

A micro-computed tomographic evaluation of dentinal microcrack alterations during root canal preparation using single-file Ni-Ti systems

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Abstract. The aim of the present study was to evaluate the length of dentinal microcracks observed prior to and following root canal preparation with different single-file nickel-titanium (Ni-Ti) systems using micro-computed tomography (micro-CT) analysis. A total of 80 mesial roots of mandibular first molars presenting with type II Vertucci canal configurations were scanned at an isotropic resolution of 7.4 μm . The samples were randomly assigned into four groups (n=20 per group) according to the system used for root canal preparation, including the WaveOne (WO), OneShape (OS), Reciproc (RE) and control groups. A second micro-CT scan was conducted after the root canals were prepared with size 25 instruments. Pre- and postoperative cross-section images of the roots (n=237,760) were then screened to identify the lengths of the microcracks. The results indicated that the microcrack lengths were notably increased following root canal preparation ($P<0.05$). The alterations in microcrack length in the OS group were more significant compared with those in the WO, RE and control groups ($P<0.05$). In conclusion, the formation and development of dentinal microcracks may be associated with the movement caused by preparation rather than the taper of the files. Among the single-file Ni-Ti systems, WO and RE were not observed to cause notable microcracks, while the OS system resulted in evident microcracks.

Introduction

The major purpose of root canal therapy is to reduce the intracanal microorganisms. Chemo-mechanical preparation is an essential and indispensable step in disinfecting the root canal system (1). During endodontic treatment, the roots are susceptible to dentinal damage. Various factors, including the physical properties of the teeth, the endodontic instruments and the preparation technique used, contribute to this damage (2).

In addition to stainless steel hand files, several rotary nickel-titanium (Ni-Ti) file systems have been introduced for the preparation of root canals (3). Ni-Ti instruments have numerous advantages over conventional files, including increased flexibility and a shorter working time (3). However, these systems have different tip designs, tapers and cutting blade configurations that place stress on the root canal walls and may lead to microcracks or craze lines, which may develop into fractures due to repeated stress from occlusal forces (4) and may then lead to tooth loss. Three recently introduced single-file Ni-Ti systems, including WaveOne (WO), OneShape (OS) and Reciproc (RE), enable canal preparation using only one instrument and require less time in comparison with full-sequence rotary instrument systems. However, Kishen (5) reported that cracks may also form in untreated teeth due to the patient age (6), gender, masticatory function or occlusal trauma. Certain studies have indicated that root fracture is connected with dentinal removal (3,7,8), whereas other researchers have not supported this theory (9,10). The movement caused by the preparation method, the design and taper of the file, and the preparation time lead to different degrees of microcracks (9,11-13).

The primary techniques currently used to observe dentinal microcracks are stereoscopic microscopy (14), scanning electron microscopy (SEM), staining, infrared imaging and micro-computed tomography (micro-CT). SEM is typically used to observe the slice of a root (11,15,16); however, cracks may be formed at the root during both sample preparation and the period of observation. Since microcracks can extend through every slice or remain on the surface, SEM may miss microcracks in the slices, which limits its use in dentinal

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microcrack observation. Furthermore, stereoscopic microscopy, staining and infrared imaging do not reveal cracks with a micro-scale resolution (17,18).

Micro-CT is a multi-functional three-dimensional scanning method that offers high resolution; thus, the use of micro-CT in dental analyses is increasing. In recent years, micro-CT has enabled novel possibilities for endodontic research by allowing nondestructive volumetric quantitative and qualitative assessments prior to and following different endodontic procedures (9,19).

The present study evaluated the alterations observed in dentinal microcracks following root canal preparation with three different single-file Ni-Ti file systems using micro-CT analysis. A hand k-file system was used as a reference technique for comparison.

Materials and methods

Selection of the specimens. A total of 100 human mandibular first molars with completely separated roots, which were extracted for reasons unrelated to the present study, were obtained from a pool of teeth between April 2016 and June 2016 from a total of 92 patients at the Guanghua School and Hospital of Stomatology (Guangzhou, China). Teeth were stored in 0.9% normal saline at 5°C. All patients (aged 20-70 years old; 52 male:40 female) provided informed consents, and the experiments were approved by the local ethics committee of Guanghua School and Hospital of Stomatology, Sun Yat sen University.

