

ORIGINAL ARTICLE

Shaping ability of novel nickel-titanium systems in printed primary molars

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Abstract

Background: Manual or mechanized instruments can be used for root canal preparation. Manual instrumentation using K-files is widely used in primary teeth, but there are many limitations. Mechanized root canal preparation can lead to easy access to all canals, decrease instrumentation time, and result in more funnel-shaped root canals, resulting in a more predictable uniform paste fill.

Aim: This study aimed to evaluate the shaping ability and instrumentation time of VDW.ROTATE™ and EdgeTaper Platinum™ during the preparation of resin-printed primary molars. Hand K-files were used as a reference for comparison.

Design: Sixty-six resin-based maxillary second primary molars, obtained from extracted tooth cone-beam computed tomography (CBCT) image and printed on a three-dimensional printer, were divided into three groups: VDW.ROTATE™, EdgeTaper Platinum™, and K-files. The specimens were scanned using CBCT imaging before and after root canal preparation. Images were registered using a dedicated software, and changes (Δ) in the canal area, volume, and untouched canal surface were calculated. Instrumentation time was evaluated. Data were statistically analyzed using the SPSS program.

Results: There was no significant difference among the tested file systems for Δ canal volume and area ($p > .05$). VDW.ROTATE™, however, showed significantly lower untouched canal surface area than other systems in all roots ($p < .001$). The VDW.ROTATE™ was found to be significantly faster (6.47 ± 0.39 min) than EdgeTaper Platinum™ (7.71 ± 0.73 min) and K-files (8.22 ± 0.72 min), ($p < .05$).

Conclusions: The shaping ability and the instrumentation time were directly influenced by the root canal instrumentation system used during the preparation of resin-printed primary molars, with VDW.ROTATE™ being the faster system and associated with the lower amount of untouched canal surface area.

KEYWORDS

K-file, primary molars, printed resin teeth, rotary systems, three-dimensional printing

1 | INTRODUCTION

Maintenance of primary teeth until physiological exfoliation is essential for children's function, esthetics, and phonation. Therefore, pulpectomy is a standard endodontic treatment for primary teeth, like permanent ones.¹ The success of pulpectomy depends on complete debridement, chemical and mechanical preparation, and three-dimensional seal.² Manual or mechanized instruments can be used for root canal preparation. Manual instrumentation using K-files is widely used in primary teeth, but there are many limitations regarding the effective cleaning of root canals, ledging, perforations, zipping, dentine compaction, canal transportation, and instrument fracture.³ Mechanized root canal preparation can lead to easy access to all canals, decrease working time, and more funnel-shaped root canals, resulting in a more predictable uniform paste fill.⁴

VDW.ROTATE™ (VDW) is a rotary system manufactured with a proprietary heat-treated “blue-wire” nickel-titanium (Ni-Ti) alloy and a double-bladed adapted S-shaped cross-section design. According to the manufacturer, the design of the instruments and their increased flexibility reduce canal transportation and preserve root canal anatomy.⁵ EdgeTaper Platinum™ (EdgeEndo) is another Ni-Ti rotary file system made with a heat-treated Ni-Ti alloy named Fire-Wire. This system has a bloated triangular cross section with a progressive changing taper, which according to the manufacturer has high flexibility able to work safely in 90° curves.⁶ Although some studies have evaluated the performance of VDW.ROTATE™ and EdgeTaper Platinum™ in permanent teeth,^{7,8} little is known about the shaping ability of these novel Ni-Ti rotary instruments on primary teeth.

The anatomy and structure of the root canals during the shaping procedures could affect the disjunctive features in these file systems. Although microcomputed tomographic (micro-CT) imaging is the best way to perform an accurate assessment,^{9,10} by using cone-beam computed tomography (CBCT), shaping ability could also be evaluated without causing any harm to the tooth structure. Moreover, CBCT could provide advantages like reducing cost and allowing faster data acquisition and is of relatively easier access for many investigators.^{11–14}

Laboratorial endodontic studies in primary teeth are challenging to establish an adequate and standard sample group since most of the primary teeth with an indication for extraction have physiological or pathological root resorption.¹⁵ With the development of 3D printing technology, 3D-printed resin replicas of primary teeth may be an alternative to overcome some problems with extracted

Why this paper is important to paediatric dentists?

