Synchrotron radiation-based micro-computed tomographic analysis of dentinal microcracks using rotary and reciprocating file systems: An in vitro study

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Abstract

Background: Advances in both X-ray sources and X-ray optics have dramatically improved the feasibility of these techniques in various fields.

Aims: We aimed to evaluate the frequency of dentinal microcracks observed after root canal preparation with ProTaper Gold (PTG), Hyflex electrodischarge machining (HEDM), Reciproc (RPC), and WaveOne Gold (WOG) using synchrotron radiation-based micro-computed tomographic (SR-µCT) analysis.

Subjects and Methods: Forty mandibular molars were assigned to 4 experimental groups (n = 10) according to the file system used for the root canal preparation: Group 1: PTG (25/0.08), Group 2: HEDM (25/0.08), Group 3: RPC (25/0.08), and Group 4: WOG (25/0.07). The specimens were scanned on SR-µCT system before and after root canal preparation. The pre- and postoperative cross-sectional images (N = 183,200) of the mesial roots were screened to identify the presence of dentinal microcracks. The number of microcracks was determined as a percentage for each group.

Statistical Analysis: There was no necessity of statistical analysis in the present study. The number of microcracks was determined as a percentage for each group.

Results: Among the four file systems tested, HEDM (0.9%) has shown a fewer number of new dentinal microcracks.

Conclusions: SR-µCT can be used as a reliable diagnostic tool for further implications.

Keywords: Dentinal microcracks; root canal preparation; synchrotron

INTRODUCTION

Endodontically treated teeth are more susceptible to vertical root fractures which eventually lead to tooth or root extraction.¹ The biomechanical preparation of root canals causes stress concentration in the root dentin, which creates a gateway to dentinal microcracks, leading to failure of treatment.²,³ Complexities in canal preparation may be attributed to variation in design of cutting instruments, taper, and composition from which it is made.⁴ With the inception of nickel–titanium (NiTi) rotary instrumentation, root canal treatment has transfigured to a more convenient and time-saving procedure which also

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The specimens were decoronated to remove any visible preexisting craze lines, cracks, and root canal obstructions. Only teeth with fully formed roots, closed apices, and two mesial root canals with independent foramina having moderate curvature (10°–20° curvature angle) analyzed through Schneider’s technique were selected. The specimens were decoronated to remove the distal roots using a low-speed saw (Isomet; Buehler Ltd., Lake Bluff, NY, USA) with water cooling, leaving mesial roots with approximately 12 ± 1 mm in length to prevent the introduction of any confounding variables. All the roots were inspected with a dental operating microscope (Carl Zeiss, Jena, Germany) at ×24 magnification to detect any new cracks formed. Four teeth with such findings were excluded and replaced. Canal patency was established with a size 10 K-file in the presence of Glyde (Dentsply Maillefer, Ballaigues, Switzerland). All teeth in which canal patency could not be established were excluded from the study and replaced with similar patent teeth. The specimens selected were then stored in 0.1% thymol solution at 5°C.

**Experimental setup**
To attain an overall outline of the mesial roots and structural variations in pre- and postprocessing conditions with different file systems, micro-CT experiments have been performed at X-ray Imaging Beamline (BL-04), Indus-2 Synchrotron Source, Raja Ramanna Centre for Advanced Technology, Indore, India. Indus-2 is a third-generation synchrotron source operating at 200 mA current and 2.5 GeV energy which provide broad radiation extending from infrared to hard X-ray. This beamline extracts a hard X-ray monochromatic beam using a silicon Si (111) double-crystal monochromator to expose the teeth samples. The projection images were acquired at 25 keV X-ray-beam energy with a rotation step of 0.2°. The bright- and dark-field images were also acquired to correct the image noise. A very high-resolution (pixel size: 4.5 μm) photonic science charge-coupled device camera having field of view 18 mm × 12 mm is used to acquire the X-ray projection images. The sample was mounted on two high precision linear stages along with one angular stage on the top to rotate the sample. The detector was mounted on three linear translation stage systems to align and optimize the imaging system. The acquired projection images were preprocessed, normalized, and reconstructed using a filtered back-projection algorithm to get the 3D slice images. Further quantitative analysis of all slice images was done using the ImageJ software.

