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Durability and efficacy of a root canal filling remover after repeated use: an in vitro study

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1. Title

Durability and Efficacy of a Root Canal Filling Remover after Repeated Use: An In Vitro Study

2. Running title

Durability and Efficacy of Remover in Retreatment

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4. Keywords

Cyclic Fatigue, Dental Instruments, Micro-Computed Tomography, Nickel-Titanium Alloys, Root Canal Therapy.

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7. Ethics Approval and Consent to Participate:

This study was approved by the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee, decision number 2022/3236. All teeth had been extracted for therapeutic reasons unrelated to this research, and informed consent for their use in scientific studies was obtained from all patients. The study was conducted in full accordance with the principles of the Helsinki Declaration.

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ABSTRACT

Durability and Efficacy of a Root Canal Filling Remover after Repeated Use: An In Vitro Study

Background: Many nickel-titanium (NiTi) rotary instruments are recommended for single use, yet they are often reused for economic reasons. This study aimed to evaluate how repeated use affects the cyclic fatigue resistance and root canal wall cleanliness of the Micro-Mega Remover file.

Methods: A total of 40 new Remover files were divided into four groups based on the number of uses: Control Group (unused), Group I (1 use), Group II (2 uses), and Group III (3 uses), with 10 files in each group. Sixty extracted mandibular molars with curved canals were obturated using the single-cone technique and then randomly allocated to the experimental groups in accordance with the designated usage frequency (10 teeth for 1 use, 20 teeth for 2 uses, and 30 teeth for 3 uses). Following the retreatment procedures, all files—both from the control and experimental groups—were subjected to cyclic fatigue testing using a stainless-steel artificial canal with a 60° curvature and a 5 mm radius. The time to fracture (in seconds) was recorded, and the number of cycles to fracture (NCF) was calculated. The fractured fragment lengths (in mm) were measured, and selected samples were examined under a scanning electron microscope (SEM). Additionally, micro-computed tomography (µCT) analysis was performed to assess the volume of residual canal filling material remaining on the root canal walls after the retreatment procedure. Statistical analysis was performed using the Kruskal–Wallis and Dunn tests ($p < 0.05$).

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Results: Fracture time and NCF differed significantly among the groups ($p <$ 0.001). Group I exhibited significantly higher values than Groups II and III, while the control group also performed better than Group III ($p < 0.001$). No significant differences were observed between the control group and Groups I or II, nor between Groups II and III. Fragment lengths were significantly shorter in Group I compared to Groups II and III ($p < 0.001$). µCT analysis revealed no significant differences in residual filling volume among the groups. SEM observations demonstrated fracture initiation lines, rough surfaces, and voids consistent with cyclic fatigue.

Conclusions: In this in vitro study, the cyclic fatigue resistance of Remover files was found to be similar to the control group after the first and second uses; however, a significant decrease was observed from the first to the second use. Nevertheless, the third use showed lower resistance compared to the control group and was associated with an increased risk of fracture. In addition, µCT analysis revealed residual filling material remaining on the root canal walls in all groups, with no significant differences among them.

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Keywords: Cyclic Fatigue; Dental Instruments; Micro-Computed Tomography; Nickel-Titanium Alloys; Root Canal Therapy.

INTRODUCTION

Successful root canal treatment requires an appropriate treatment plan, proper canal shaping, and adequate obturation [1]. Despite its demonstrably high success rate, post-treatment failures may still occur, necessitating retreatment in some cases [2]. Effective removal of filling material from the root canal system is fundamental to successful retreatment procedures [3]. However, the complete

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removal of root canal filling material remains a challenge in endodontic retreatment [4].

The Micro-Mega Remover (Micro-Mega, Besançon, France), a novel instrument designed for canal retreatment, is manufactured using electropolishing and a proprietary thermomechanical process called C-Wire, which enhances cyclic fatigue resistance. This single-use, asymmetrically designed instrument (ISO size 30, 0.07 taper, available in 19 mm and 23 mm lengths) operates in continuous rotary motion at a recommended speed of 400–800 rpm with a torque up to 2.5 N·cm, and is asserted by the manufacturer to effectively remove canal filling material without the need for solvents [5]. Despite the superior performance of nickel-titanium (NiTi) files, their cost makes reusability a desirable feature. However, this desire for reuse should be carefully considered in light of the structural limitations of NiTi files.

A significant limitation of NiTi files is their susceptibility to sudden fracture without any visible signs of defect. The two primary causes of such fractures are torsional failure, when the file tip binds while the shank continues to rotate, and cyclic fatigue, resulting from repeated tension–compression stresses in curved canals, with the latter considered the most prominent [6]. Inan et al. [7] noted that well-condensed filling material offers resistance to endodontic instruments. Consequently, files selected for retreatment are expected to exhibit high resistance to both cyclic and torsional fatigue.

Therefore, determining the safe usage limits of instruments and the number of cases in which they can be effectively employed is a critical requirement for clinical success. Previous studies have investigated the effects of repeated use on the cyclic fatigue resistance of NiTi files, demonstrating that multiple use and sterilization can significantly reduce their resistance [8,9]. However, there is still

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insufficient data regarding the cyclic fatigue resistance and filling material removal efficiency of the newly developed retreatment instrument, the Remover file, under multiple-use conditions. Thus, evaluating the structural durability and effectiveness of contemporary retreatment systems such as the Remover under repeated-use scenarios is of great importance for both instrument safety and treatment success.