- This study is the first study which assessed the shaping ability and instrumentation time of K-files and two novel different heat-treated Ni-Ti rotary systems in printed primary teeth.
- The study combined the advantages of the standardized endodontic resin blocks with the benefits of the CBCT images by evaluating standardized printed dental replicas made of a radio-opaque resin.
- Although the VDW.ROTATE™ instrument showed superior results for instrumentation time and untouched canal surface area, neither technique could completely prepare the resin-printed primary molars.

teeth: standardization, difficulty getting enough numbers, and potential cross-infection risks.¹⁶ Using CBCT data from an extracted tooth associated with 3D printing technology, it is possible to obtain a model of a natural tooth with the same external and internal morphology.^{17,18} Thus, 3D printing technology allows one to compare endodontic instruments' shaping abilities in standardized primary teeth samples.

This study aimed to assess the shaping ability and instrumentation time of EdgeTaper Platinum™ and VDW.ROTATE™ Ni-Ti rotary systems during the preparation of resin-printed primary molars. Hand K-files (VDW) were used as a reference for comparison. The null hypotheses tested were that the type of instrumentation, manual or rotary, (i) does not interfere with the final quality of the mechanical preparation and (ii) in the instrumentation time during the preparation of resin-printed primary molars.

2 | MATERIALS AND METHODS

2.1 | Sample size calculation

A power calculation was performed using G*Power 3.1 (Heinrich Heine University) software with $\alpha = 0.05$ and $\beta = 0.80$. The calculation indicated 22 teeth for each group.¹⁹ Therefore, 22 resin replicas of the maxillary second primary molar were included for each group. Ethics committee approval of the study was obtained from the local ethics committee (no. 2011-KAEK-2/2020/279).

2.2 | Description of printed teeth

2.2.1 | Obtaining a three-dimensional model of an extracted tooth

One sound extracted human maxillary second primary molar with three roots, and no physiological resorption was selected for the study. After decoronation of the crown, a #10 size K-file was inserted into each root canal until the tip of the instrument was just visible in the apical foramen. A radiography was obtained using an XMind unity DC X-ray device (Acteon Satelec), with settings set at 60 kVp and 7 mA, and exposed for 0.25 s, with the distance from the source to the film set at 20 cm obtained using the parallel technique. No calcification was observed in any of the root canals. All three roots were determined as Vertucci type 1 according to the root canal morphology classification,²⁰ and root canal curvatures were $<20^\circ$ as verified by the Schneider method.²¹

The working length (WL) was set to 1 mm shorter than the apex. The root canals were prepared with K-files up to size #20, rinsed with distilled water, and dried with paper points.²² Since the resolution of the resin layers used in the 3D printer is between 16 and 32 μm , the initial diameter of the root should not be less than ISO 15 size.²³ Then, the tooth was scanned using a CBCT (GXDP-700; Gendex Dental Systems, Hatfield, MA, USA). All images were

obtained at 90 kV, 13 mA, field of view: 6 \times 8 cm, and exposure time: 12 s.

Cone-beam computed tomography data were saved using the Digital Imaging and Communications in Medicine (DICOM) format on a computer workstation. The DICOM slice data were converted into 3D volumetric images using RealGUIDE 5.0 software (3diemme Software Corp). As described in previous studies,^{24,25} data were segmented using this software's "Tooth Segmentation" option (Figure 1).

2.3 | 3D printing of primary molar replicas

The three-dimensional model of the tooth, in .stl format, was exported to the 3D printer (Accuretta FreeShape 120; Accuretta Technologies). To achieve radiopacity, 10 g of 12.5% barium sulfate powder (KeyVest, Keystone Ind.) was mixed with 100 ml of resin (Accuretta CURO Guide resin, Hardness: Shore D 75; Accuretta Technologies), keeping in mind that the radiopacity of the replicas had to be reevaluated after each manufacturing process as the printer constantly adds fresh resin into the printer's reservoir. Then, the replicas were separated from the support structure and cleaned with alcohol for 5 min in an ultrasonic bath. Subsequently, the replicas were inserted

