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Influence of voxel size on dentinal microcrack detection by micro-CT after root canal preparation

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Abstract: The aim of this study was to assess the influence of micro-computed tomography (micro-CT) voxel size on dentinal microcrack detection after root canal preparation using rotary heat-treated nickel-titanium files. Curved mesial root canals (n = 24) of mandibular molars were prepared using ProDesign Logic 30/.05 (PDL) or HyFlex EDM 25/.08 (HEDM). The specimens were scanned by micro-CT at 5 µm voxel size before and after root canal preparation. The percentage of microcracks was evaluated in images at 5, 10 and 20 µm voxel size, by two examiners at two moments. The Kappa and McNemar tests (α = 0.05) were used. The percentage of dentinal microcracks was similar before and after PDL and HEDM preparations, at 10 and 20 µm ($p > 0.05$). HEDM showed a higher percentage of dentinal microcracks in the middle third at 5 µm after preparation ($p < 0.05$). The detection of dentinal microcracks before and after instrumentation using PDL was more accurate at 5 µm than at 20 µm, in all thirds ($p < 0.05$). Within the limitations of this ex vivo study, as expected, the results showed that different resolutions influence the micro-CT analysis of microcracks. The highest accuracy in detecting microcracks was observed for analyses performed at 5 µm voxel size. HyFlex EDM caused even more microcracks to develop in the middle third, detectable only by visualization of images made at 5 µm voxel size.

Keywords: Endodontics; Dentin; Root Canal Preparation; Dental Pulp Cavity.

Introduction

Nickel-titanium (Ni-Ti) files have improved root canal preparations by enabling enlargement and maintenance of the canal trajectory.[1,2] Although mechanized root canal preparation presents several advantages, it has also been reported to cause potential dentinal microcracks.[3,4,5,6] The performance of endodontic files during root canal preparation is related to their cross-sectional design, taper, and thermal or surface treatment.[7,8] Heat treatments applied to NiTi files have been associated with lower risk of dentinal microcracks.[9] These microcracks could potentially promote a vertical root fracture,[10] especially in roots with reduced dentin thickness.[11] Owing to contrasting information in the literature, there is still no consensus on whether root canal preparation

using continuous or reciprocating motion induces the formation of microcracks.[4,9,12,13,14]

High precision tools are required to detect microcracks, such as micro-computed tomography (micro-CT), which allows the defect to be located with great precision.[9] Microcracks can be evaluated for endodontic instrumentation at different resolutions, using scanning voxel sizes of 10 µm,[14] 14 µm,[15,16] 17.42 to 21 µm,[9,12,17] and 30 µm.[13,18] Although most studies using micro-CT have not observed any correlation between root canal preparation with NiTi files and microcrack formation,[14,15,16,17,18] some studies have correlated the presence of microcracks with root canal preparation using rotary NiTi files.[12,13]

ProDesign Logic - PDL (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) are Ni-Ti files with control memory (CM).[19] PDL allow shorter working time associated with resistance to cyclic fatigue.[20] In addition, preparation with these instruments has not been associated with the formation of new dentin defects.[17] Although Hyflex EDM – HEDM files (Coltène / Whaledent, Allstätten, Switzerland) also have CM characteristics, they are manufactured using electrical discharge machining.[18,21] HEDM instruments maintain their integrity after multiple use, and are associated with high resistance to cyclic fatigue.[20] There is contrasting evidence regarding microcracks after HEDM root canal preparation.[13,18]

There are no studies that assessed the influence of micro-CT voxel size on microcrack evaluation, much less any assessing this influence at 5 µm. Therefore, this study aimed to evaluate the influence of micro-CT voxel size on microcrack detection after instrumentation with CM files. The null hypotheses were that there would be no difference among the voxel sizes, or between the dentinal microcracks present after preparation with PDL and HEDM.

Methodology

Specimen selection

The sample size for this study was calculated using a specific software (G * Power 3.1.7 for Windows, Heinrich-Heine-Universität Düsseldorf). The chi-square test was used, with an alpha type error of 0.05, beta power of 0.90, and effect size of 0.93. The ideal sample size was reported to be six specimens per group.

