

# Finding Appropriate Nickel-Titanium Instruments for Lingual Canals in Mandibular First Premolars with Two Canals: A Micro-CT Study

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## Research Article

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# Abstract

*Background:* To find appropriate Nickel-Titanium instruments for lingual canals in mandibular first premolars with two canals.

*Methods:* A total of 42 extracted mandibular first premolars with two canals were selected after micro-CT scanning. The teeth were matched and divided into three groups, and three types of Nickel-Titanium instruments (M3, HyFlex CM and XP-endo Shaper) were used to prepare the lingual canals of the sample teeth. After instrumentation, micro-CT scanning was used again to reconstruct the three-dimensional (3D) teeth model, and then it was geometrically aligned with the original model before instrumentation. Two-dimensional (2D) and three-dimensional parameters of teeth models were measured to evaluate the ability of cleaning and maintaining the original canal shape by the three different instruments.

*Results:* Compared with HyFlex CM and XP-endo Shaper, the apical transportation of M3 was significantly greater within the apical 3 mm ( $P < 0.05$ ), while there was no significant difference between HyFlex CM and XP-endo Shaper ( $P > 0.05$ ). There was no significant difference in centering ability among three types of instruments ( $P > 0.05$ ). In general, HCM group and XPS group performed better than M3 group in the 2D and 3D analysis ( $P < 0.05$ ).

*Conclusions:* Preparation of the lingual canal in mandibular first premolar was more challenging than that of the main buccal canal. All the three nickel-titanium instruments are safe for canal preparation, and generally, the HyFlex CM and XP-endo Shaper performed better than the M3.

## Background

Previous studies have demonstrated the presence of a characteristic mesial radicular groove in mandibular first premolars (MFPs) which are collectively referred to as Tome's roots[1]. It has been shown the differences among ethnic populations worldwide with a higher incidence in Sub-Saharan Africans (>25%), followed by Chinese population (24%-27.8), and West Eurasia (<10%)[2-4]. It was reported that 64.04% of MFPs had only one canal (Vertucci's type I canal), while 34.27% possessed two canals (Vertucci's type III or type V system) and 1.69% had three canals (Vertucci's type VIII system) in a Chinese population.[5]. The incidence of C-shape morphology in MFPs was 2.3% [6]. Sikri et al [7]reported the existence of C-shaped canals in 14% of MFPs in an Indian population, and Baisden[8] reported an incidence of 14% in an American population, and Lu[9] et al found a higher incidence of 18% in a Taiwanese population.

Previous scholars had demonstrated that the existence of a radicular developmental grooves in MFPs was associated with a complex multi-canal or C-shaped canal structure, and moreover, a complete C-shaped canal may finally developed into Fan's C2, C3 or Vertucci's type 1-2 canal form due to continues deposit of secondary dentin at the isthmus zone [4, 10].The cross-sectional shape of these complex root canals may change at different root levels, causing dead space that instruments cannot reach and increasing the difficulty of root canal therapy[11]. Vertucci's type V canals in MFPs are mostly "h" shaped (Fig1), and the lingual canals are usually severely curved at the bifurcation region. The average angle between the lingual canal and the main canal (buccal root canals) is 33.54 °[12]. During root canal preparation, the most challenging and key step for the clinicians is to guide the instruments to enter the lingual canal orifice and approach the apical portion of the canal[13]. In most of the cases, the orifice is located on the lingual wall of the main canal at the middle 1/3 of the root level[14] which makes it more difficult to locate, negotiate and prepare with a mental instrument. In addition, the deep radicular groove results in a very thin dentin wall and overzealous instrumentation with conventional mental instruments may easily lead to strip perforation or weakening of the lingual canal wall at the dangerous zone (bottom of the groove)[10]. Therefore, it is very important to

