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The Influence of Brushing Motion on the Cutting Efficiency of Two Heat-Treated Endodontic Files: An In-Vitro Micro Computed Tomography Study

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Abstract: Background: To assess the cutting efficiency of two heat-treated endodontic files with a brushing motion in oval-shaped canals. **Methods**: A total of 10 intact lower molars with a single oval distal canal were selected and randomly divided into two groups according to the system used for shaping: 2Shape and Hyflex CM. The instrumentation was performed in two parts: a first shaping according to the manufacturer's instructions and final shaping using 5 brushing motions on the distal, lingual, and buccal walls, avoiding the danger zones. Micro computed tomography (micro-CT) scans before and after the brushing motion were superimposed at all three levels: coronal, middle, and apical. Canal changes in buccolingual (ΔBL) and mesiodistal (ΔMD) dimensions were measured. Data were statistically analyzed by repeated-measures analyses of variance and the student *t*-test ($p < 0.05$). **Results**: For ΔMD, no significant difference emerged between the 2Shape and Hyflex CM in the apical and coronal thirds. However, the cutting efficiency was significantly greater with the 2Shape in the middle third ($p < 0.05$). Regarding ΔBL, no statistically significant difference was detected between 2Shape and Hyflex CM comparing the anatomical thirds, while 2Shape had a significant higher cutting efficiency considering the total BL diameter ($p < 0.05$). **Conclusions**: Under the limits of a vitro study, the shaping procedure with 2Shape and brushing motion was more efficient than the Hyflex CM in the midroot levels in terms of ΔMD, and in total canal space for ΔBL. Both files ensured an effective mechanical preparation.

Keywords: brushing motion; cutting efficiency; heat treatment; micro-CT; oval canal

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1. Introduction

Cutting action is a fundamental characteristic of endodontic files, which permits the dentin to be efficiently removed and to obtain a funnel-shaped canal, facilitating the obturation procedure. In addition, an efficient cutting action is essential to minimize the file torsional stress and consequently the risk of breakage [1]. Because of the pseudoelastic characteristics, NiTi instruments have to be machined rather than twisted, which may cause surface defects within the cutting surfaces with a negative impact on their cutting efficiency [2].

Despite the advanced technologies and improvements in nickel-titanium (NiTi) files, it is still challenging to mechanically prepare and disinfect the entire root canal system due to its anatomical complexities, especially in oval root canals [3]. Forty percent of oval root canal walls remain untouched after chemo-mechanical preparation, rendering the mechanical debridement of this oval anatomy difficult [4]. Anatomical complexities of oval configurations including buccal and lingual extensions are challenging to be instrumented and filled. It is questionable if the NiTi instruments are able to guarantee a complete and effective preparation of these areas. Indeed, it has been reported that the middle and coronal cross-sections of oval root canals exhibit zones not adequately prepared [5]. Thus, NiTi rotary instruments should be used in a "brushing" motion especially in oval canals with the aim to attain more canal walls to make the preparation more efficient [5]. Currently, as reported in previous studies, no technique ensures a full preparation of the walls of oval-shaped canals [6,7].

Within this context, heat-treated instruments have been introduced to improve shaping procedures with NiTi endodontic instruments [8]. The 2Shape system (Micro Mega, Besançon, France) includes TS1 and TS2 files with a 25 tip, a 4% and 6% taper, respectively. The instruments are T-Wire heat-treated and possess a modified triple helix cross-section exhibiting an off-centered blade [9]. The HyFlex CM (Coltene, Cuyahoga Falls, OH, USA) files are made from a thermomechanical process of NiTi alloy with the property of "Controlled Memory". These instruments are in martensitic condition at body temperature and possess two types of cross-sections: a quadrangular for the 25.04 file and a triangular for the 25.06 [10]. Heat treatments are able to influence the all-mechanical properties of NiTi instruments such as flexibility and fatigue resistance. The above-mentioned treatments act in enhancing the mechanical behaviour of the instruments due to modifications in microstructure of NiTi alloy [1]. Even if the heat treatments and new metallurgy increase the flexural properties, it was suggested that heat treatments may impair the cutting efficiency [11].

The cutting efficiency of 2Shape and HyFlex CM rotary systems and the supplementary effect of a brushing motion have not yet been studied.