For the selection of samples, the roots were initially inspected by stereomicroscopy under a magnification of x12 to exclude teeth with pre-existing craze lines or cracks. A digital radiography scan in a buccolingual direction was performed to determine the curvature angle of the mesial root using an open-source image analysis program (Fiji version 1.47n software; Fiji, Madison, WI, USA). Only teeth with a moderate curvature of the mesial root (ranging between 10° and 20°) were selected. Teeth without patency for the canal length, as determined by a size 10 k-file (Dentsply Maillefer, Tulsa, OK, USA), were also discarded. The coronal portions and distal roots of all teeth were removed using a low-speed saw (IsoMet; Buehler, Lake Bluff, IL, USA) with water cooling. Mesial roots of ~11±1 mm in length were left to prevent the introduction of confounding variables. As a result, 100 specimens were selected and stored in 0.9% normal saline at 5°C.

Micro-CT scanning. In order to obtain an overall outline of the anatomic configuration of the mesial canals, specimens were pre-scanned at a relatively low isotropic resolution (70 μm) using a micro-CT scanner (μ CT 50; Scanco Medical, Brüttisellen, Switzerland) at 70 kV and 114 mA. Based on this pre-scan set of images, 80 specimens with type II Vertucci canal configurations were selected. These specimens were scanned again at an isotropic resolution of 7.4 μm. Flat-field correction was performed prior to the scanning procedure in order to correct for variations in the camera pixel sensitivity. Scanning was performed by 360° rotation around the vertical axis with a rotation step of 0.5°. The X-ray source was an air-cooled, sealed, microfocuss X-ray tube with a focal spot size of 5 μm. X-rays were filtered with a 0.5-mm aluminum

filter, and the X-ray tube was operated at 70 kV and 228 μA. The X-ray detector comprised a 2,048x2,048 pixel, 16-bit charge-coupled device camera with fiber-optic coupling to an X-ray scintillator. The system, which was controlled with a PC workstation running the Microsoft Windows XP Professional operating system (Microsoft Corp., Redmond, WA, USA), was used to acquire 1,300-1,600 transverse cross-sections per tooth in a bitmap format.

Root canal preparation. A thin film of polyether impression material was used to coat the cement surface of the roots to simulate the periodontal ligament. Each specimen was placed coronal-apically inside a custom-made epoxy resin holder (diameter, 18 mm) to further streamline the co-registration processes. Apical patency was determined by inserting a size 10 k-file (size 10, 0.02 taper) (9,15) into the root canal until its tip was visible at the apical foramen, then the length of the file was measured from the apical foramen to the cross section, and the working length (WL) was 0.5 mm shorter than the length of the file. Subsequent to establishing glidepaths with a length up to the WL using a size 15 k-file (size 15, 0.02 taper) (Dentsply Maillefer), the specimens were randomly assigned to three experimental groups and a control group (n=20 per group) according to the system used for root canal preparation. The groups were as follows: WO group, in which the WO Ni-Ti reciprocating instrument (Dentsply Maillefer, Tulsa, OK, USA) was used; OS group, in which the OS Ni-Ti rotary instrument (Micro-Mega, Besançon, France) was used; RE group, in which the Reciproc Ni-Ti reciprocating instrument (VDW GmbH, Munich, Germany) was used; and the control group, in which a stainless steel root canal file (Dentsply Maillefer) was used.

In all groups, irrigation was performed using 40 ml (5.25%) sodium hypochlorite. Instruments were driven with the X-Smart plus motor (Dentsply Maillefer) according to each manufacturer's protocol, and a single experienced operator performed all the preparations. The apical sizes and tapers of the Ni-Ti preparation systems are shown in Table I.