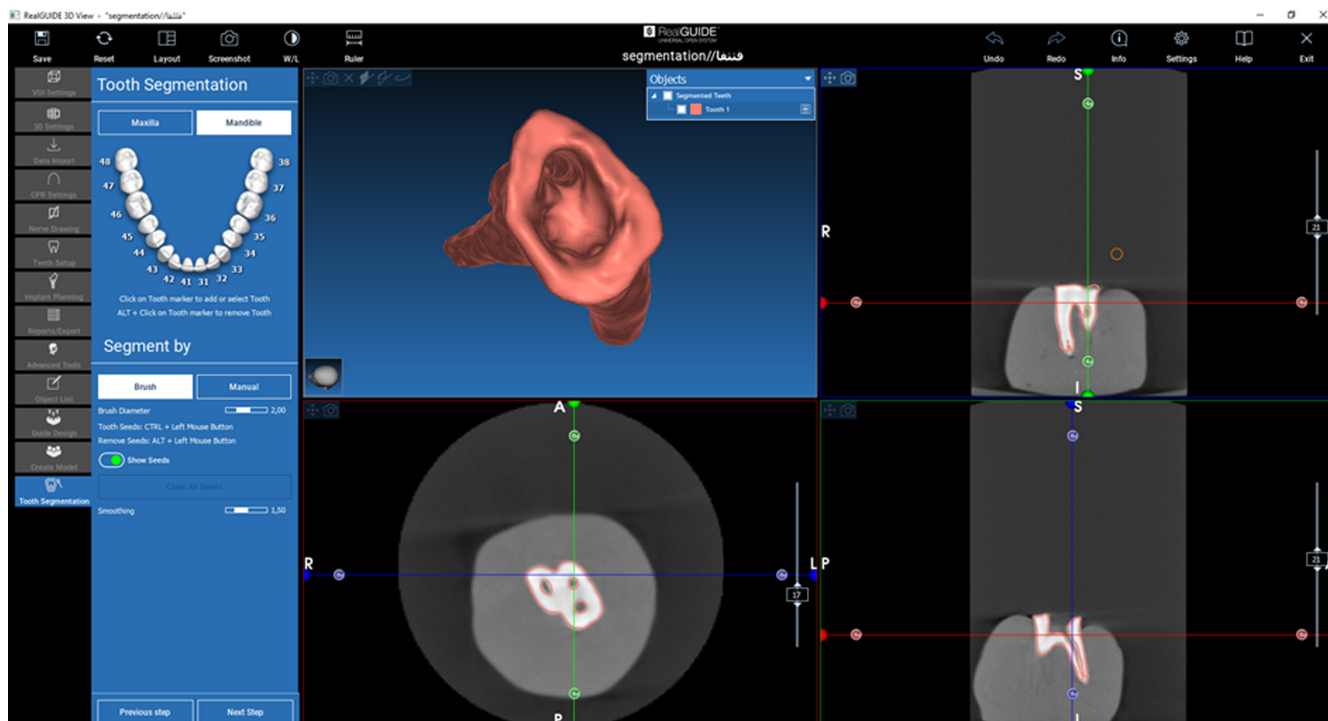


FIGURE 1 3D modeling in software

into an Ackuretta Finishing Kit UV Oven (Ackuretta Technologies) for 3 min. A #15K-file was used to control the apical patency in the resin root canals.

2.4 | Root canal preparation

All instrumentation was performed by a single experienced paediatric dentist. The apical foramen of the roots was blocked with modeling wax. All the resin teeth were randomly assigned into three experimental groups ($n = 22$ resin teeth/66 root canals; mesiobuccal, distobuccal, and palatal).²⁶ Each instrument was used for one tooth model (three canals), and they were used according to the manufacturer's instructions. All mechanized Ni-Ti instruments were operated by using an endodontic motor (VDW Gold). After three gentle in-and-out motion strokes to the apical direction, the instrument was removed and cleaned. This procedure was repeated until it reached the WL. After each step, irrigation was performed using 30-Gauge IrriFlex needle (Produits Dentaires SA) taken up to 2 mm short of the WL using a total of 20 ml of distilled water. Apical patency with a size #15 K-file was also performed between the use of each instrument.

2.4.1 | VDW.ROTATE™ group

Root canals were prepared by VDW.Rotate™ 20.05 and 25.06 files taken up to the WL. Instruments were used at 300 rpm and 2 N.cm torque.

2.4.2 | EdgeTaper Platinum™ group

Root canals were prepared by EdgeTaper Platinum™ S2 (20.06v), F1 (20.05v) and F2 (25.05v) files enlarged up to the WL. Instruments were used at 300 rpm and 3 N.cm torque.

2.4.3 | Manual K-file group

Preparation was performed using hand K-files (VDW) in a step-back manner. The canals were prepared to #25 MAF (Master Apical File) followed by stepping back to #40.