choose appropriate Ni-Ti instruments. Nevertheless, the severe bending of the lingual canals can also lead to fracture of instruments due to cyclic fatigue[15, 16]. In this experiment, three types of Ni-Ti rotary systems were used to prepare lingual canals: M3, HyFlex CM, XP-Endo Shaper. They were selected because all of them are very flexible and can be pre-curved to enter the lingual canal orifice. M3 is manufactured using an advanced memory alloy technology that can increase cyclic fatigue resistance and it allows quick and safe preparation of root canals, especially of curved root canals[17]. It is claimed that Hyflex CM instruments were suitable to prepare curved root canals and possess a superior centering ability compared with conventional NiTi instruments benefiting from a specific sequence of heat treatment[18]. XP-endo Shaper consists in a rotary snake-shaped instrument made of a proprietary alloy that makes the file change its shape according to the temperature. We hypothesized that these Ni-Ti instruments could be safe and create fewer procedural errors during preparation of curved root canals[19].

At present, numerous studies have made detailed descriptions and reports on the root canal morphology of mandibular premolars with radicular grooves[20, 21]. Additionally they have also provided some suggestions in management of the lingual canals in MFPs[13]. Since most of the extra lingual canals cannot be found with direct vision, we can search them with the help of stereomicroscope system and pre-curved hand files. Removing the dentin collar over the orifice by long-neck round burs is also helpful to expose the extra canal. However, many suggestions and recommendations were more based on theoretical assumption or practice in empirical sense. Therefore, the purpose of this ex-vitro study is to use high-resolution micro-CT, to evaluate the ability of three different Ni-Ti instruments (M3, HyFlex CM, XP-Endo Shaper) in maintaining the original shape and cleaning of the lingual root canal in MFPs.

## Methods

### Sample collection and grouping

This experimental study was approved by the Ethics Committee of the Affiliated Stomatological Hospital of Nanjing Medical University (PJ-2017-054-001). During 2017-2020, a total of 125 extracted MFPs with radicular developmental grooves were collected from Department of Oral and Maxillofacial Surgery, Affiliated Stomatological Hospital of Nanjing Medical University due to irreparable dental caries, trauma, periodontitis and orthodontics. After removing the surface calculus and other tissues by an ultrasonic tooth cleaning machine, the sample teeth were numbered and stored in 10% neutral formalin solution at room temperature. All sample teeth were scanned by micro-computerized tomography (micro-CT) (Skyscan 1174v2: Bruker microCT, Kontich, Belgium). The inclusion criteria were as follows: (1) MFPs with two canals confirmed by micro-CT images (type V); (2) MFPs with fully formed apices; (3) No root resorption or longitudinal fracture was identified; (4) The root canals had not been instrumented or filled previously. A total of 42 samples were included in this experiment.

The micro-CT scanning parameters were set as followings: voltage 50 kV, current 800  $\mu$ A, resolution of 18  $\mu$ m, 180° rotation around the vertical axis, rotation step of 0.7°, 1-mm-thickness aluminum filter. NRecon (v.1.6.9, Bruker-microCT) was used to reconstruct the images, and Mimics (v19.0; Materialise, Leuven, Belgium) software was used to measure the root canal morphology. The 3D measurements including volume, and surface area of the canal wall (apical 2/3 portion); the 2D measurements including cross-sectional root canal area, perimeter, roundness, major and minor diameter (at a 5-mm interval from the foramen) and level of canal bifurcation were recorded using Mimics. The morphologic data of root (length, volume of dentin); and RG (length, depth, external and internal dentin thickness in the middle slice) was achieved as well.

According to the above morphological characteristics, 42 sample teeth were equally divided into three groups: M3 group, HCM group and XPS group (n=14 for each group). Different Ni-Ti instruments were used to prepare the root

canals. After checking the normality assumption (Shapiro-Wilk test), the degree of homogeneity (baseline) of the three groups was confirmed using Tukey HSD test ( $\alpha=5\%$ ).