In the WO group, the WO instrument (size 25, 0.08 taper) was moved in the apical direction using a slow in- and -out pecking motion of ~3 mm in amplitude with light apical pressure in a reciprocating motion until the WL was reached. The instrument was then removed from the canal and cleaned. The specimens in the OS group were prepared with the OS instrument (size 25, 0.06 taper) using rotary motion to reach 2/3 of the WL value, the WL-3 mm and the WL. In the RE group, the Reciproc instrument (size 25, 0.08 taper) was moved as described for the WO group. The control group was also prepared in a standard manner with a stainless steel root canal file until the WL was reached. The following sequence was used: A size 20 k-file (size 20, 0.02 taper) and a size 25 k-file (size 25, 0.02 taper). Subsequent to four steady strokes, the instrument was removed from the canal. Next, 17% EDTA was used to wipe off the smear layer, and 0.9% normal saline was used to finish the preparation. Micro-CT scans of all samples were then performed using the aforementioned parameters.

Dentinal microcrack measurement. The cross-section images of the mesial roots from the furcation level to the apex (n=297,200) were observed with ImageJ image processing

Table I. Apical sizes and tapers of different nickel-titanium preparation systems.

Preparation system	Apical size (mm)	Taper (%)
WaveOne	0.25	8
OneShape	0.25	6
RE	0.25	8

Table II. Length of microcracks in all groups.

Group	Microcrack length		P-value
	No preparation, μm	Prepared to size 25, μm	
WO	576.097 \pm 233.310	1745.492 \pm 293.933	0.12
OS	456.928 \pm 200.030	2763.932 \pm 333.685 ^a	0.011
RE	626.044 \pm 259.122	1822.519 \pm 370.132	0.078
Control	657.710 \pm 202.638	1521.711 \pm 392.589	0.054

^aP<0.05 vs. no preparation. WO, WaveOne; OS, OneShape; RE, Reciproc.

software (National Institutes of Health, Bethesda, MD, USA) in order to analyze the type and distribution of microcracks. The images were screened by three ImageJ trained examiners to measure the dentinal microcrack lengths according to the length of the black line in the slice that was measured, as demonstrated in Fig. 1. To validate the screening process, image analyses were repeated twice at 2-week intervals. In cases of disagreement among the examiners, the images were re-examined until agreement was reached. The samples were divided evenly into the coronal, medial and apical parts. The percentage, which was determined by the microcrack length of one part divided by the length of the entire sample, was quantified as the distribution of microcracks.

Statistical analysis. The data were statistically analyzed using SPSS software (version 19.0 for Windows; IBM Corp., Armonk, NY, USA). All data were presented normal distribution and homogeneity of variance. The lengths of preoperative dentinal microcracks were analyzed using analysis of variance. Alterations in microcrack lengths prior to and following preparation within the same group were analyzed using a paired t-test. Differences in the dentin microcrack lengths were assessed using a Student-Newman-Keuls (SNK) test. P<0.05 indicated that the differences were statistically significant.

Results

Microcrack classification. ImageJ processing software was used to observe the morphology of each sample. As shown in Fig. 2, the microcracks were classified as follows: Complete, originating from the root canal and extending to the root wall (black arrow); incomplete, originating from the root canal and

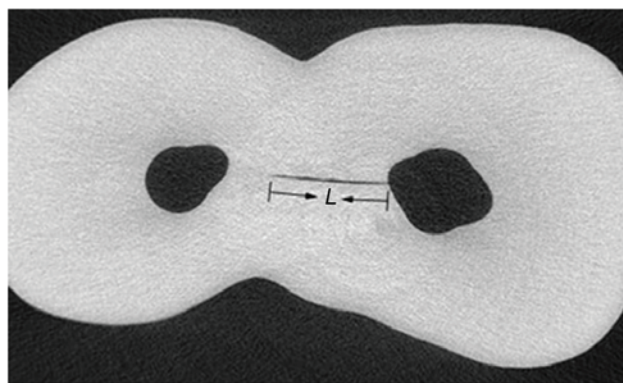


Figure 1. Micro-computed tomography image used for the measurement of the microcrack length, indicated by L.