All procedures were approved by the dental school ethics committee (CEP no. 64736116.4.0000.5416). The roots were inspected under a stereomicroscope at 12× magnification to exclude any roots with external dentinal defect or immature apices. The study used human mandibular first and second molars previously stored in 0.1% thymol solution at 5°C. The inclusion criteria required teeth with two independent mesial root canals according to Vertucci's type IV classification,[23] angle of curvature between 25° and 35° based on the Schneider method,[24] and radius of curvature smaller than 10 mm, following the Pruett method,[25] in addition to complete apical formation, and absence of root fractures, calcifications or internal resorptions. Compliance with the criteria required that the specimens be radiographed using a digital system (RVG 6100; Kodak Dental Systems, NY, USA), and scanned with a micro-CT device (SkyScan 1176; Bruker-microCT, Kontich, Belgium). The first scanning was performed at low-resolution (35 µm voxel size) under the following settings: copper and aluminum filters, 87-millisecond exposure time, frame averaging of 3, 180° rotation around the vertical axis, rotational step of 0.5° at 80 kV and 300 µA. After excluding all the teeth that did not comply with the inclusion criteria, 12 mesial roots of mandibular molars were selected, totaling 24 root canals.

The mesial root canals of the specimens were divided into two experimental groups randomly (n = 12), with stratified random sampling, considering the preoperative volume of the root canals. The root length was standardized at 18 mm, with a tolerance of ± 1 mm of discrepancy. The specimens were embedded in condensation silicone (Oranwash, Zhermack SpA, Badia Polesine, Italy) to simulate the periodontal ligament.

Root canal preparation

Conventional access cavities were performed, and the root canals were explored by using a size #10 K-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland). The working length was established at

1 mm short of the apical foramen of each specimen. Afterwards, a trained operator instrumented all the specimens with an operating microscope (MC-M1232, DF Vasconcellos, Valença, Brazil) at 13× magnification, using an endodontic motor (VDW Silver, VDW, Munich, Germany).

PDL preparation: A 30/.01 file was used in continuous rotation at 350 rpm speed and 1 Ncm torque, using in-and-out movements up to the working length. Then, a 30/.05 file was used at 600 rpm speed and 4 Ncm torque.

HEDM preparation: A 10/.05 file was used in continuous rotation at 300 rpm speed and 1.8 Ncm torque, using in-and-out movements up to the working length. Then, a HEDM 25/.08 file was used at 500 rpm speed and 2.5 Ncm torque.

Root canal irrigation was performed with 5 mL of 2.5% sodium hypochlorite (NaOCl), using a 30G side-vented needle (NaviTip, Ultradent Products, South Jordan, UT) adapted to a 5 mL syringe (Ultradent Products), which was placed 2 mm short of the working length. The final irrigation used 2 mL of 17% EDTA, followed by 5 mL of distilled water, as described above.

Micro-CT analysis

The micro-CT analysis was performed by scanning the specimens at 5 μm voxel size in a micro-CT device (SkyScan 1272. Bruker, Kontich, Belgium), before and after instrumentation, under the following settings: copper filter, 180° rotation around the vertical axis and rotational step of 0.2° at 100 kV and 100 μA. The images were reconstructed using a NRecon software program (NRecon v.1.6.3; Bruker), and superimposed with geometric alignment using a DataViewer software program (Data Viewer v.1.5.1, Bruker). The qualitative analysis was performed using a CTAn software program (CTAn v.1.14.4, Bruker). The original images were resized isotropically to perform analyses with different voxel sizes. Images at 10 and 20 μm were obtained for qualitative analysis. This was achieved by resizing the previously scanned 5 μm images by 2 and 4, respectively, using a CTAn software program, while the dataset previously superimposed in DataViewer software was being loaded.[26] Three dimensional images were made with a CTVox software program (CTVox v.3.2; Bruker).

The analyses were performed in the middle and apical thirds of the roots, considering 3 mm for each third. The microcracks were evaluated by opening the cross-sectional images before and after instrumentation, and evaluating them at the same time. The analyses were performed in three voxel sizes, totaling 14,400 slices of cross-sectional images at 5 μm, 7,200 at 10 μm, and 3,660 at 20 μm, per third in each group. The images were then compared twice by two blinded examiners, at two different times. Microcracks were defined as lines or defects extending from inside the root canal to the dentin, or from the outer root surface into the dentin (Figure 1). The distribution of microcracks was expressed as a percentage of the total cross-sectional images.