**Subjects and Methods**

**Sample selection**
Forty freshly extracted human mandibular first and second molars from patients of 40 to 60 years which were selected for different clinical reasons were used in the present study. Teeth were extracted by an experienced oral surgeon using an atraumatic technique. In brief, an intrasulcular incision was used to separate the mucoperiosteum from the root and bone. Periotomes were used to sever the periodontal ligament from the root surface. Extraction was completed with luxators and forceps. All roots were initially inspected with digital radiographs to detect and exclude teeth with any visible preexisting craze lines, cracks, and root canal obstructions. Only teeth with fully formed roots, closed apices, and two mesial root canals with independent foramina having moderate curvature (10°–20° curvature angle) analyzed through Schneider’s technique were selected. The specimens were decoronated to remove the distal roots using a low-speed saw (Isomet; Buehler Ltd., Lake Bluff, NY, USA) with water cooling, leaving mesial roots with approximately 12 ± 1 mm in length to prevent the introduction of any confounding variables. All the roots were inspected with a dental operating microscope (Carl Zeiss, Jena, Germany) at ×24 magnification to detect any new cracks formed. Four teeth with such findings were excluded and replaced. Canal patency was established with a size 10 K-file in the presence of Glyde (Dentsply Maillefer, Ballaigues, Switzerland). All teeth in which canal patency could not be established were excluded from the study and replaced with similar patent teeth. The specimens selected were then stored in 0.1% thymol solution at 5°C.

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**Root canal preparation**
The surface of the roots was coated with a thin film of polyvinyl siloxane impression material to simulate the periodontal ligament and placed corono-apically inside a custom-made jig to further streamline the coregistration process. Apical patency was determined by inserting a size 10 K-file into the root canal until its tip was visible at the apical foramen, and the working length (WL) was set 1.0 mm shorter of this measurement. After glide paths were established with a size 15 K-file (Dentsply Maillefer) up to the WL, the specimens were randomly assigned to 4 experimental groups (n = 10):

- **Group 1** – PTG (25/0.08): Root canals were prepared in a crown-down fashion with the aid of an X-Smart Plus electric motor with torque control (Dentsply Maillefer) at 300 rpm. The PTG shaping SX, S1, and S2 and finishing F1 and F2 files were sequentially used with a continuous in-and-out movement until the WL was reached. Torque and other parameters...
for each file were set as per the manufacturer’s recommendation

- **Group 2 – HEDM (25/0.08):** The canals in this group were shaped using X-Smart Plus electric motor with torque control (Dentsply Maillefer) with HEDM file at 500 rpm and 2.5 Ncm torque in accordance with the manufacturer’s instructions up to the WL.

- **Group 3 – RPC (25/0.08):** The R25 instrument was used with X-Smart Plus electric motor with torque control (Dentsply Maillefer) using in-and-out pecking motion in the “RECIPROC ALL” mode until reaching the WL of the canals. According to the manufacturer’s instructions, gentle apical pressure was applied to the file.

- **Group 4 – WOG (25/0.07):** The canals in this group were shaped with X-Smart Plus electric motor with torque control (Dentsply Maillefer) using the WOG primary NiTi file in the “WAVEONE ALL” mode until reaching the WL. According to the manufacturer’s instructions, gentle apical pressure was applied to the file. In all the four groups, canal irrigation was consistent with 2 ml 2.5% NaOCl and saline alternatively. After preparation, a postoperative micro-CT scan of each specimen was performed using the aforementioned parameters.

**Dentinal microcrack evaluation**

Octopus 3D imaging reconstruction software was used for superimposition that coregistered the image stacks of the specimens pre and post the canal preparation. The cross-sectional images of the mesial roots were screened ($N = 81,747$) to identify the presence of dentinal microcracks. To start the analysis, the postoperative images were scanned and the number of the cross sections in which dentinal microcracks had been noticed was recorded as showed in Figure 1. This was proceeded by the analysis of preoperative corresponding images to verify the new dentinal microcracks that were observed in the postoperative equivalent. For the present study, dentinal microcracks included all lines and cracks observed, which were extended or not extended to the external root surface.

**RESULTS**

On examining the 81,747 cross-sectional images, 11,555 (14%) images showed new dentinal microcracks. The presence of dentinal microcracks was observed in 1.96% ($n = 399$), 0.90% ($n = 200$), 1.32% ($n = 256$), and 1.04% ($n = 210$) in the PTG, HEDM, RPC, and WOG groups, respectively, as shown in Table 1. All dentinal defects identified in the postoperative scans were already present in the corresponding preoperative images, and new microcracks were observed after root canal instrumentation with the tested systems.