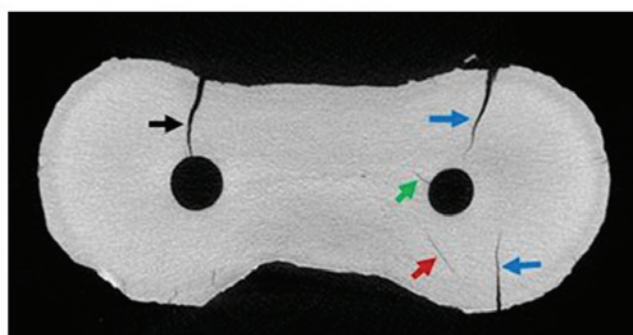


Figure 2. Micro-computed tomography image used to examine the morphology of the dentinal microcracks. Complete microcrack, originating from the root canal and extending to the root wall (black arrow); incomplete microcrack, originating from the root canal and not extending to the root wall (green arrow); or in-dentine microcrack, indicating microcracks present only in the dentine or originating from the root wall without reaching the root canal (red and blue arrows, respectively).

not extending to the root wall (green arrow); or in-dentine, indicating microcracks present only in the dentine or originating from the root wall without reaching the root canal (red and blue arrows, respectively) (20).

Microcrack length. The lengths of the dentin microcracks without preparation were not statistically significant (P>0.05). A paired t test was used to analyze changes in the microcrack length prior to and following preparation. When compared with the length prior to preparation, the OS group length was significantly increased after preparation (P<0.05), whereas there were no significant changes in the WO, RE and control groups (P>0.05; Table II). Furthermore, as determined by the SNK test, the differences in the dentin microcrack lengths when prepared to size 25 between the three groups (WO, RE and control) and the OS group were statistically significant (P<0.05; Table II). Similarly, Figs. 3 and 4 also indicated the lengths of microcracks were markedly increased in the OS after preparation compared with the other groups (Fig 3).

Distribution of microcracks. Samples were trisected, the length of each section was collected and the distribution of microcracks as a percentage of the total length was expressed. As illustrated in Table III, the distribution changes of

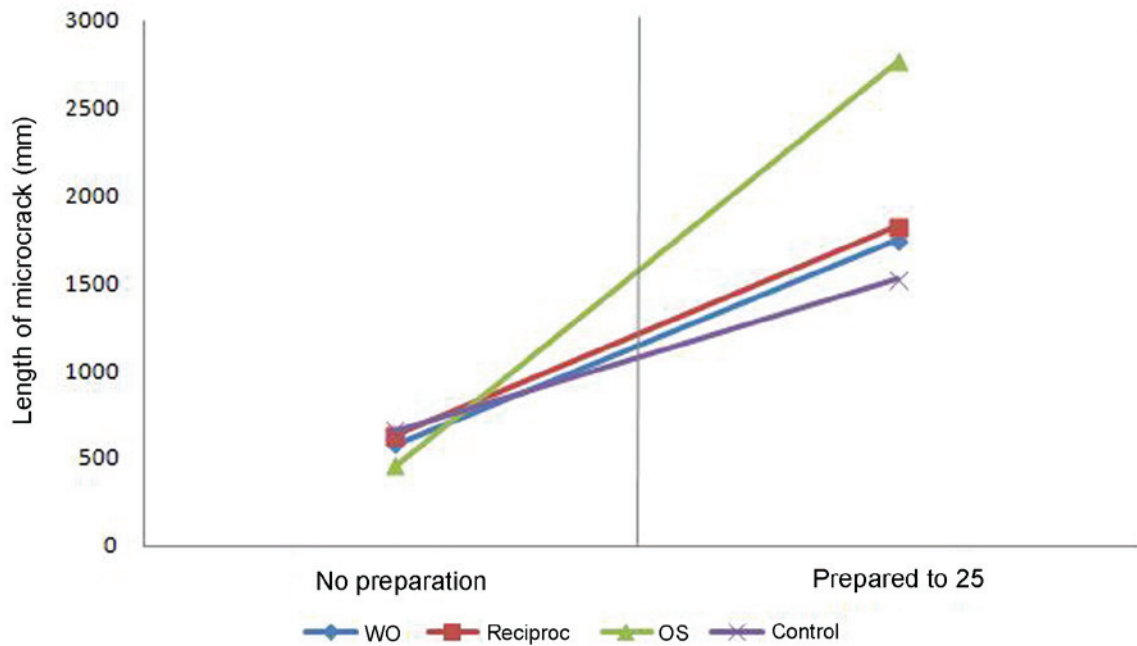


Figure 3. Length of microcracks prior to and post-preparation.

microcracks in the OS group was primarily observed in the apical and coronal parts of the samples. Others groups exhibited no distribution changes in the apical, coronal or medial parts.