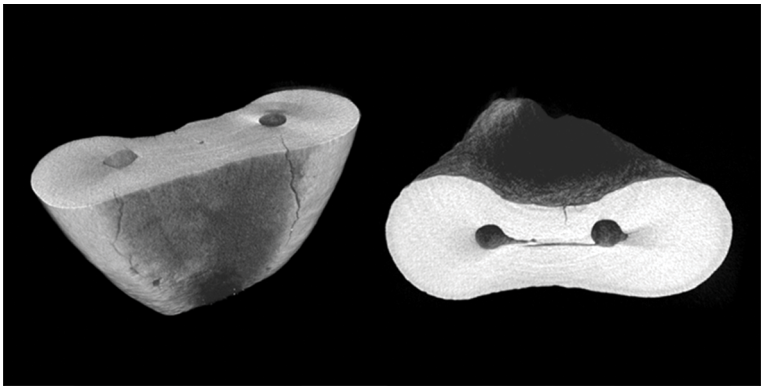

Figure 1. 3D reconstructions showing microcracks in the mesial roots of mandibular molars.

Statistical analysis

The results were expressed as a percentage for each group. Kappa statistics were used to ascertain intra- and extraexaminer agreement. The McNemar test was used to determine significant differences before and after instrumentation, and at different voxel sizes in the same group. The level of significance was set at 5%.

Results

The kappa values were greater than 0.81 for all parameters assessed, thus confirming intraexaminer and extraexaminer agreement. The detection of dentinal microcracks in the PDL group, before and after instrumentation, was more accurate at 5 μm than at 20 μm in all thirds ($p < 0.05$) (Table). Figure 2 shows a thinner continuity of microcracks based on different voxel sizes.

No differences were observed in the apical third before or after instrumentation in any of the voxel sizes (5, 10 and 20 μm), in the HEDM group, ($p > 0.05$). The comparison between before and after instrumentation with PDL and HEDM showed that the percentage of dentinal microcracks was similar for both groups at 10 and 20 μm ($p > 0.05$). However, the percentage of dentinal microcracks after instrumentation with HEDM was higher in the middle third at 5 μm ($p < 0.05$) (Table). Figure 3 shows cross-sectional images revealing microcracks before and after instrumentation with the PDL and HEDM systems.

Discussion

Tooth fracture is a critical issue in endodontics, owing to its direct relation to long-term survival rates.[27] In the present study, a higher percentage of microcracks was observed at a small voxel size (5 μm). Hence, our first null hypothesis was rejected, considering that microcrack detection was influenced by the voxel size.

Many factors may influence microcrack evaluation, such as different scan settings, number of cross-sectional images, different voxel sizes, and misinterpretation of ring artifacts.[18]. The influence of voxel size has been previously assessed for cone beam computed tomography.[28,29] Similarly, previous studies have observed that analysis of trabecular bone structure can be significantly affected due to micro-CT voxel size.[26,30,31] In endodontics, Orhan et al.[32] evaluated the presence of voids in filled root canals, at different voxel sizes. The authors detected more voids at smaller voxel sizes (5.2, 8.1 and 11.2 μm) than at 16.73 μm. In the current study, voxel size was the only variable evaluated, since the samples were scanned at 5 μm, and the reconstructed images were resized to acquire images at 10 and 20 μm voxel sizes.[26] This method allows the micro-CT images to be resized to higher voxel sizes, which represent an average of the previous voxels.[26,33] Therefore, the smallest voxel size used during the scanning procedure can be considered the gold standard for determining the level of accuracy under limited resolution scenarios.[31,34]

Table. Percentage of sections with dentinal microcracks before and after preparation, based on different voxel sizes.

Variable	ProDesign Logic 30.05			HyFlex EDM 25.08		
	5 μm	10 μm	20 μm	5 μm	10 μm	20 μm
Before preparation						
Middle	3.04^{a}	2.92^{ab}	1.85^{b}	19.94^{a*}	18.80^{b}	17.32^{b}
Apical	3.19^{a}	2.35^{b}	2.32^{b}	1.40^{a}	1.13^{a}	1.13^{a}
After preparation						
Middle	3.36^{a}	3.02^{a}	2.09^{b}	24.11^{a*}	20.04^{b}	18.16^{b}
Apical	3.45^{a}	2.44^{ab}	2.38^{b}	1.52^{a}	1.23^{a}	1.20^{a}

Different lowercase letters on the same line indicate statistical difference among the different analysis resolutions of the same group. *represents statistical difference between before and after preparation in same group and third (McNemar test, 5% significance).