2.5 | Measurement of instrumentation time

A digital chronometer was used for the measurement of instrumentation time. The time of active instrumentation,

instrument changes within the sequence protocol, and irrigation were included in the total instrumentation time. The nearest 0.1% second was noted for each root and total time for a tooth.

2.6 | Methods for evaluating shaping ability

After root canal preparation, the root canals were dried with absorbent paper points and the teeth were submitted to a new CBCT scan. The specimens were mounted on a cylindrical basis holder manufactured for resin models from the 3D printer in the same position in both initial and final scans (Figure 2). For standardization, each specimen was scanned with the previous CBCT unit using the same parameters.

2.6.1 | Three-dimensional analysis of root canals

Then, new DICOM images were rendered in 3D in RealGuide software again. The 3D model of each tooth was imported onto Rapidform software (INUS Technology, Inc.) with the same coordinate system. With the relative segmentation mask, only the 3D version of the canals was obtained. After superposing 3D canal models, the differences between root canals before and after preparation were evaluated (Figure 3). Using a specific function of the software, "Surface deviation command" allowed finding a deviation of the normal from the specified direction for the selected surface. The colored image of the face allowed viewing the deviation of the overall surface area. The software automatically calculated the best fitting between the

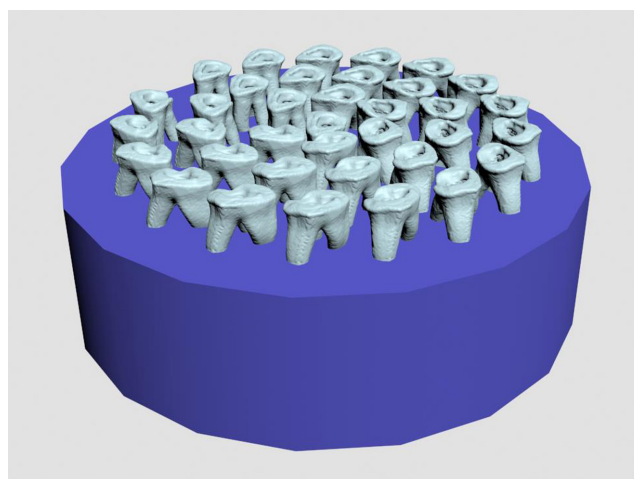


FIGURE 2 Images of a cylindrical base designed for cone-beam computed tomography imaging with printed roots.