Thus, the primary aim of this study was to assess the influence of brushing motion on cutting efficiency of 2Shape and Hyflex CM in oval canals using micro computed tomography (micro-CT) imaging. The secondary aim was to compare the percentage of untouched canals generated by the two rotary systems. The first null hypothesis is that there would be no influence of brushing motion on the cutting efficiency of 2Shape and Hyflex CM in oval canals. The second is that the percentage of untouched canals between the two rotary files would not be significantly different.

2. Materials and Methods

2.1. Teeth Selection

The Ethics committee of Saint Joseph University, Beirut, Lebanon provided the ethical approval (FMD184). Following a previous pilot study, sample size was determined for ensuring a power of 80% and $\alpha = 0.05$ using G*Power 3.1.9.2 software (Heinrich-Heine University at Dusseldorf, Dusseldorf, Germany). Based on these parameters, a number of 10 samples for group was determined. Hence, a total of 20 intact human mandibular molars recently extracted for periodontal reasons were included. The selection criteria consisted of teeth with intact crowns exempted from fracture lines or cracks, caries, any previous restoration, and molars with one distal root with completely formed apices and an oval shape 5 mm below the root apex. This parameter was confirmed by periapical radiography in the mesial-distal (MD) and buccal-lingual (BL) directions. The canals were considered as oval when the BL diameter was 2.5 times larger than the MD diameter [12] (Figure 1).

In addition, preoperative micro-CT images were used to corroborate the teeth selection. The molars were radiographed in a BL direction and the angle of curvature of the distal root was calculated using Sopix 2 software (Acteon, Marignac, France). Only roots with curvature from 10° to 20° as established by Schneider's classification were selected [13]. The teeth collected were cleaned and maintained in 0.1% thymol solution at 4 °C until the experiment was performed.

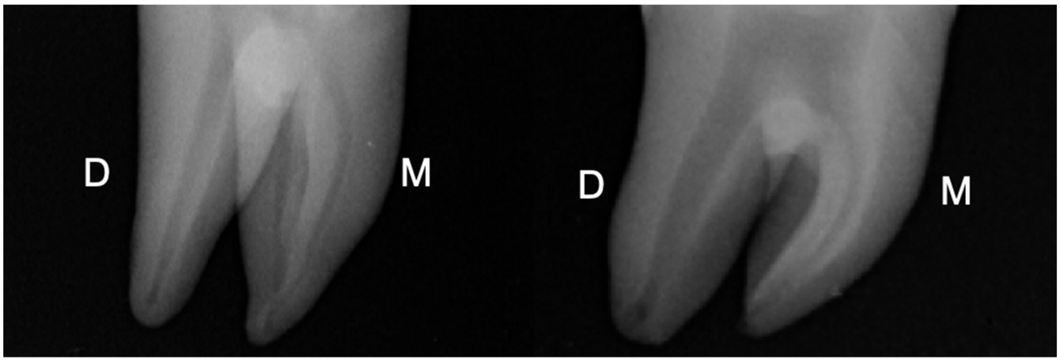

Figure 1. Periapical radiographs of two mandibular molars selected for the study. D: distal, M: mesial.

2.2. Micro-CT Scanning Protocol and Reconstruction

The molars were scanned with a high-resolution micro-computed tomographic scanner (SkyScan 1172; Bruker microCT, Kontich, Belgium) three times: before procedures for guaranteeing the homogeneity of samples and identifying oval-shaped canals; after instrumentation; and after applying the brushing motion. The x-ray tube was activated at 130 kV and 61 μA (1 mm-thick Al filter), and the scanning was made by 360° rotation around the vertical axis with a rotation step of 0.5°, a resolution of 5.66 μm, and 1800 ms exposure. The images were converted into cross-sectional slices by the SkyScan software interface (NRecon v.1.6.4; Bruker micro-CT) with standardized indices for beam hardening (100%), ring artifact correction of 10, and setting of standardized min and max contrast levels (0–0.3).