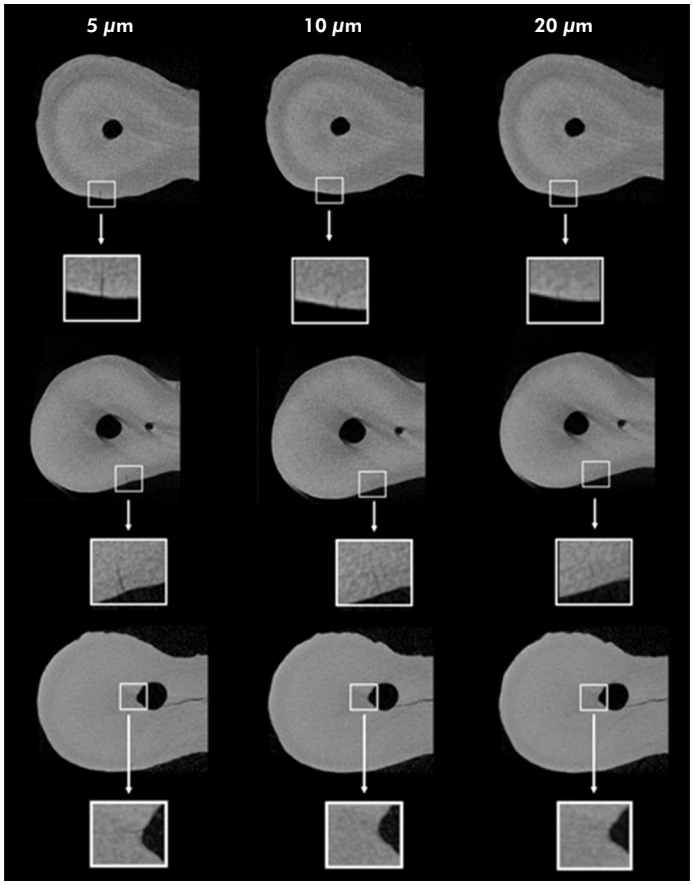

Figure 2. Representative cross-sectional micro–CT images showing a thinner continuity of microcracks based on different voxel sizes.