Discussion

A novel technique that uses reciprocating motion has been previously proposed for root canal preparation (21). This approach relieves the stress on the instrument through counterclockwise and clockwise movements and, therefore, increases its resistance to cyclic fatigue compared with the traditional continuous rotation motion (22,23). The WO and RE instruments, which were designed by different manufactures, are the main examples of commercially available single-file reciprocating Ni-Ti systems for root canal preparation that alternate between different values of counterclockwise and clockwise rotation movements, which allows for 360° preparation subsequent to a series of reciprocating movements (24,25). In addition, the OS instrument was designed using a single file and a rotary movement to complete preparation.

Previous studies have demonstrated a high rate of dentinal defects caused by the mechanical preparation of root canals (15,26). Bürklein *et al* (12) demonstrated that root canal preparation with both rotary and reciprocating instruments resulted in dentinal defects. In addition, at the apical level, reciprocating files produced significantly more incomplete dentinal cracks as compared with those produced by full-sequence rotary systems. By contrast, Liu *et al* (13) used a similar methodology and observed that the ProTaper multiple-file rotary system caused an increased number of cracks on the apical root surface and in the canal wall in comparison with single-file rotary or reciprocating systems. Ashwinkumar *et al* (15) also observed that canal preparation with ProTaper rotary files was associated with significantly

more microcracks compared with the WO reciprocating system.

Studies correlating mechanical preparation and the development of dentinal defects are based only on root-sectioning methods and direct observation by optical microscopy (11,15,16). These methods undoubtedly have significant limitations associated with the destructive nature of the experiment, as reported in previous studies (11-13,15,16). In previous results in which unprepared teeth were used, their control groups appeared to be validated as effective control groups; however, as no dentinal defects were detected, this type of control does not consider the potential damage produced by the interplay among the four sources of stress on the root dentin, including mechanical preparation, a chemical attack with sodium hypochlorite-based irrigation, sectioning procedures and dehydrogenation drying procedures (27).

In the present study, micro-CT imaging technology was used to evaluate the length of dentinal defects at the baseline and to compare the thickness of the dentine. This highly accurate and non-destructive method enables the assessment of specimens prior to preparation. Therefore, pre-existing cracks can be detected, and it is possible to determine the precise region in which they were created and/or propagated. However, it may be argued that any dentin damage occurring between pre- and post-preparation conditions would be below the spatial resolution threshold of the micro-CT system, and thus may be overlooked. The full extension of dentinal microcracks visualized under conventional stereomicroscopy may also be observed in micro-CT cross-sectional images, which confirms the reliability of this novel technology for detecting dentin defects. Notably, while conventional sectioning techniques allow the evaluation of only a few slices per tooth with the possibility of missing several defects along the root, hundreds of slices of each tooth can be analyzed with

Table III. Distribution of microcracks prior to and following preparation (%).

Time point	WO	OS	RE	Control
Prior to preparation				
Coronal part	35	35	36	35
Medial part	37	44	37	36
Apical part	28	21	27	29
Following preparation				
Coronal part	35	41	36	35
Medial part	37	36	37	36
Apical part	28	23	27	29

WO, WaveOne; OS, OneShape; RE, Reciproc.