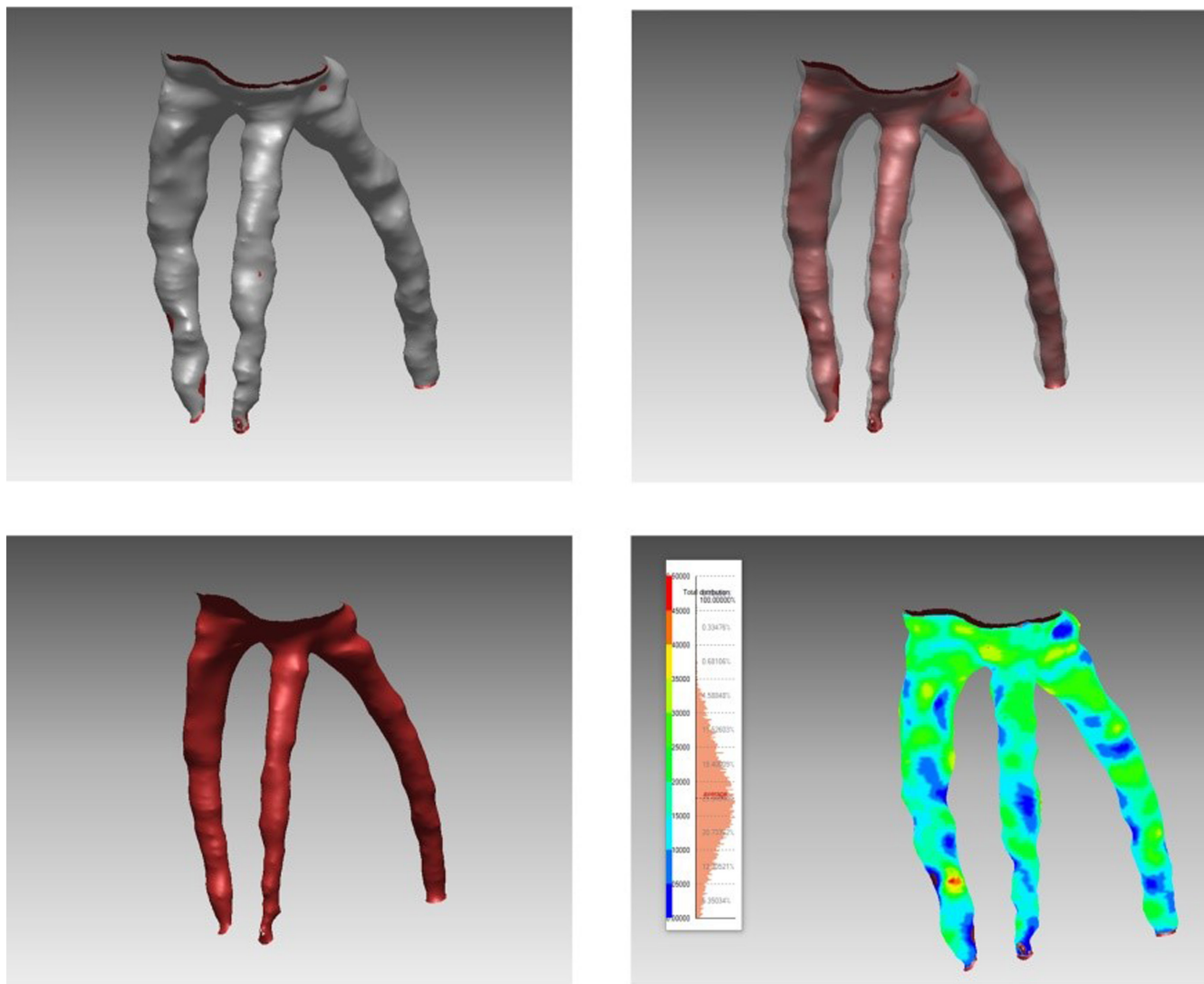


FIGURE 3 Root canal preparation analysis in the superposition of the pre- and postpreparation of the root canals. The colored map shows the dentine wall wear (minimum blue, maximum red).

two canal models to be registered and generated a set of linear transformation values.²⁷ The changing canal area (Δ canal area), changing canal volume (Δ canal volume), and untouched canal surface area were calculated by a single blinded, calibrated, experienced operator (the intraobserver agreement was found substantial for Δ canal volume and untouched surface area, almost perfect for Δ canal area; [Figure 4](#)). According to the analysis, the distances between corresponding areas of the pre- and postpreparation models were computed and complemented by visualization of the 3D color-coded maps in which the blue to red fields indicated that the postpreparation of the model was wider than the pre-preparation model. The range of tolerance was set at ± 0.50 mm. In addition, the deviation analysis was carried out, and the percentages of untouched canal surface area within the tolerance range were calculated. Volumetric assessment of root canals was

also calculated for each tooth investigated. These values represented the degree of matching between the pairs of pre- and postpreparation models.

2.7 | Statistical analysis

The data for each root were tabulated and analyzed statistically using SPSS version 26.0 (IBM SPSS, Inc.). The distribution of continuous numeric variables was determined by the Kolmogorov–Smirnov test. The homogeneity of variance was tested with the Levene test. Descriptive statistics for parametric variables, mean and standard deviation, and for nonparametric variables, median and range, were given. Intergroup comparisons of continuous variables were performed using the one-way ANOVA, one-way ANOVA (Welch), and Kruskal–Wallis H test. Post hoc