The aim of this study is to evaluate the effect of repeated use on the cyclic fatigue resistance of Micro-Mega Remover files and on the three-dimensional volumetric amount (mm^3) of filling material remaining on the root canal walls. The null hypothesis of the study is that different numbers of uses of the Remover files do not result in a statistically significant difference in the cyclic fatigue resistance of the files or in the volume of filling material remaining on the root canal walls.

MATERIALS AND METHODS

This study was approved by the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee, decision number 2022/3236. All teeth had been extracted for therapeutic reasons unrelated to this research, and informed consent for their use in scientific studies was obtained from all patients. The study was conducted in full accordance with the principles of the Helsinki Declaration.

Sample Selection and Group Formation

The sample size was determined using the G*Power 3.1 software (Heinrich Heine University, Düsseldorf, Germany). An a priori power analysis was performed for the primary outcome (cyclic fatigue resistance), with a significance level of $\alpha = 0.05$, a test power of 80%, and an assumed large effect size ($\eta^2 = 0.25$) based on previous studies [3]. This analysis indicated that 36 Remover files would be

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sufficient. To compensate for potential sample loss, four groups consisting of 10 files each (n = 40) were used.

The files were divided into four groups based on the number of uses (Figure 1):

Control Group: 10 unused files

Group I: 10 files used once (1 use per file)

Group II: 10 files used twice (2 uses per file)

Group III: 10 files used three times (3 uses per file)

A total of 200 extracted mandibular molars were initially assessed for inclusion in the study. The teeth were pre-screened based on the following criteria: comparable root lengths, fully developed apices, absence of caries, developmental anomalies, resorption, calcification, visible fractures or cracks, and extraction due to periodontal reasons. Teeth that met these initial criteria were further evaluated using digital periapical radiographs taken in both buccolingual and mesiodistal directions to confirm root canal anatomy. Only those in which two mesial canals (Vertucci type IV configuration) and one distal canal were clearly identified were advanced to the next stage, and the curvature angles of their root canals were subsequently measured. The curvature angles were determined on periapical radiographs using the method described by Schneider [10]. Teeth were included in the study if the mesial canals had curvature angles between 20° and 40°, and the distal canals between 0° and 15°. Teeth with four or more canals or a history of previous root canal treatment were excluded. A total of 60 teeth that met all inclusion criteria were stored in 0.1% thymol solution at 4°C for disinfection and preservation.

Sixty teeth with completed root canal fillings were randomly assigned to Remover file groups according to the predetermined number of uses for filling material removal, as follows:

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Group I: 10 teeth (1 use per file × 10 files)

Group II: 20 teeth (2 uses per file × 10 files)

Group III: 30 teeth (3 uses per file × 10 files)

Cyclic fatigue testing was performed on the Remover files from the control group and the experimental groups (Group I, Group II, and Group III). Additionally, a second sample set consisting of three groups of 10 teeth each was prepared to evaluate the volumetric amount (mm^3) of residual filling material remaining on the root canal walls after retreatment using micro-computed tomography (µCT).

Group I-µCT: 10 teeth in which retreatment was performed with a Remover file on its first use

Group II-µCT: 10 teeth in which retreatment was performed with a Remover file on its second use

Group III-µCT: 10 teeth in which retreatment was performed with a Remover file on its third use

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Root Canal Preparation

Sixty teeth meeting the inclusion criteria were randomly numbered from 1 to 60. A conventional access cavity preparation was performed for all teeth under ×10 magnification using a dental operating microscope (DOM) (Densim Optics, Bratislava, Slovak Republic). Canal patency was confirmed by negotiating all canals with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) to the apical foramen. The working length was determined under a DOM by visually observing a #10 K-file at the apical foramen and subtracting 1 mm from this length. Coronal flaring of the teeth was performed using One Flare (Micro Mega, Besançon, France) files. A glide path was created in all teeth at the working length with a

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#15 K-type hand file (Dentsply Maillefer, Ballaigues, Switzerland). The canals of the mandibular molar teeth with established glide paths were shaped with One RECI (Micro Mega, Besançon, France) (25/0.6) files using reciprocating motion under the preset the WAVEONE ALL program of the VDW Gold Endomotor (VDW, Munich, Germany). Canals were irrigated with 2 mL of 2.5% NaOCl after each instrumentation during the shaping process. The procedure was completed when the file reached the working length. Final irrigation was performed with 1 mL of 17% EDTA for 1 min, followed by 2 mL of distilled water as an intermediate rinse, then 1 mL of 2.5% NaOCl for 1 min, and finally 2 mL of distilled water.

Obturation of Prepared Teeth and Retreatment

The canals of the prepared teeth were dried with paper points and obturated using the single-cone technique with Dia-Pro ISO-25.06 gutta-percha cones (DiaDent, Seoul, South Korea) and AH Plus (Dentsply DeTrey, Konstanz, Germany) sealer. The quality of the root canal fillings was confirmed by digital periapical radiographs to ensure adequate length, density, and absence of voids. The root canal orifices were sealed with a temporary filling material, and the teeth were stored in a 100% humid environment for 2 weeks to allow the sealer to set before gutta-percha removal. After the designated 2-week period, a One Flare (Micro-Mega) file was used at 400 rpm and 2.5 Ncm torque, according to the manufacturer’s recommendations, to remove the coronal filling material and access the root canal. A rotary Remover file (Micro-Mega) was used for root canal retreatment as this is a recently introduced instrument with limited data available in the literature. Forty new files were examined with a DOM at ×16 magnification at Inonu University Faculty of Dentistry, Department of Endodontics, and no fractures, cracks, or deformations were observed. During the retreatment process, the endomotor was set to 400