2.3. Root Canal Shaping and Postinstrumentation Scanning of Teeth

Access cavities were performed by using an 856-diamond bur (Komet Italia SRL, Milan, Italy) with a high-speed handpiece under running water. The root canal apical patency was verified applying a size 10 K-type file (Flexofile; Dentsply Sirona, Ballaigues, Switzerland) until it was detectable at the apical foramen. The working length (WL) was established 0.5 mm short of this length. Then a glide path was obtained using One G 14.03 (Micro Mega, Besançon, France).

Once the comparability of samples was checked, the ten molars were randomly assigned to the 2 groups (n = 10) on the basis of the shaping system used. Distal canals in Group 1 were prepared with 2Shape and HyFlex CM in Group 2. The technical features of the analyzed instruments are listed in Table 1 and cross-sections reported in Figure 2.

Table 1. Technical features (manufacturer, size (mm), conicity (%), cross section, cutting edge and NiTi alloy) of analyzed instruments.

Instrument	Manufacturer	Size (mm)	Conicity (%)	Cross Section	Cutting Edge	NiTi Alloy
2Shape	Micro-Mega, Besançon, France	0.25	4	Triangular asymmetrical	2 main and 1 secondary	T-wire
		0.25	6	Triangular asymmetrical	2 main and 1 secondary	T-wire
HyFlex CM	Coltene-Whaledent, Altstatten, Switzerland	0.20	4	Quadrangular	4	CM-wire
		0.25	6	Triangular	3	CM-wire

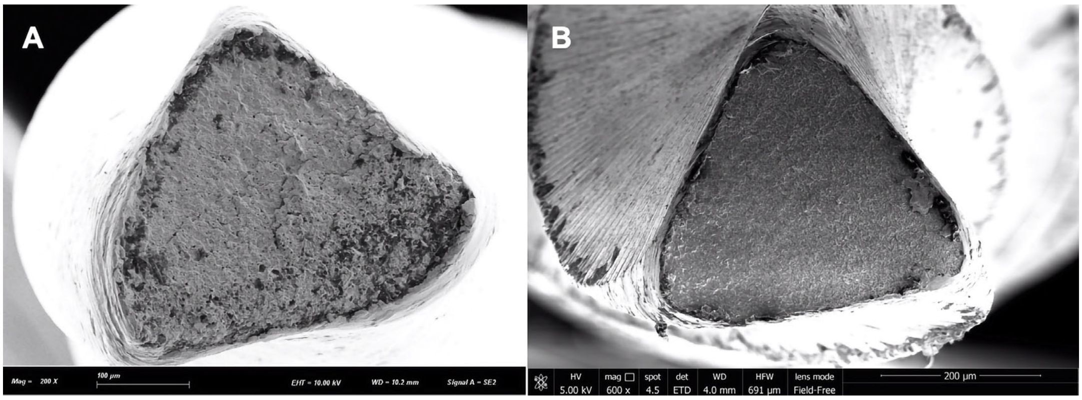

Figure 2. (**A**,**B**) The scanning electron microscope (SEM) images of the cross-sections of the tested files captured at 5-mm from the tip. (**A**) 2Shape TS2 (#25.06); (**B**) HyFlex CM (#25.06).

Group 1: The TS1 (25.04) was introduced until the WL, followed by TS2 (25.06) with the same dynamic movement in a step-down motion. For both files, the torque was set to 2.5 Ncm and the speed to 300 rpm using an electronic motor MM Control (Micro Mega, Besançon, France). The instruments were used in a pecking motion with an amplitude of 3 mm. With a sterile gauze, the instruments were cleaned after each movement and inspected. This procedure was carried out until the WL was achieved [14]. Each set of instruments was employed for the preparation of 1 canal and then discarded.

Group 2: Hyflex CM 20.04 was used in a gentle in-and-out motion until WL, followed by the Hyflex CM 25.06. For both files, the torque was set to 2.5 Ncm and speed to 500 rpm on the MM Control motor according to manufacturer recommendations.

A second micro-CT acquisition was done after initial shaping.

Afterward, in Group 1, the TS2 file was used in five lateral brushing motions on the buccal, distal and lingual walls respectively and in Group 2, Hyflex CM 25.06 was then used in five lateral brushing motions on the buccal, distal and lingual walls respectively. A third micro-CT scan was performed in the same way to determine the effect of the brushing motion applied in both groups.