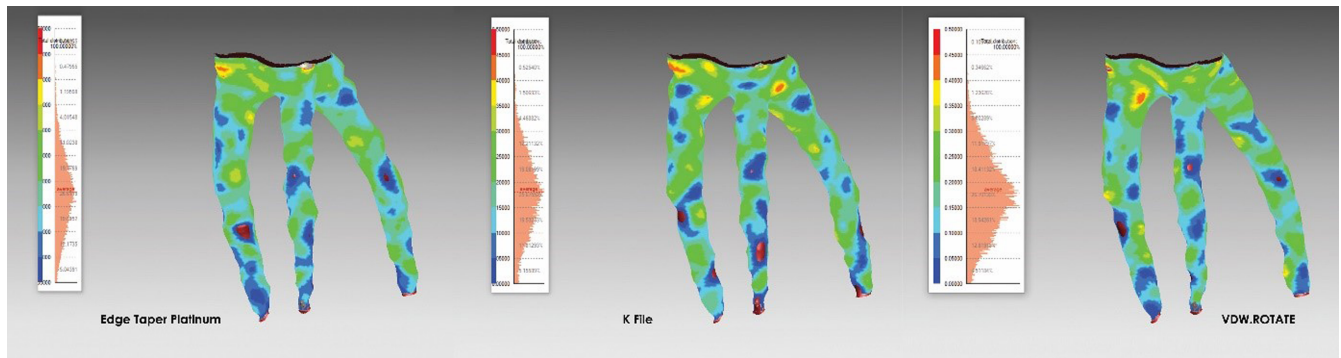


FIGURE 4 Software superposed images of root canals before and after preparation; EdgeTaper Platinum™, K-file, and VDW.ROTATE™.

comparisons (Tamhane 2, Tukey, and Dunn tests) were done for statistically significant parameters. The level of significance was set at $p < .05$.

3 | RESULTS

The changes (Δ) in canal volume, canal area, and untouched area parameters after preparation are shown in Table 1. The lowest value for the Δ canal area in all roots was found in the K-file group, but the difference between the groups was not statistically significant ($p > .05$). Similarly, the lowest value for the Δ canal volume in all roots was found in the K-file group, but the difference between the groups was not statistically significant ($p > .05$).

The difference between all groups in terms of untouched canal surface was statistically significant for each root ($p < .001$). VDW.ROTATE™ performed significantly lesser untouched canal surface area than other systems in all roots (27.09 ± 8.95 for mesial root, 27.51 ± 8.34 for distal root, and 25.21 ± 9.12 for palatinal root). The K-file showed significantly less untouched canal surface area than EdgeTaper Platinum™ ($p < .05$).

Regarding the instrumentation time, VDW.ROTATE™ file system was significantly faster (6.47 ± 0.39 min) than EdgeTaper Platinum™ (7.71 ± 0.73 min) and K-files (8.22 ± 0.72 min) ($p < .05$). K-file was significantly slower (3.05 ± 0.35) than other mechanized files during mesial root preparation ($p < .05$). Besides that, the mean instrumentation time for K-file was found similar to EdgeTaper Platinum™ during distal and palatinal root preparation (Table 2).

4 | DISCUSSION

This study assessed the shaping ability and instrumentation time of K-files and two different heat-treated Ni-Ti rotary systems in printed primary teeth. There was no significant

difference among VDW.ROTATE™, EdgeTaper Platinum™, and K-file for Δ canal volume and area, but VDW.ROTATE™ showed significantly lower untouched canal surface area. In addition, the VDW.ROTATE™ file system was found to be significantly faster than the other groups. Therefore, the first and second null hypotheses tested were rejected.

Kuo et al.²⁸ demonstrated in their clinical study with a 12-month recall examination that root canal preparation in primary molars with an apical diameter of #20–30 and a mean conicity of 0.04–0.06 could be performed safely and efficiently. Based on the clinical and radiographic results of this study, the success rate was 96%.²⁸ Per the paediatric pulpectomy guidelines, the recommended apical size for primary molars has been #25–30.²⁹ For this reason and to provide a standardization of the experimental groups of this study, the final apical diameters and conicities were set to #25.06 for both tested rotary systems. Moreover, in the hand file group, root canals were prepared to #25 MAF. Residues and bacterial biofilm on the untouched surfaces of root canals after enlargement reduce the success of the treatment and may cause permanent infections.^{30,31} Although some progress has been made in the manufacturing process and design of Ni-Ti instruments, to date no system has been shown to be able to touch all surfaces of the root canals.^{19,32} This might be explained mainly due to the root canal geometry with anatomical areas that cannot be touched regardless of the used instrument.³³ In this study, none of the instrumentation systems could touch the total surface of the canal walls. Even so, VDW.ROTATE™ system provided less untouched surface area. The characteristics of instruments such as tip and taper, cross-section design, and cutting flutes can explain these differences. The EdgeTaper Platinum™ files have beveled triangular section that can increase the adaptability of the file edges to the canal walls and produce smooth surfaces. In contrast, the VDW.ROTATE™ system has the double-bladed adapted S-shaped cross

TABLE 1 Mean and standard deviations of canal area and untouched canal surface; and median, minimum, and maximum values of canal volume according to different file systems