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rpm and 2 Ncm torque, as per the manufacturer's instructions. During the retreatment procedure, Remover files were used exclusively with 2–3 mm pecking and brushing motions. After every three pecking motions, the file was removed from the canal, and the canal was irrigated with 2 mL of 2.5% NaOCl delivered slowly over approximately 30 seconds. This procedure was repeated until the file reached 3 mm short of the working length, and no filling material was observed on the flutes of the file. For the removal of the filling material in the apical 3 mm, a new ProTaper Next file was used with the same 2–3 mm pecking motion. The file was advanced until it reached the working length, and no filling material was observed on its flutes; after every three pecking motions, the file was removed, and the canal was irrigated with 2 mL of 2.5% NaOCl delivered slowly over approximately 30 seconds. All endodontic procedures were performed by the same endodontist. The files were sterilized in an autoclave at 134°C for 20 minutes after each use.

Cyclical Fatigue Resistance Testing Device

The artificial canal used in our study was designed in a metal block with a curvature angle of 60°, a curvature radius of 5 mm, a distance of 5 mm from the center of curvature to the apex of the canal, and an internal diameter of 1.5 mm. A static test device was designed to measure the cyclic fatigue resistance of the Remover files used in our study. The files in the control group were subjected to cyclic fatigue testing without any prior use. All files in the control and test groups (1, 2, and 3 uses) were used at a speed of 400 rpm and a torque of 2 Ncm during the cyclic fatigue test. All files were rotated in a rotational motion until a fracture occurred. The working length of the files in the artificial canal was fixed at 19 mm with the aid of silicone stoppers. To minimize frictional forces generated by the files during testing, a synthetic lubricant, WD-40 (WD-40 Company, Milton

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Keynes, England), was applied to the interior of the artificial canal [11]. The time until fracture was recorded using a stopwatch. The moment of fracture was confirmed by video camera recording. The time was recorded in seconds. All these measurements were performed by an evaluator who was blinded to the group allocation. The number of cycles to failure (NCF) for the files in the test groups was calculated using the following formula [3]:

NCF = (time to fracture × rpm) / 60

The lengths of the fractured fragments of each file were measured using digital calipers.

Examination of Fracture Surfaces with SEM

After the cyclic fatigue test, a total of 8 files, 2 from each group, were examined under a scanning electron microscope (SEM) (LEO EVO 40 (LEO Ltd., Cambridge, England)) at magnifications of ×450 and ×1500 at the Test and Analysis Unit of Inonu University Scientific and Technological Research Center to examine the fracture surfaces and determine fracture types.

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Image Acquisition with µCT Device After Canal Retreatment

Samples were scanned with a micro-computed tomography (µCT) device at 90 kV, 111 µA, and a pixel size of 21.6 µm and a rotation step of 0.6° over 180° (Bruker Skyscan 1272, Kontich, Belgium) to obtain images after canal retreatment. The evaluator conducting the analysis was blinded to the experimental groups. The raw data obtained were reconstructed using NRecon 1.6.10.5 (Bruker-microCT, Kontich, Belgium) software. For quantitative measurements, the data were transferred to CTAn (CT Analyser) software (version 1.16.4.1, Bruker µCT, Skyscan). The residual filling volume remaining on the root canal walls after canal

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filling removal was determined using CTAn software. Values were obtained in mm^3.

Statistical Analysis

IBM SPSS V23 (IBM Co., Armonk, NY, USA) was used for statistical analysis of the data. The normality of data distribution was evaluated with the Shapiro-Wilk test. Descriptive statistical data are presented as mean ± standard deviation and median (Q1 - Q3). Because the data were not normally distributed, the Kruskal–Wallis test was used for group comparisons and the Dunn test with Bonferroni correction for pairwise comparisons. Effect sizes were also calculated to evaluate the magnitude of differences: for Kruskal–Wallis tests, eta squared (η^2) values were reported. $P < 0.05$ was considered statistically significant.

RESULTS

Median fracture time values differed significantly among the groups (Kruskal–Wallis: H = 22.13, $p < 0.001$, $\eta^2 = 0.53$). There were statistically significant differences between Group I and Group II, Group I and Group III, and the control group and Group III. Group I values were significantly higher than those of Group II and Group III. Control group values were significantly higher than those of Group III. No significant difference was observed between Groups II and III (Table 1).

Median NCF values differed significantly among the groups (Kruskal–Wallis: H = 22.13, $p < 0.001$, $\eta^2 = 0.53$). Group I showed higher values than Groups II and III, and the Control group showed higher values than Group III. No significant difference was observed between Groups II and III (Table 2). Median fractured fragment length also differed significantly among the groups (Kruskal–Wallis: H = 19.77, $p < 0.001$, $\eta^2 = 0.47$). Group I produced shorter fragments than Groups

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II and III, while the other pairwise comparisons were nonsignificant (Table 3). Median µCT residual volume showed no statistically significant differences among the groups (Kruskal–Wallis: H = 2.66, $p = 0.274$, $\eta^2 = 0.02$), although Group III had the highest residual filling volume (Table 4, Figure 2A–F). Images obtained from SEM analysis (×450 and ×1500) revealed fracture initiation lines indicative of cyclic fatigue, rough surfaces, and pits and/or voids created by the initial stages of fracture on these surfaces (Figure 3A–H).