During shaping procedures, the canals were irrigated with 3 mL of 5.25% sodium hypochlorite (Vista Apex Dental Products, Racine, WI, USA) using a side-vented 27-G needle (Endo-Eze Irrigator, Ultradent Products, South Jordan, UT, USA). After instrumentation, the root canals were irrigated with 17% EDTA solution delivered at a 1 mL/min rate for 5 min. Then, a 5-min 5-mL rinse with distilled water was applied and the canals were dried using paper points (Meta Biomed Inc., Colmar, PA, USA). All instrumentation procedures were performed by a single expert operator to avoid any operator bias.

2.4. Image Analysis

The preoperative and postoperative 3D images of the canals were co-recorded using the 3D Amira 5.3.2 software (Mercury Systems, Andover, MA, USA). The matched images were examined to determine the proportion of non-touched canal areas and the volume of the canal before and after the brushing motion. This procedure was performed by the number of static voxels before and after instrumentation.

The resultant modifications in the maximum buccolingual (ΔBL) and mesiodistal dimension (ΔMD) were obtained in pixels at the apical, middle, and coronal sections of the canal, following the protocol described by Alattar et al. [14].

$$\Delta MD = MDPostInst\ 2 - MDPostInst1$$

$$\Delta BL = BLPostInst\ 2 - BLPostInst1$$

with

MD: Mesiodistal

BL: Buccolingual

[PostInst1]: Root canal instrumentation was performed according to the manufacturer's recommendations for each instrument.

[PostInst2]: A brushing motion was performed.

2.5. Statistical Analysis

The SPSS program 25.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical elaborations, with p-Value set at 0.05. After verifying the normality of the data ($p > 0.05$; Shapiro–Wilk test), the homogeneity level within the groups (concerning the root length, curvature degree of the distal root, and preoperative volume of the canals) was statistically verified by one-way ANOVA ($p > 0.05$). Repeated-measures analyses of variance were used to compare the MD and BL cutting efficiency between groups within levels, followed by multiple comparisons tests. The t-test was applied to evaluate the percentage of untreated surfaces between the two groups.

3. Results

Table 2 reports mean and standard deviation of change (Δ) in maximum mesiodistal and buccolingual dimensions.

Table 2. Mean and standard deviation (SD) of change (Δ) in maximum mesiodistal and buccolingual dimensions considering each anatomical level and total root canal space.

	Δ			
Group	**MD Diameter**			
	Coronal	**Middle**	**Apical**	**Total**
2Shape	0.1356 [a] ± 0.0128	0.1190 [a*] ± 0.0332	0.1110 [a] ± 0.0293	0.1202 ± 0.0342
Hyflex CM	0.1584 [a] ± 0.0302	0.0734 [b] ± 0.0268	0.0948 [b] ± 0.0773	0.1123 ± 0.0467
	BL Diameter			
	Coronal	**Middle**	**Apical**	**Total**
2Shape	0.0904 [a] ± 0.0613	0.0710 [a] ± 0.0262	0.1114 [a] ± 0.0364	0.1219 * ± 0.0268
Hyflex CM	0.0690 [a] ± 0.0721	0.0458 [a] ± 0.0207	0.0658 [a] ± 0.0430	0.1089 ± 0.0597

Δ BL, variation in the maximum buccolingual dimension; Δ MD, change in the maximum mesiodistal dimension. Different superscript letters indicate significant difference between the different root canal thirds of a same instrument. * Indicates significant difference between the two instruments in the same root canal third.

In the MD direction, the cutting efficiency was not significantly different between 2Shape and Hyflex CM in the apical and coronal thirds while it was significantly greater for 2Shape in the middle third ($p < 0.05$). Considering the total root canal space, the two systems were not significantly different ($p > 0.05$). The cutting efficiency of 2Shape was not significantly dissimilar between the anatomical thirds ($p > 0.05$) while it was significantly higher in the coronal for Hyflex CM ($p < 0.05$).

In the BL direction, 2Shape and Hyflex CM had no statistically significant differences in the all-anatomical thirds ($p > 0.05$). Considering the total root canal space, 2Shape had significantly higher cutting efficiency compared with Hyflex CM ($p < 0.05$). The cutting efficiency of 2Shape was not significantly different in all anatomical thirds ($p > 0.05$). Likewise, no statistically significant difference was detected between the different levels for Hyflex CM ($p > 0.05$).