	Roots	Parameters	VDW. ROTATE™	EdgeTaper Platinum™	K-file
Canal area (mm ²)	MR [†]	Initial area	35.23	35.23	35.23
		After instrumentation	48.2 ± 5.18	48.23 ± 7.01	44.4 ± 5.52
		Δ	12.97 ± 5.18	13 ± 7.01	9.17 ± 5.36
	DR [†]	Initial area	35.52	35.52	35.52
		After instrumentation	48.22 ± 5.43	47.09 ± 7.22	44.4 ± 6.89
		Δ	12.7 ± 5.42	11.57 ± 7.12	8.88 ± 6.34
	PR [†]	Initial area	35.67	35.67	35.67
		After instrumentation	48.33 ± 5.86	47.92 ± 7.28	44.5 ± 5.67
		Δ	12.66 ± 5.86	12.25 ± 7.28	8.83 ± 5.62
Canal volume (mm ³)	MR [‡]	Initial volume	7.13	7.13	7.13
		After instrumentation	13.8 (8.1–18)	14.09 (9.9–17.9)	12.17 (7.4–15.6)
		Δ	6.67 (1–10.9)	6.96 (2.7–10.8)	5.04 (0.3–8.4)
	DR [‡]	Initial volume	8.24	8.24	8.24
		After instrumentation	14.34 (8–19.5)	12.7 (10.6–18)	11.8 (5.4–16)
		Δ	6.1 (0.2–11.3)	4.46 (2.3–9.7)	3.52 (0.2–7.7)
	PR [‡]	Initial volume	8.62	8.62	8.62
		After instrumentation	13.78 (8.4–18)	13.87 (8.8–18.9)	12.93 (7.4–17.9)
		Δ	5.16 (0.2–9.7)	5.25 (0.6–10.6)	4.31 (0.8–9.6)
Untouched canal surface (%)	MR [§]		27.09 ± 8.95 ^a	43.9 ± 6.6 ^b	36.41 ± 9.62 ^c
	DR [†]		27.51 ± 8.34 ^a	44.04 ± 6.72 ^b	37.72 ± 9.29 ^c
	PR [†]		25.21 ± 9.12 ^a	44.37 ± 7.39 ^b	36.42 ± 8.61 ^c

Note: For each row, values with superscript letter are not significantly different at $p < .05$.

Abbreviations: Δ, The changing amount of parameter; DR, distal root; MR, mesial root; PR, palatal root.

[†]One-way ANOVA test.

[‡]Kruskal–Wallis H test.

[§]One-way ANOVA/Welch test.

TABLE 2 Distribution of the mean and standard deviations (SD) of instrumentation time according to different file systems

Parameter	Roots	VDW.ROTATE™ (mean minute ± SD)	EdgeTaper Platinum™ (mean minute ± SD)	K-file (mean minute ± SD)
Instrumentation time (minutes)	MR	2.21 ± 0.16 ^a	2.5 ± 0.24 ^b	3.05 ± 0.35 ^c
	DR	2.11 ± 0.17 ^a	2.56 ± 0.32 ^b	2.67 ± 0.41 ^b
	PR	2.15 ± 0.17 ^a	2.64 ± 0.36 ^b	2.49 ± 0.44 ^b
	Total time for one tooth	6.47 ± 0.39 ^a	7.71 ± 0.73 ^b	8.22 ± 0.72 ^b

Note: For each row, values with superscript letter are not significantly different at $p < .001$, one-way ANOVA (Welch) test.

Abbreviations: DR, distal root; MR, mesial root; PR, palatal root.

section, which may provide an effective cutting ability to the instrument blades. Moreover, VDW.ROTATE™ has an off-centered design, which might improve the amount of touched root canal walls.

Compared with the results of our study in terms of the evaluated parameters, there are a few studies, which evaluated the efficacy of manual and rotary instrumentation in primary molar teeth.^{34,35} In contrast to the present

findings, Esenturk et al.³⁴ compared rotary instrumentation systems (OneShape and Revo-S) and K-files and demonstrated that most of the root canal walls remained untouched (76.5%) irrespective of the preparation system used. Whereas one rotary system (Revo-S) showed better results than hand K-files, the other (OneShape) had a similar untouched surface area when compared to hand K-files. Dalzell et al.³⁵ reported that the untouched canal

surface areas in both nonfused and fused primary teeth were 62% and 69.2% with Mtwo, Reciproc Blue, and K-files root canal preparation without differences between the root canal preparation protocols. These contradictory results may be explained by differences in method, preparation technique, and sample selection.