DISCUSSION

NiTi rotary files are often reused by clinicians, primarily for economic reasons. One study indicated that 25.6% of general dentists and 36.1% of endodontists reuse rotary files up to 6–10 times [12]. While NiTi rotary files offer more effective shaping in curved root canals than stainless steel files because they preserve their original form [13], the primary concern is their potential for sudden fracture during clinical use, even without visible signs of permanent deformation. File fractures can hinder apical access, leading to complications that affect the success of root canal treatment. This issue has consequently received significant attention in the literature [14]. In addition, file fractures are not only considered technical failures but also stressful events that impose emotional and professional burdens on clinicians [15].

In clinical practice, root canal retreatment has a considerably lower success rate in molar teeth compared to anterior teeth [16]. In a retrospective study, Patnana et al. [17] reported a higher incidence of instrument fracture in mandibular molars compared to other teeth. They noted that the mesial roots of mandibular molars, often characterized by narrow canals curved in 2 planes, can increase the risk of complications such as strip perforation and transportation during shaping

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procedures. Therefore, this study used mandibular molar teeth with 20–40 degree curvatures in the root canal retreatment procedure.

Manufacturers, researchers, and clinicians have explored various methods for evaluating the cyclic fatigue resistance of NiTi instruments to ensure their safe and efficient use. Ideally, testing cyclic fatigue resistance would involve instrumenting curved canals in natural teeth. However, teeth can only be used once in such tests, and the root canal shape changes during instrumentation, making it impossible to standardize experimental conditions. Consequently, various devices, like glass or metal tubes, have been employed in these tests [18]. Nevertheless, there is no internationally standardized laboratory protocol for investigating the cyclic fatigue fracture resistance of NiTi rotary endodontic instruments.

Thermal cycling can influence the cyclic fatigue of engine-driven NiTi instruments during post-use sterilization [19]. While the literature remains inconclusive on the effects of autoclave sterilization on the cyclic fatigue resistance of heat-treated NiTi instruments, the files in our study were sterilized after each use to simulate clinical practice.

Recent advancements in alloy technology have enabled the development of next-generation files with enhanced mechanical properties through advanced thermomechanical processing and production techniques. The recently introduced Remover is a rotary file system that undergoes a proprietary heat treatment known as C-Wire and electropolishing. Designed for removing root canal filling material during retreatment procedures, the Remover has, to our knowledge, been employed in only a limited number of studies [5]. While limiting it to a single use is recommended by the manufacturer, as is common for root canal treatment files, adherence to this guideline can be challenging in resource-

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constrained settings and is often questioned by clinicians. Therefore, investigating how many teeth a file can safely be used on before fracturing is necessary, and studies have explored the changes in cyclic fatigue resistance of files after repeated use in root canals, both in vivo and in vitro [20, 21].

Consistent with our findings, studies investigating the cyclic fatigue resistance of various file brands before and after repeated use have reported significant decreases in cyclic fatigue after repeated use. One study reported surface deformations on Reciproc and WaveOne files after 3 uses in a single canal, but no plastic deformation was observed [22]. A study with GT and GTX files, used in 3 and 4 molar teeth, respectively, and then tested for cyclic fatigue resistance in stainless steel canals, reported that clinical use decreased their cyclic fatigue resistance [23]. Pessoa et al. [24] evaluated 36 RaCe rotary NiTi files for changes in cyclic fatigue resistance after being divided into groups of 1, 3, and 5 uses in simulated curved root canals. Their results indicated that clinical use of RaCe files negatively impacted their fatigue resistance, particularly with 5 uses. Duque et al. [25] assessed the cyclic fatigue resistance of ProDesign R, Reciproc Blue, and WaveOne Gold files after shaping 3 curved, single-canal teeth. While the cyclic fatigue resistance of ProDesign R and WaveOne Gold decreased, that of Reciproc Blue remained unchanged. Another study showed that using Reciproc REC25 files in 4 or more molar canals significantly reduced their cyclic fatigue resistance [26]. After a certain point, the cyclic fatigue resistance of files decreases significantly, in inverse proportion to the number of uses [25, 27].

The impact of repeated use on cyclic fatigue resistance during root canal shaping has been investigated by numerous researchers utilizing various files and test designs. However, to our knowledge, only one study has examined the effect of retreatment procedures on cyclic fatigue resistance. Serefoğlu et al. [3] evaluated

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cyclic fatigue resistance after repeated use of Reciproc Blue files in the retreatment of mandibular molars. They reported no significant decrease in cyclic fatigue resistance after the first use but significant decreases after the second and third uses. Our findings showed that the cyclic fatigue resistance of Remover files was similar to the control group after the first and second uses. However, a significant reduction was observed between the first and second uses. In addition, no significant reduction was detected between the second and third uses, indicating that the instruments stabilized at a lower resistance level after the second use. Nevertheless, the third use recorded significantly lower values compared with the control group. This finding suggests that the third use further increases the risk of fracture and may exceed the safe threshold. Although there was no significant difference in fracture time or cyclic fatigue data between the control group and Group I (1 use) in our study, Group I values were unexpectedly higher. A possible explanation for the higher cyclic fatigue resistance of used files compared to unused files may be related to the properties of the tested file. A study by Cheung et al. [28] showed that repeated torsional preloads in NiTi rotary files improve the superelastic behavior of NiTi materials without causing surface defects or microcracks. They reported that torsional preloads below the superelastic limit can increase the cyclic fatigue resistance of files. This effect is similar to the improvement of fatigue resistance by appropriate heat treatment [29]. Our files being subjected to some torsional preload during the gutta-percha removal process may have increased their cyclic fatigue resistance. El Abed et al. [30] examined cyclic and torsional fatigue resistance by dividing 60 One Curve files into 3 groups: new, used for simulated canal shaping, and sterilized after use. The One Curve (Micro-Mega) file used in the study is also a single-file rotary system that undergoes a heat treatment and electropolishing process called C-Wire, similar to the Remover file, which was recently introduced. The cyclic

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fatigue resistance of One Curve rotary files increased slightly, though not statistically significantly, after a single use. Similarly, another study conducted in a clinical setting reported an increase in the cyclic fatigue resistance of One Curve files following their use [31]. The findings of that study are similar to those of ours in this respect.