The percentage of untouched areas was similar between the two rotary systems ($p > 0.05$) (Table 3). As reported in Figure 3, no significant differences in the distribution of untouched areas (i.e., green zone in the superimposed images) were appreciable between the samples prepared with 2Shape and Hyflex CM.

Table 3. Percentage of untouched areas by 2Shape and HyFlex CM rotary instrumentation.

	Percentage of Untouched Areas (%)			
Instrument	**Mean**	**SD**	**Minimum**	**Maximum**
2Shape	4.6023 [a]	3.18867	1.77	8.59
Hyflex CM	3.7965 [a]	2.79915	0.76	7.13

Different superscript letters indicate significant difference between different files ($p < 0.05$).

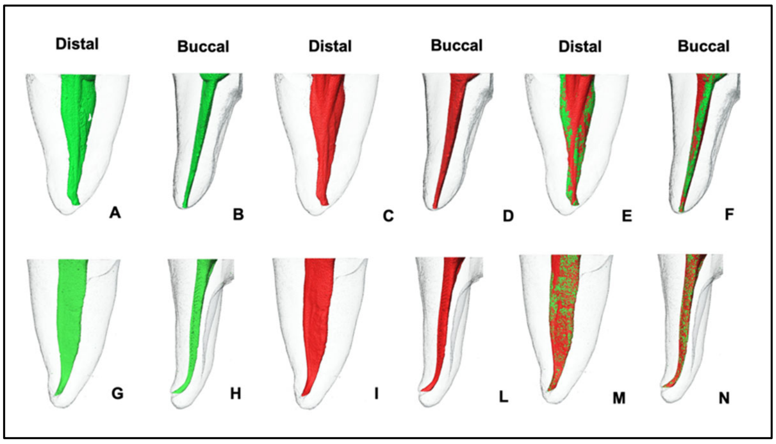

Figure 3. Illustrative 3D images of the root canals of distal root of mandibular molars before (in green) and after (in red) canal shaping associated with brushing motion. The non-touched canal wall areas were reported in green in the superposition view ((**A**–**F**) = 2Shape; (**G**–**N**) = HyFlex CM).

The 2Shape increased significantly canal volume by 11.1% after brushing ($p < 0.05$) whereas Hyflex CM by 8.5% ($p < 0.05$) in percentages not statistically significant between the two files ($p > 0.05$) (Table 4).

Table 4. Root canal volume pre- and post- brushing with 2Shape and HyFlex CM rotary instruments.

	Root Canal Volume (mm^3)		
	Groups	**Mean**	**SD**
Volume pre	**2Shape**	5.1348 [a]	1.89759
	Hyflex CM	6.6940 [a]	4.09832
Volume post	**2Shape**	5.7057 [a]	1.84434
	Hyflex CM	7.2657 [a]	3.90572

Different superscript letters indicate significant difference between different files ($p < 0.05$).

4. Discussion

Cutting efficiency is an important mechanical property because it affects the ability of instrumentation to safely prepare the root canal system [14]. Several factors affect the cutting ability of NiTi instruments, such as rake angle and helical angle, depth and number of grooves, cross-sectional area and design (which appears to be a decisive feature of the tip), chip elimination ability, hardness, kinematics, and manufacturing processes, including heat treatments [1,14]. An additional brushing motion could also modify the cutting behavior of NiTi files [14].

This study aimed to evaluate the effect of brushing motion on the cutting efficiency of 2Shape and Hyflex CM rotary systems. 2Shape and Hyflex CM are two heat-treated files with a similar design but a different heat treatment [15,16]. To evaluate the cutting efficiency, the use of a suitable substrate is necessary. Acrylic and plexiglass blocks with simulated canals were employed to determine cutting efficiency and other mechanical properties of endodontic files. In the current investigation, the human dentin (i.e., extracted mandibular molars) were essentially chosen for two reasons. First, natural dentin appears more appropriate than the artificial canals to simulate clinical conditions because of the different surface texture and hardness [9]. Moreover, the heat generated by rotary instruments may melt the resin and influence the results obtained [14]. Second, distal roots of mandibular molars often consist of oval canals especially in the apical third [6,7,17,18]. Some authors suggested that cutting behavior of endodontic NiTi files should be investigated using plexiglass models because of their standardized production [19]. Yet, it has been previously reported that the hardness of plexiglass is significantly lower than the dentine one and thus not guaranteeing a reliable and adequate simulation of clinical conditions [1].