This study evaluated changes in canal volume and canal area and untouched surface area parameters. The evaluation of centering ability and transportation, however, was not the focus of this study. Although it may suggest that no system abruptly changed the trajectory of root canals with the current parameters, future studies evaluating such parameters for the systems tested are still needed.

One of the biggest problems of laboratorial studies regarding root canal treatments of primary teeth is to obtain appropriate sample collection. Laboratorial studies with extracted human teeth can better reflect clinical conditions. Still, it is challenging to find a sufficient number of standardized primary teeth with the same canal length, canal configuration, and root inclination.^{22,36} It, however, is difficult to establish an adequate and standard sample group since most primary teeth with an indication for extraction have physiological or pathological root resorption.¹⁵ Also, using extracted natural human teeth has some disadvantages, such as ethical considerations and the possibility of cross-infection risk.²² Today, with the developing technology, it is possible to evaluate the efficacy of different instrumentation systems and treatment methods by obtaining standardized resin-based models with 3D printing technology using CBCT.¹⁸ In this study, resin-based teeth were obtained from a CBCT scan image of extracted human teeth to overcome the difficulties previously discussed. In previous studies, it was reported that 3D-printed resin teeth were very promising and have the potential for educational purposes and standardization in studies.³⁷ In the study of Cui et al.,³⁸ no differences were found in the postpreparation volume, surface area, or transportation between the 3D-printed teeth and the extracted teeth, and the authors suggest that 3D-printed teeth could be suitable for comparing the shaping abilities of different Ni-Ti instruments. Therefore, this study combined the advantages of the standardized endodontic resin blocks with the benefits of the CBCT imagistic investigation by evaluating standardized printed dental replicas made of a radio-opaque resin.^{18,39} It is a fact that printed teeth increase this study's internal validity, with reduced selection and assessment bias. In addition, all assessments were blinded to the experienced operator, decreasing the risk of detection bias.

There, however, are discussions in the literature about the difference in radiopacity and hardness between resin and human dentine.²³ The 3D-printed resin used in this study has a Shore D hardness of 75, which is higher than the reported value of acrylic resin.⁴⁰ Due to Shore D

hardness testing limitations, only one study compared the mechanical properties of 3D-printed materials with those of natural teeth. Reymus et al.⁴¹ reported that no commercially available ones could mimic human dentine in hardness and radiopacity. It has been stated that the critical reason for the hardness difference is 3D technology. The resin should have a low viscosity. Additives such as filler particles can be used to support the base resin to improve material properties, but they will also increase its viscosity. Therefore, the possibility of increasing the hardness by using fillers is limited.⁴² The change in the resin content, however, also may affect the radiopacity. In order to reduce the hardness and radiopacity differences between resin and dentine, printing materials that can better mimic human dentine should be developed. In addition, in this study, distilled water was used instead of NaOCl as the irrigation solution during preparation because no infection control was needed and NaOCl can dissolve only organic debris.⁴³

In paediatric dentistry, instrumentation time is crucial regarding children's anxiety, cooperation in the dentist's chair, and fatigue of both the operator and the child.⁴⁴ Therefore, reduced instrumentation time is required for safe and optimal root canal treatment.²⁷ In most previous studies, rotary systems were found to be significantly faster than manual files in endodontic treatments of primary and permanent teeth.^{27,44,45} In this study, both rotary file systems reduced total instrumentation time, but significant reductions were observed for only the VDW. ROTATE™ system for all root canal preparation and also for the three different roots, separately. The instrumentation time can be affected by the study's design, the use of different types of rotary instruments, file sequences, and instruction manuals.⁴⁶ The difference between the two systems performing the same rotation movement in this study may have a direct relation to the number of files used, two in the VDW.ROTATE™ group and three for the EdgeTaper Platinum™ group.

Under the conditions of this laboratorial study, it can be concluded that the shaping ability and the instrumentation time were directly influenced by the root canal instrumentation system used during the preparation of resin-printed primary molars, with VDW.ROTATE™ being the faster system and associated with a lower amount of untouched canal surface area.

AUTHOR CONTRIBUTIONS

B.G.T., S.F., and E.I. conceived the ideas and designed the study; B.G.T. and S.F. collected the data; B.G.T. analyzed the data; S.F., E.S., and M.G. interpreted the data, B.G.T., S.F., and E.S. led the writing, and E.S. and M.G. revised the manuscript. All the authors revised and approved the final paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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