When comparing the lengths of fractured fragments based on the number of uses, the fractured fragment length in the single-use group was significantly lower than that in the second and third use groups. The reason for the difference may be surface cracks and deformations occurring at different levels in the working sections of the files due to repeated use in root canal retreatment procedures.

During root canal shaping, wear and deformation of NiTi rotary instruments can reduce their cutting efficiency. Sağlam et al. [32], using atomic force microscopy and SEM, investigated surface alterations occurring after repeated use (three or five times) of three different retreatment systems (ProTaper Retreatment, R-Endo, and Mtwo Retreatment). All three NiTi rotary retreatment file systems exhibited significant structural degradation—including cracks, damage, and alterations in the spiral design—following three and five uses. In a study by Cırakoğlu et al. [33], the levels of wear after one, two, and three uses of Reciproc Blue, WaveOne Gold, and ProTaper Next file systems were compared. High-resolution microscopic images were analyzed using AutoCAD software, and statistically significant apical diameter reductions and wear patterns were identified, particularly in the apical region after clinical use. These findings suggest that multiple uses affect not only the mechanical integrity of instruments but also their surface morphology. Repeated use and sterilization cycles can significantly reduce cutting efficiency by inducing wear and deformation on file surfaces [33,34]. Such surface changes may adversely impact the ability of

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instruments to remove filling materials from canal walls, thereby potentially compromising retreatment outcomes. Despite these concerns, there remains a significant gap in the literature regarding the effect of multiple uses on the filling removal efficiency of NiTi retreatment instruments. Some previous studies have evaluated the efficiency of filling removal using two-dimensional methods such as stereomicroscopic or digital image analysis, quantifying the remaining filling material as surface area (mm^2) [35,36]. In contrast, the present study employed µCT to assess the residual filling material on the root canal walls in three dimensions (mm^3), allowing a more precise and volumetric evaluation. Although no statistically significant difference was observed among the groups, the highest volume of residual filling material was found in the group using the Remover file for the third time (Group III-µCT). Our findings indicate that repeated use of the Micro-Mega Remover file does not result in a significant difference in the volume of residual filling material on the root canal walls.

To the best of our knowledge, this study is the first to simultaneously evaluate the Remover file after multiple uses in terms of both cyclic fatigue resistance and the volumetric assessment of residual filling material on the root canal walls within the same experimental design. In addition, restriction of canal curvatures within narrow anatomical ranges, simulation of clinical conditions through sterilization of the instruments after each use, and the performance of all procedures by a single experienced operator constitute the strengths of the present study. Nevertheless, the obtained findings should be interpreted in light of certain methodological limitations.

First, as this is an in vitro study, the experimental conditions may not fully reflect the dynamics of the clinical environment. Since the Remover files were used only up to 3 mm short of the working length in accordance with the manufacturer’s

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recommendations, supplementary instrumentation with new ProTaper Next files was required in the apical 3 mm, which may have influenced the volumetric results. In addition, as pre-operative µCT scanning was not performed, the initial root canal filling volume could not be normalized on an individual basis, and therefore the proportional evaluation of residual filling material could not be performed. To minimize these effects, the same preparation protocol and obturation technique were applied to all specimens, canal curvatures were restricted within narrow anatomical ranges, and the samples were randomly allocated to ensure balanced group distribution. Future studies incorporating matched pre-operative and post-operative µCT analyses would allow more precise volumetric comparisons. Finally, the limited sample size and the inclusion of only mandibular molar teeth restrict the generalizability of the findings; therefore, further studies are required to better support the clinical relevance of these results.

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CONCLUSIONS

In this in vitro study, the null hypothesis that repeated use of the Remover file does not affect its cyclic fatigue resistance was rejected, whereas the null hypothesis regarding residual filling volume was accepted. The Remover files demonstrated cyclic fatigue resistance similar to the control group after the first and second uses; however, a significant decrease in fatigue resistance was observed between the first and second uses. Beyond this point, no further reduction was detected between the second and third uses, indicating a plateau effect. Nevertheless, the third use exhibited lower resistance compared with the control group and was associated with an increased risk of fracture. In addition, µCT analysis revealed that residual filling material remained in all groups, with no significant differences among them. These findings indicate that repeated use of Remover files may reduce their cyclic fatigue resistance and that the filling

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material on the root canal walls could not be completely removed. Further in vivo and comparative studies are needed before definitive clinical recommendations can be made.