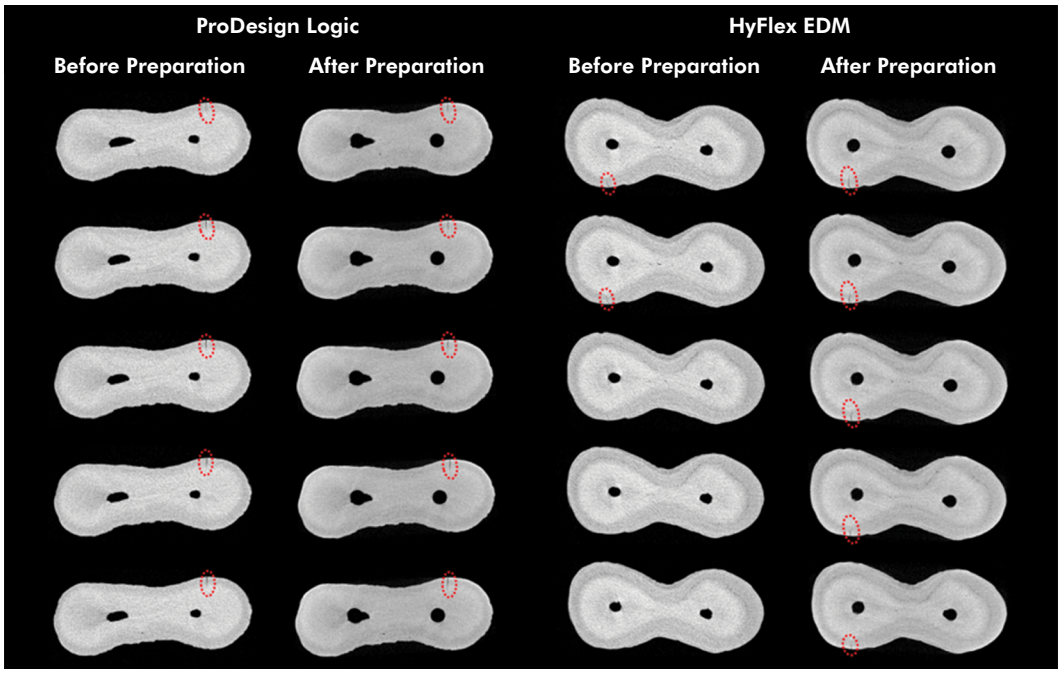

Figure 3. Representative cross-sectional micro–CT images, at 5 µm voxel size, showing microcrack continuity with a 50 µm interval between slices, before and after instrumentation with the ProDesign Logic and HyFlex EDM systems. Microcracks are circled in red.

The analyses at 10 and 20 μm were similar regarding the presence of microcracks before and after the preparation with HEDM files. However, assessment at 5 μm showed a higher percentage of microcracks in the middle third after instrumentation, partially rejecting the second null hypothesis. The highest number of slices in images at 5 μm may have contributed to better capacity to detect the thinner continuity of microcracks, since micro-CT high-resolution scans were recommended to provide more accurate analysis of microstructures.[30] On the other hand, considering that statistical significance is dependent on the sample size[35] and that each cross-sectional image was used for statistical qualitative tests,[9,12,14] the larger number of slices observed at the higher resolution (5 μm) may have contributed to the statistical significance observed in the analyses before and after preparation with HEDM performed at 5 um.

In a previous study, HEDM did not cause microcracks in mandibular premolars with straight root canals.[18] However, Mandava et al.[13] showed a higher percentage of microcracks after HEDM instrumentation in mesial curved canals of mandibular molars. The root canal anatomy may have contributed to producing discordant observations between the two studies, since mesial curved canals may favor microcracks formation.[13] The increased percentage of microcracks could be attributed to the taper,[7,9] the hard surface induced by the surface treatment,[21,36,37] the tip design, the cross-sectional geometry, the variable pitch or the flute form.[7,9,27] Specifically, the greater taper of the first 4 mm of the 25/.08 HEDM file, despite its progressive taper reduction to 0.04,[13,21,38] may have caused a more aggressive instrumentation than the constant taper of the 30/.05 PDL file. The propagation of microcracks in HEDM instrumentation was probably caused by tensions inside the root canal, which were transmitted to the external root surface, thus increasing a preexisting defect. A previous study reported that this effect is caused by the collapse of the dentin bonds around the external root surface.[10]

After PDL preparation, no new microcracks were observed, even at 5 μm. Moreover, no correlation was observed between the instrumentation and the formation/propagation of microcracks after preparation with PDL files.[17] Although PDL also has CM heat treatment,[19] its reduced taper could influence the differences observed in regard to the HEDM group, even at the similar speed and torque values used in both systems.

In the present study, the images revealed that microcracks were already present in all the groups before instrumentation, thus corroborating previous investigations that used larger voxel sizes.[12,15,16,18] These defects in uninstrumented teeth could be attributed to the forces at play during extraction, excessive occlusal functional loads, tooth age[39] or dentin dehydration.[16] The authors decided to reduce this methodological bias by carefully selecting the specimens, and storing them in 0.1% thymol, as part of our methodology. This resulted in a low percentage of pre-existing microcracks. The majority of microcracks were observed from the external root surface extending to the root canal, corroborating a previous study.[9] In the current study, the preoperative volume of root canals was considered in selecting the sample. The HF-EDM group presented a higher percentage of microcracks than the PDL group. Micro-CT analysis allows visualization of preexisting and post-operative microcracks, thereby allowing each specimen to act as its own control.[40] However, the greater presence of preexisting microcracks may have induced a greater formation of microcracks during the preparation by HF-EDM. Therefore, within the limitations of this *ex vivo* study, it can be concluded that the voxel size should be considered as a potential factor for detecting microcracks when assessing micro-CT images. Further studies are needed to evaluate detection in lower micro-CT voxel size scans.

Conclusions

As expected, the results showed that different resolutions influenced the micro-CT analysis of microcracks. The highest accuracy in detecting microcracks was observed for analyses performed at 5 μm voxel size. HyFlex EDM caused even more microcracks to develop in the middle third, detectable only by visualization of images made at 5 μm voxel size.

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