Since no standardized method to measure cutting efficiency is available, several methodologies have been described to investigate the cutting efficiency of instruments in oval-shaped root canals, such as reassembly [20], histological sections [21], and weight loss from acrylic blocks or extracted teeth [22]. Anyway, the above-mentioned methods have been proven to have limitations because they are two-dimensional, imprecise, invasive, and therefore less accurate [14]. Three-dimensional evaluation of cutting efficiency has been introduced for a more detailed understanding of cutting behavior. Micro-CT was reported to be the most accurate technique for detecting non-instrumented areas and examining cross-sectional images [21,23–25]. Recently, sophisticated measurement software has been introduced that enables the evaluation of basic geometric parameters, such as the volume of dentin removed, ensuring reliable and reproducible measurements [25].

The cutting efficiency of 2Shape and Hyflex CM was compared after applying 5 brushing movements in oval canals. Indarika et al. (2018) applied 3 brushing movements [26]. However, Alattar et al. (2015) found that the greater the number of strokes, the greater the cutting efficiency [14]. Yet, an increased number of strokes can weaken the root structure. Consequently, 5 strokes were considered as a suitable compromise in consideration of the danger zones [14]. The cutting efficiency is generally evaluated at the middle and apical thirds, with the coronal third prepared by a pre-flaring instrument [27]. In this study, no coronal pre-flaring was applied so the entire root canal area was assessed [14]. The mean thickness in the mesial portion of the distal root is smaller in comparison to all other portions of the roots [28]. Thus, the mesial wall was excluded from brushing motion to avoid further weakening a wall with reduced thickness.

We have evaluated the cutting efficiency of two heat-treated files when used in combination or not with a supplementary brushing motion. The cutting efficiency of each file was expressed by the resultant modifications in the maximum BL and MD dimensions pre and post instrumentation. According to our results, the cutting efficiency with brushing motion was significantly greater with the 2Shape in the middle third, in the mesiodistal direction. Also, considering the total space in the buccolingual direction (corresponding to direction of brushing action), 2Shape was more efficient than Hyflex CM. Thus, the first null hypothesis can be rejected.

In both directions, all groups reported similar cutting efficiency at apical levels, as previously reported [14]. The difference observed at midroot level could be linked with the helix angle of the file [14]. Moreover, it has been reported that 2Shape is principally in the Austenitic phase at room temperature [15]. According to Gao et al. [29] a more flexible instrument would require less pressure against root canal walls and this might justify the higher cutting efficiency observed for stiffer files as 2Shape [9,10,15] in brushing motion [14]. In addition, 2Shape has a triplehelix cross-sectional consisting of two main and one secondary cutting edge with the two cutting edges demonstrating an excellent cutting performance [30].

In the mesiodistal direction, 2Shape exhibited a homogenous preparation in all root canal levels while Hyflex CM had significantly greater cutting efficiency at the coronal third. This result is consistent with the investigations of Morgental et al. (2013) and Peters et al. (2014) where Hyflex CM proved to be more effective in the coronal section probably because of the alloy thermo-treatments and the design of its flutes [31,32].

The influence of rotational speed on mechanical properties of NiTi files has been investigated, but no definitive consensus about its effect on cutting ability has been reached [31]. In the current study, both files were used according to manufacturer recommendations. More specifically, 2Shape was activated at 300 rpm while HyFlex CM at 500 rpm. Morgental et al. [31] determined how the rotational speed and number of uses influenced the cutting ability of four nickel-titanium files for coronal flaring. They obtained that an increase in speed resulted in greater cutting efficiency. Even if that study is not comparable with our investigation because of the different methodological conditions, the operating speeds applied for the two tested files could have influenced the results obtained. Further investigations are required to explore how rotational speed modifies the cutting behavior of NiTi rotary files.

Canal volume after bushing was significantly increased with both rotary systems, confirming that mechanical preparation with heat-treated files is effective and safe [11].