Abbreviations:

DOM Dental operating microscope

NiTi Nickel Titanium

NCF Number of cycles to fracture

SEM Scanning electron microscope

µCT Micro-computed tomography

Author Contributions:

The authors have actively contributed to the work as shown below: Gizem Fatma Özden: data acquisition, investigation, writing- original draft, conceptualization, methodology. Levent Akıncı: visualisation, supervision, writing – review and editing, conceptualization, methodology. Neslihan Şimşek: visualisation, supervision, writing – review and editing, conceptualization, methodology. **Ethics Approval and Consent to Participate:**

This study was approved by the Inonu University Health Sciences Non-Interventional Clinical Research Ethics Committee, decision number 2022/3236. All teeth had been extracted for therapeutic reasons unrelated to this research, and informed consent for their use in scientific studies was obtained from all patients. The study was conducted in full accordance with the principles of the Helsinki Declaration.

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Data Availability:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary Material:

The data used in this study are derived from the content presented within the manuscript. Additional data supporting the findings of this study can be made available upon reasonable request to the corresponding author.

Conflicts of Interest:

The research team did not receive financial support from any public, commercial, or non-profit funding agencies for this study. Additionally, all authors declare no conflict of interest.

Consent for Publication:

Not applicable.

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Figure Legends

Figure 1. Depicts the flow diagram of group formation.

Figure 2. The raw images obtained through µCT scanning after the root canal fillings of Group I-µCT, Group II-µCT, and Group III-µCT were removed are shown in A, B, and C, respectively. The µCT cross-sectional images obtained after root canal fillings were removed from Group I-µCT, Group II-µCT, and Group III-µCT are shown in D, E, and F, respectively.

Figure 3. SEM micrographs of the axial views at ×450 magnification of unused (A), first time used (B), second time used (C), and third time used (D) Remover files. The white arrows indicate the fracture initiation lines that point to cyclic fatigue. SEM micrographs of the axial views at ×1500 magnification of the unused (E), first time used (F), second time used (G), and third time used (H) Remover files.

Table Legends

Table 1. Fracture Time According to Group (sec)

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**Kruskal–Wallis test, SD: standard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

Table 2. Number of Fracture Cycles Among Groups

**Kruskal–Wallis test, SD: standard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

Table 3. Fractured Fragment Length According to Group (mm)

**Kruskal–Wallis test, SD: standard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

Table 4. Residual Volume Values According to Group (mm^3)

