and PTUR systems were significantly faster and left less residual gutta-percha compared to the manual group, demonstrating the superiority of NiTi rotary systems over manual instrumentation. While both studies confirm that NiTi systems perform hand files in terms of speed and efficiency, our study also revealed no statistically significant difference between the 2 NiTi systems (PTUR and REM), but REM was faster. Jaiswal et al. (6) investigated the efficacy of H-files, PTUR, Mtwo, and R-Endo using a clearing technique and found that while H-files were more effective in removing gutta percha, they required significantly more time compared to the rotary systems. However, our study's findings contradict these conclusions. Several earlier investigations demonstrated that rotating NiTi files could remove gutta-percha more quickly than manual instruments. On the other hand, Imura et al. (47) examined mandibular premolars, they found that the average retreatment time varied significantly between the groups. The H-file group needed significantly less time than the Quantec rotary group. This result was associated with the elimination of gutta-percha in bigger pieces. However, when it came to removing gutta-percha, all NiTi files were noticeably faster than H-file in this investigation. In clinical practice, retreatment procedures with rotary files can be preferred because they take less time and are more effective. This allows for extended irrigation processes to be allocated, which contributes to better elimination of bacteria. As a result, it can be predicted that patient comfort will increase, and the chair time will be reduced.

Conclusion

Each of the files kept a particular residual material content inside the root canal. Comparatively to PTUR and H-files, the REM file system produced less remaining gutta-percha and sealer-yield residues under the experimental conditions. However, this difference had no statistical significance. The rotary file systems also completed the removal procedure significantly faster than the H-file group.

In clinical practice, clinicians may be encouraged to incorporate irrigation and additional cleaning protocols into their procedures when performing retreatment, as it is known that mechanical preparation alone may not be able to remove the entire filling. In addition, even if complete cleaning is not achieved, faster retreatment with rotary instruments may create additional time for additional cleaning methods.

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Ethical Approval: The study utilized 45 freshly extracted maxillary incisors from humans, which were approved by the Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University (date: 18.09.2020 protocol no: 23-15).

Informed consent: Written informed consent was obtained from patients who participated in this study.

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