No significant difference emerged between 2Shape and Hyflex CM files for the percentage of untouched canal walls. Thus, the second null hypothesis cannot be rejected. No instrument was able to prepare the entire oval canal, as previously reported [21,26]. Thus, to enhance the cleaning and disinfection phase, attention must be paid to optimize the efficient delivery of the irrigating solutions in the root canal space. Indeed, effective irrigant delivery and agitation are required to favor the canal disinfection and debris elimination from the most irregular anatomical configurations with a significant improvement of the endodontic treatment outcomes. To date, many systems for mechanical activation of irrigants are available such as special brushes, sonic and ultrasonic tools as well as strategies to increase the intracanal bacterial decontamination including ozone, direct laser action or light-activated disinfection [33]. Beyond the method used, what is important to emphasize in this context is that different irrigation systems can compensate for the limits associated with mechanical shaping, helping clinicians in everyday clinical practice.

Because no previous study compared the cutting efficiency of 2Shape and HyFlex CM with brushing motion, no direct comparison with our results is possible.

Considering the files separately, two previous studies assessed the cutting behavior of Hyflex CM rotary files [34,35]. Yet, the results obtained are not directly comparable with our findings because there are various methodological differences. In the study of Seago et al. [34], the measuring system and general methodological approach applied to determine cutting efficiency ability are different. The cutting ability in that study was expressed by measuring the necessary load to guarantee a constant feed rate while simulated canals were prepared. In addition, the purpose of that study was to explore the effect of repeated simulated clinical use and sterilization on mechanical properties including cutting ability of Hyflex CM rotary instruments. Finally, cutting behavior of Hyflex CM was not compared with other NiTi files. One other study investigated the cutting properties of HyFlex CM [35]. Also in that case, the purpose and methodological approach were not comparable with our investigation. More specifically, the aim was to assess the effect of deep dry cryogenic treatment on the mechanical properties including cutting efficiency of martensitic SM NiTi files, including HyFlex CM. Moreover, cutting efficiency was quantified by measuring the loss of weight in plexiglass simulators.

Faisal et al. [30] compared the shaping ability of two NiTi rotary files (i.e., 2Shape and NeoNiTi) in severely curved canals determining also the volume of dentin removed by micro-CT. Even if the findings cannot be directly compared with ours due to the methodological differences, the authors confirmed that 2Shape was relatively safe to shape root canals and preserve the original canal anatomy. The results are in agreement with other studies [15,36,37].

Although the reduced number of sample size could be considered a limitation, the results obtained are deemed reliable considering the evaluation method (i.e., micro-CT) and the similar sample size to some previous studies [6,30]. Further studies on larger samples are desirable to confirm these results. Despite the standardized methodology, other limitations have to be considered regarding the NiTi instrument type, anatomical configuration of distal root of mandibular molars included and the protocol of brushing motion applied. Consequently, clinicians should consider these findings with caution, taking into account that clinical performance of NiTi instruments can be affected by several factors including operator ability, NiTi instruments properties and anatomical complexities. Within the limits of a laboratory study, these findings are clinically relevant because they show that brushing motion associated with heat-treated files could guarantee an effective mechanical preparation of root canal space.

Future investigations should be focused on how the brushing motion affects the cutting efficiency of NiTi rotary files in different contexts including the usage of rotary files after multiple clinical uses and sterilization procedures or the use of instruments with different heat-treatments and geometrical characteristics. Moreover, it is important to emphasize that clinical performance of NiTi instruments is the result of different mechanical properties. Thus, a comprehensive study of mechanical behavior of NiTi files including cyclic and torsional fatigue resistance is required to better understand and predict their operative use.

5. Conclusions

Under these experimental conditions, the 2Shape system was more efficient when used in a brushing motion compared to the Hyflex CM in oval canals, considering the all-root canal space in the BL direction and in midroot levels in the MD direction. No significant difference emerged between the two files regarding the untouched surface areas. It is pivotal for the clinicians to consider all the operating variables occurring in the clinical use when mechanical properties of an instrument are assessed because statistical significance could or could not directly correspond to clinical significance.

Further studies testing instruments with different metallurgical and geometrical features are warranted to corroborate these results and improve their generalizability.

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Data Availability Statement: The datasets generated during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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