**DISCUSSION**

In the present study, HEDM (Group 2) have shown to induce a fewer number of dentinal microcracks compared to other Groups 1, 3, and 4. All dentinal microcracks identified in the postoperative scans were already present in the corresponding preoperative images, and few new microcracks were observed after root canal instrumentation. A high frequency of defects caused by the mechanical preparation of root canals was analyzed through various previous studies.[13,14] Yoldas et al.[15] claimed that the tip design of rotary instruments, cross-sectional geometry, constant or variable pitch and taper, and flute form could be related to crack formation. Under the present experimental framework, the increased flexibility of HEDM and WOG may have contributed to less dentinal microcracks compared with the other file systems (RPC and PTG). HEDM is a new generation single-file system with continuous rotation motion. Throughout their entire working part, they have three different horizontal cross sections: a quadratic in

| Variables  | Total slices | Cracks | Percentage |
|------------|--------------|--------|------------|
| Group 1    | 20,301       | 399    | 1.965      |
| Group 2    | 21,984       | 200    | 0.909      |
| Group 3    | 19,310       | 256    | 1.325      |
| Group 4    | 20,152       | 210    | 1.042      |

Among the four file systems tested, HEDM (0.9%) has a shown fewer number of new dentinal microcracks comparatively. HEDM = Hyflex electrodispcharge machining.
the apical region, trapezoidal in the middle, and almost triangular in the coronal region. These files are made of a controlled memory alloy using electrodisharge machining technology, which significantly improved its flexibility. According to the manufacturer, WOG used in reciprocating motion is repeatedly heat treated and cooled, having parallelogram cross section, providing increased flexibility and cyclic fatigue resistance.

Based on the studies reviewed, the reciprocating instruments tend to create less dentinal microcracks than the continuous rotary instruments and vice versa. Few studies reported no significant difference between the motions. The differences between these previous studies could be related to different methodologies such as different sectioning techniques, imaging techniques, selection of the specimens, and different types and sizes of instruments.

Studies on dentinal microcrack formation present some limitations. This research aimed to reduce the influence of many confounding factors. The patients’ age may play an important role in the presence of dentinal microcracks; hence, teeth from a limited age group (40–60 years) were selected in accordance with a previous study. Many previous studies used single-rooted teeth, however, in the present study, mesial canals of mandibular molars were selected. The constricted anatomic configuration of these canals results in more stress on the dentinal surface during canal preparation and eventually increases the potential for crack formation. According to a previous study, root canal irrigation of mature single-rooted premolars with 5.25% NaOCl affected its dentinal properties sufficiently, altering their strain characteristics to induce more brittleness to dentin. Hence, in the present study, 2.5% sodium hypochlorite solution was used for irrigation. The presence of experimental dentinal microcracks can also be attributed to extraction process and/or the postextraction storage conditions. To minimize this, atraumatic extraction by the trained surgeon was performed, as mentioned.

It was reported that crack propagation was continuous in root slices even after 1 month of storage with no further stress over dentin, and thus, the baseline status of the sample is crucial for reliability. Hence, the storage time was minimized. Investigations using human cadaver models with a noninvasive micro-CT imaging method will provide a more profound information regarding the formation of dentinal microcracks. However, in the present study, emphasis was made in using SR-μCT as a research tool for further dental research. In the present study, hundreds of high-resolution slices per tooth for microcracks were analyzed and calculated than the conventional sectioning techniques that allow the evaluation of only a few slices per tooth with the actual possibility of missing various defects along the root, which confirms the reliability of this novel technology for detecting dentin defects.

To date, no SRCT study observed samples pre- and postinstrumentation of PTG, HEDM, RPC, and WOG. We believe that using such method will boost the efficiency on the investigation of tooth-related defects over a large number of specimens. In fact, one of the limitations of this in vitro study is the long duration which is required for series of scans per sample (pre- and postinstrumentation).

**CONCLUSIONS**

Within the limitation of this in vitro study, the few microcracks have been observed which is associated with mechanical instrumentation irrespective of the kinematics used in the study. Further ex vivo investigations should focus on the prevalence of microcracks associated with endodontic failures. Therefore, SR-μCT analysis will help the researchers to investigate the tooth-related defects more effectively and quantitatively.

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**Conflicts of interest**

There are no conflicts of interest.

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