**Kruskal–Wallis test, SD: standard deviation.*

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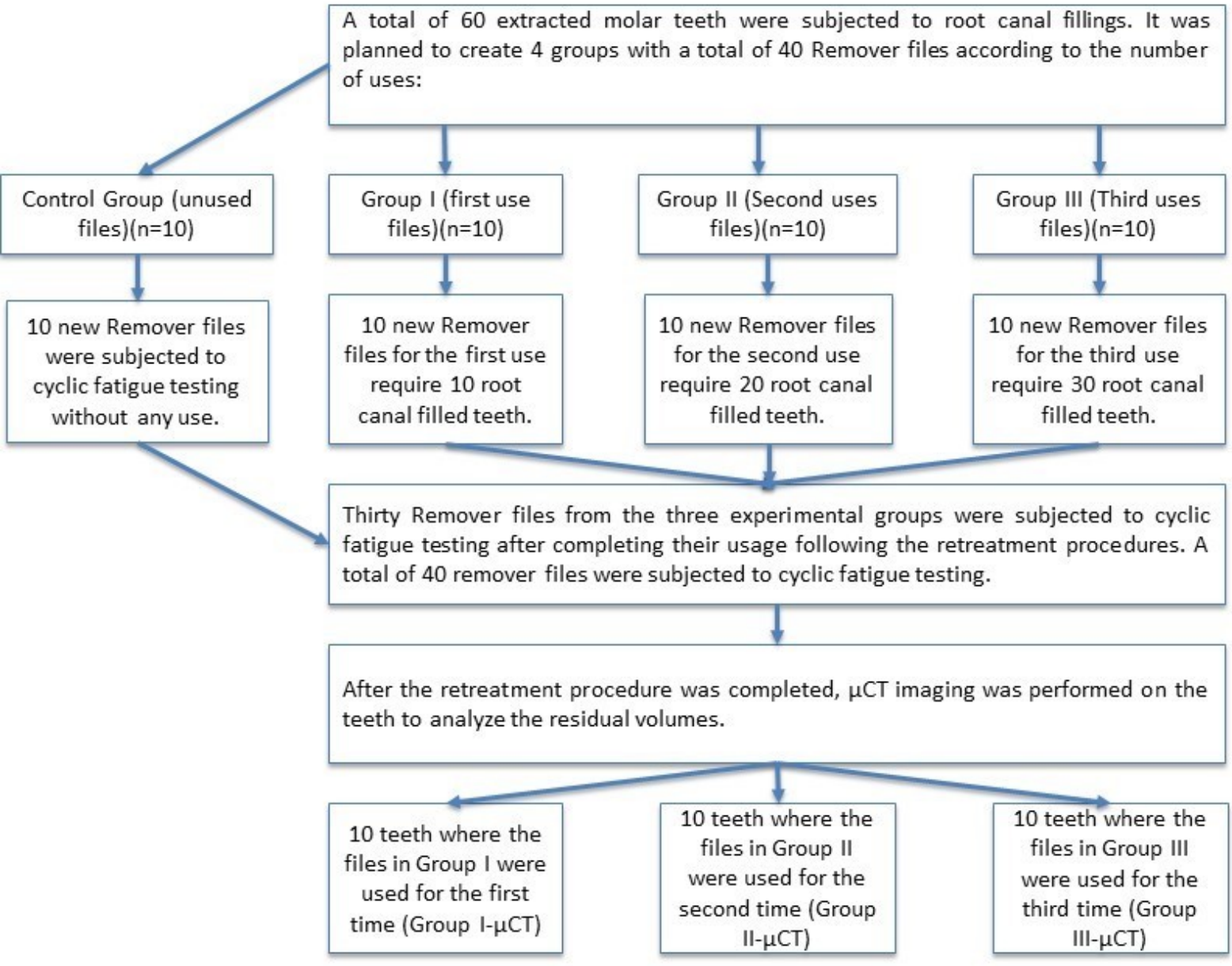
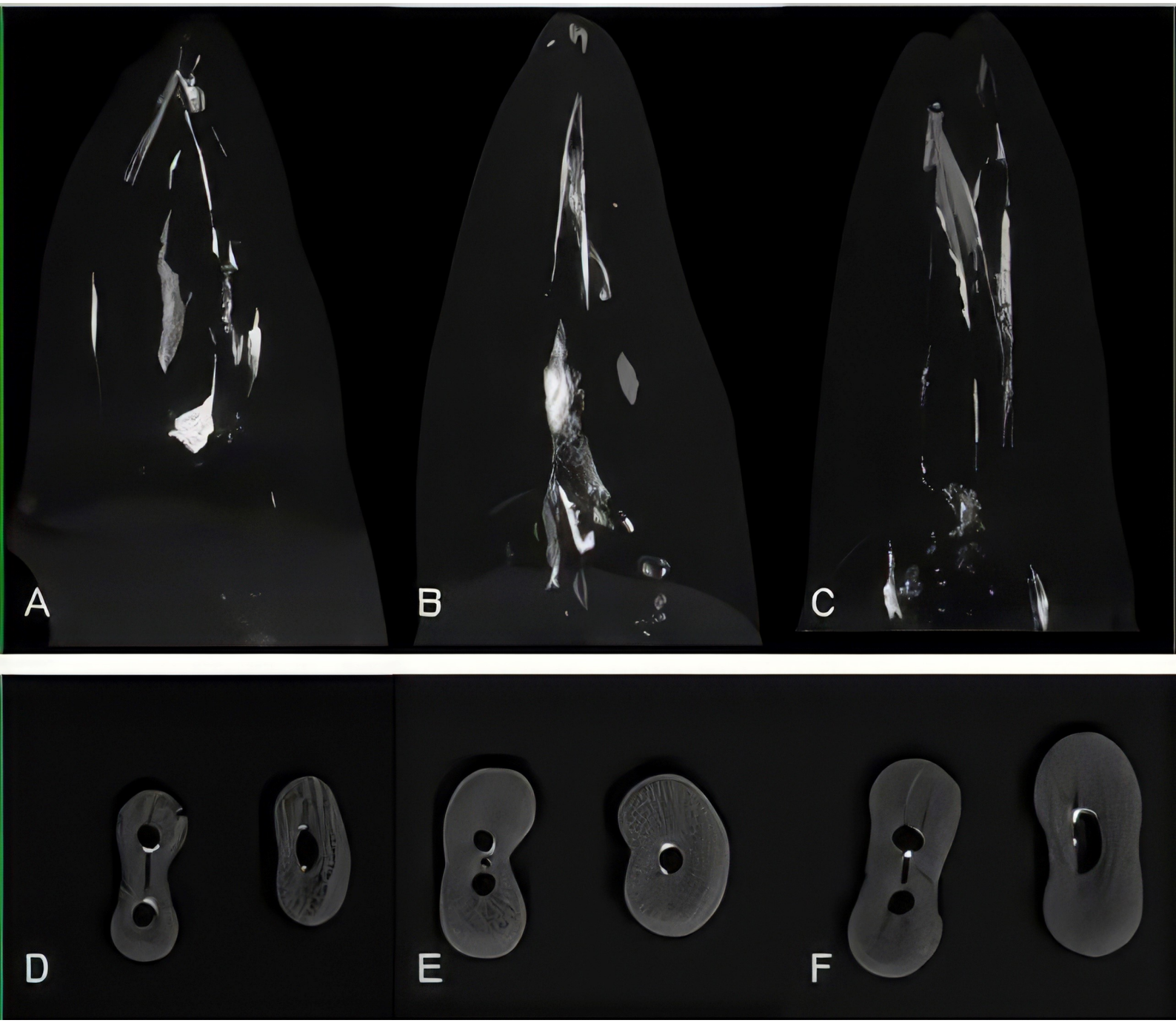