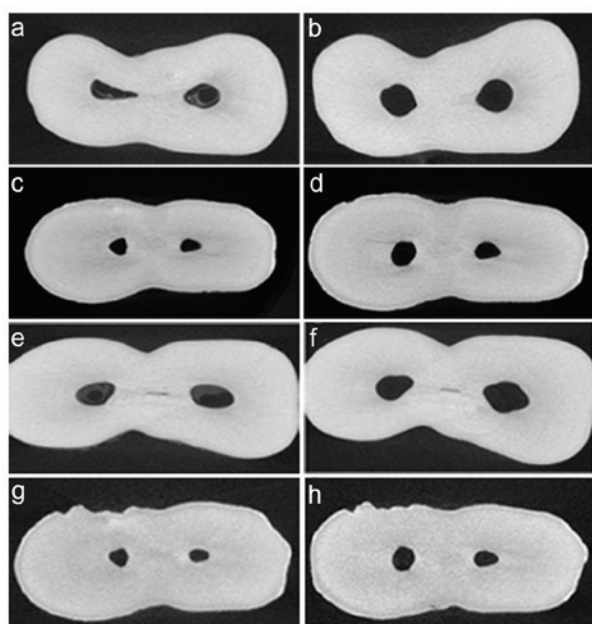


Figure 4. Micro-computed tomography images demonstrating the morphology of dentinal microcracks prior to and following preparation with different systems. (a) Prior to and (b) following preparation with the WaveOne system, microcracks were not observed. (c) Prior to preparation with the OneShape system, microcracks were observed in the dentine, and (d) microcracks increased following preparation with this system. (e) Prior to preparation with the Reciproc system, microcracks were observed in the dentine, and (f) these microcracks were not evidently increased following preparation with this system. (g) Prior to and (h) following preparation using hand files, microcracks were not observed.

micro-CT imaging (9,28). Another methodologic dissimilarity between the technique used in the present study and those of previous studies is associated with sample selection. Although the majority of previous studies used single-rooted teeth, the present study used mesial canals of mandibular molars (3,12,13,26,29). These canals have a constricted anatomic configuration that may result in increased stress on the dentinal surface during mechanical preparation and, consequently, increase the potential for cracks. Therefore, the current results demonstrated a marked contrast with the findings of previous studies. Comparing dentinal microcracks

only subsequent to preparation demonstrated that the length increased significantly. This reflected the results identified in previous studies that did not conduct pre-preparation comparisons.

Therefore, in the present study, it is hypothesized that the influence of the prepreparation dentinal microcracks on microcrack development is significant. The condition of the prepreparation microcracks is associated with the patient's age, gender, occlusion habits and occlusion force. However, the data regarding the length of microcracks prior to and following preparation exhibited normal distribution and homogeneity of variance. Therefore, a paired t-test was used to analyze the increase in the length within the same sample and the SNK test was conducted to compare the differences between the experimental and control groups (pre- and post-preparation), respectively.

The paired t-test performed in the current system indicated that the OS system resulted in the formation of evident microcracks. By contrast, the WO system, the RE system and the hand files may not form marked microcracks. It has been reported that the potential to promote dentinal defects may be associated with the design of the instrument used (11). According to Bier *et al* (3), an increased file taper of rotary instruments contributed to the formation of dentinal defects due to the increased stress on the canal walls. However, the reciprocating instruments, WO and RE systems, had larger tapers in comparison with the rotary instrument, OS system. According to a recent study, reciprocating instruments would be more likely to promote the development or propagation of dentin microcracks and dentinal damage compared with rotary movement using SEM (12). This supports the argument that root canal preparation using only a single, large-tapered reciprocating instrument, which cuts substantial amounts of dentin in a short time, tends to create or aggravate the dentinal defects when compared with the conventional preparation that allows for a more progressive and slower mechanical enlargement. In the present study, it is speculated that the number of the files, the taper and the speed and torque had no effect on the formation of microcracks, whereas the preparation movement may affect the development of dentinal microcracks.

The different morphologies of dentinal microcracks, including complete and incomplete microcracks, as well as microcracks confined in the dentine (Fig. 2), are associated with the stress intensity, concentration zone and root canal wall thickness. The OS system generated microcracks in the apical and coronal parts of the root, and the most common morphology was microcracks confined in the dentine. The thread design in the medial part of the OS system is a transition region that changes from three blades to two (24,30). This design may explain why no microcracks formed in the medial part. Furthermore, the apical part rapidly expands from size 10 to size 25 using a single file, which may have caused an increase in microcracks in this part.

In conclusion, the formation and development of dentinal microcracks may be associated with the movement caused by preparation, as opposed to the taper of the files. Among single-file Ni-Ti systems, WO and RE were not observed to cause evident microcracks, whereas the OS system resulted in increased microcracks.

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