A total of 60 extracted molar teeth were subjected to root canal fillings. It was planned to create 4 groups with a total of 40 Remover files according to the number of uses:
Control Group (unused files)(n=10)
Group I (first use files)(n=10)
Group II (Second uses files)(n=10)
Group III (Third uses files)(n=10)
10 new Remover files were subjected to cyclic fatigue testing without any use.
10 new Remover files for the first use require 10 root canal filled teeth.
10 new Remover files for the second use require 20 root canal filled teeth.
10 new Remover files for the third use require 30 root canal filled teeth.
Thirty Remover files from the three experimental groups were subjected to cyclic fatigue testing after completing their usage following the retreatment procedures. A total of 40 remover files were subjected to cyclic fatigue testing.
After the retreatment procedure was completed, µCT imaging was performed on the teeth to analyze the residual volumes.
10 teeth where the files in Group I were used for the first time (Group I-µCT)
10 teeth where the files in Group II were used for the second time (Group II-µCT)
10 teeth where the files in Group III were used for the third time (Group III-µCT)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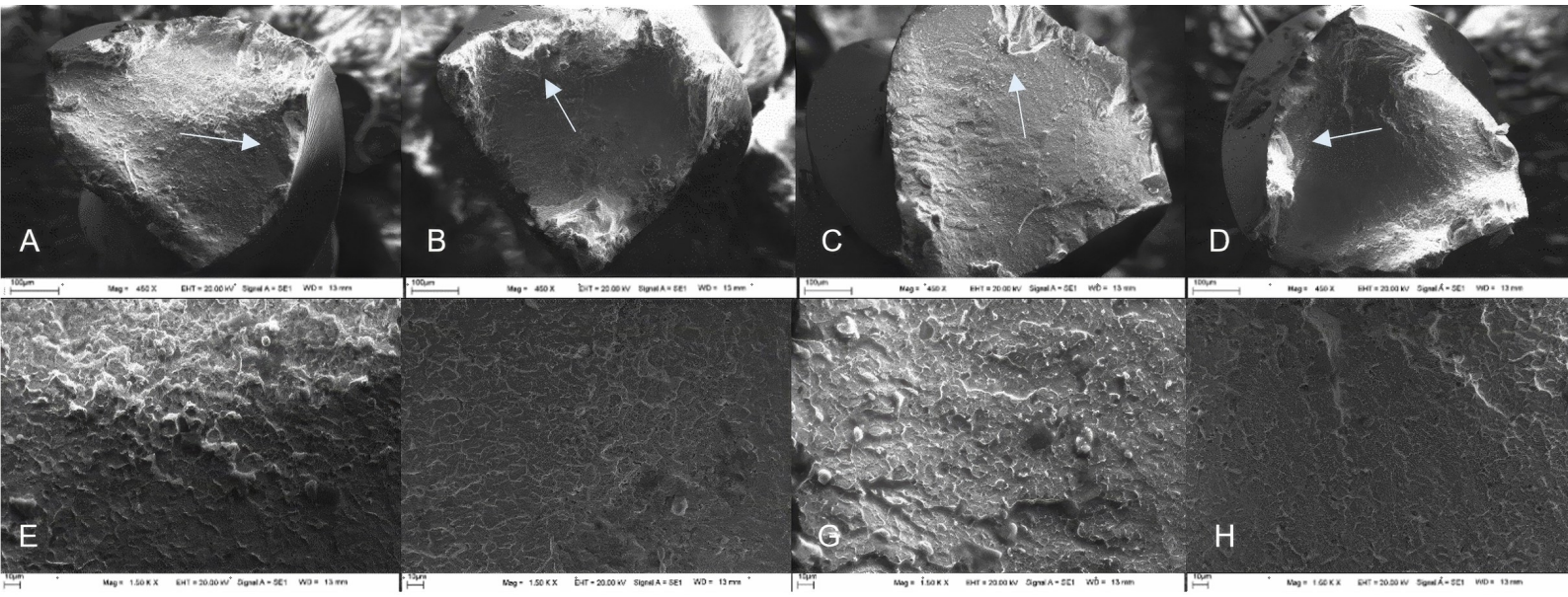
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Table 1. Fracture Time According to Group (sec)

Group	Mean ± SD	Median (Q1 - Q3)	p*
Control Group	125.89 ± 17.51	120.64 (113.28 - 143.85)[ac]	**<0.001**
Group I	147.25 ± 14.16	143.98 (134.62 - 163.57)[a]	
Group II	104.98 ± 31.88	120.22 (78.03 - 128.12)[bc]	
Group III	86.94 ± 21.26	92.67 (81.43 - 102.58)[b]	

**Kruskal–Wallis test, SD:* s*tandard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

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Table 2. Number of Fracture Cycles Among Groups

Group	Mean ± SD	Median (Q1 - Q3)	p*
Control Group	836.60 ± 116.43	803.50 (753.0 – 953.0)[ac]	**<0.001**
Group I	978.60 ± 94.24	956.50 (893.0 – 1087.0)[a]	
Group II	697.30 ± 212.46	796.00 (520.0 – 853.0)[bc]	
Group III	575.30 ± 142.00	613.50 (540.0 – 680.0)[b]	

**Kruskal–Wallis test, SD: standard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

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Table 3. Fractured Fragment Length According to Group (mm)

Group	Mean ± SD	Median (Q1 - Q3)	p*
Control Group	6.61 ± 1.23	6.15 (5.50 – 8.00)^{ab}	**<0.001**
Grup I	5.73 ± 0.21	5.70 (5.60 - 5.80)^{a}	
Grup II	7.85 ± 0.90	8.25 (7.60 - 8.40)^{b}	
Grup III	8.04 ± 0.24	8.00 (7.80 - 8.30)^{b}	

**Kruskal–Wallis test, SD: standard deviation, a–c: Different superscript letters indicate statistically significant differences between groups according to Dunn's post hoc test with Bonferroni correction. Groups sharing the same letter do not differ significantly.*

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Table 4. Residual Volume Values According to Group (mm^3)

Group	Mean ± SD	Median (Q1 – Q3)	p*
Group I-µCT	0.75 ± 0.62	0.62 (0.25 – 0.98)	0.274
Group II-µCT	0.74 ± 0.57	0.54 (0.27 – 1.16)	
Group III-µCT	1.92 ± 2.10	0.95 (0.42 – 2.85)	

**Kruskal–Wallis test, SD: standard deviation.*

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