### Root canal preparation

M3 Ni-Ti files (United Dental, Shanghai, China) were used to prepare MFPs with a apical size of #3004. The preparation sequence is as follows: 1. #10K file negotiate the root canals to the working length; 2. The glide path was established by using #1302 path file (M3-Path), #12 variable taper path file (M3 2018 [minimally invasive MIE]) and #16 variable taper path file (M3 2018 [minimally invasive MIE]). The speed was 350 rpm and the torque was 1.5 N cm. 3. The root canals were prepared with #1805 (M3 2018 [minimally invasive MIE]), #2504 (M3 2018 [minimally invasive MIE]) and #3004 (M3 2018 [minimally invasive MIE]) files to the working length, with rotational speed 350 rpm and torque 2 N cm.

In HCM group, the MFPs were prepared with HyFlex Ni-Ti files (Coltene Whaledent, Allstetten, Switzerland), and the apical size was #3004. The preparation sequence is as follows: 1. Use #10 and #15K files to negotiate the root canals to the working length; 2. the glide path is established by using #1501 and #1502 path file (HyFlex GPF), with 300rpm speed and 1.8N cm torque; 3. the teeth were prepared with #2004, #2504 and #3004 shaping files (HyFlex CM) in sequence to the working length, with rotational speed 500rpm and torque 2.5N cm.

In XPS group, canals were prepared with FKG nickel-titanium files (Dentaire, La Chaux-de-Fonds, Switzerland), and the apical size was #3004. The preparation sequence is as follows: 1. Use #10K file to negotiate the root canals to the working length; 2. the glide path was established by using #1002, #1502 and #2002 path files (ScoutRace), with a speed of 600 rpm and a torque of 1.5 N cm; 3. XP-Endo Shaper was used to prepare the root canals to the working length, with rotational speed 800rpm and torque 1 N cm.

All operations were performed by a single experienced operator (Lu Y) under a dental operating microscope. EDTA lubricating gel was used in all steps, and #10K file was used before each file change. 2.5% NaOCl and normal saline were used to rinse the root canal to ensure that the debris will not clog the canal. After the preparation, irrigation with 5 mL of 2.5% NaOCl was performed and 17% ethylenediaminetetraacetic acid (EDTA) was used to remove the smear layer. Then, EDTA was removed by irrigation with the saline solution and the root canal was dried with paper points. Finally, #3004 gutta percha point was placed into each canal to check whether they were matched, and the teeth were scanned again using micro-CT according to the settings before preparation.

### Micro-CT evaluation

The scanning data sets before and after instrumentation were co-registered using the Data Viewer (Data Viewer v.1.5.1.9, Bruker micro-CT). The centering ability of three different instruments in the lingual canals was evaluated by CTAn (v.1.15.2.2, Bruker micro-CT) in cross-sectional images of R1 (from the apical 1 mm), R bifurcation (root canal bifurcation) and R midpoint (the midpoint of both R1 and R bifurcation) before and after each preparation (Fig2). The centering ability was calculated by the method proposed by Calhoun and Montgomery[22](). The apical transportation in the lingual canals was measured by the cross-sectional images of apical 1, 2, 3 and 5 mm before and after each step of preparation (Fig3)[23], and the buccal-lingual, mesial-distal apical transportation were calculated respectively. Apical transportation was calculated according to the formula proposed by Gambill[24]: and . The closer the result is to 0, the smaller the apical transportation is.

The 3D parameters (volume, surface area of canal wall) were measured for the middle and apical third while 2D parameters (cross-sectional area, perimeter, maximum diameter, minimum diameter and roundness) were measured for the apical 5 mm. The changes of root canal volume, surface area and the proportion of unprepared surface area were calculated by Boolean operation[25](Fig4). Roundness represents the shape of the cross-sectional root canal and is an

important parameter reflecting the quality of shaping. The formula is as follows:, where “A” is the area and “dmax” is the major diameter of the root canal[26]. The maximum diameter is defined as the distance between the two farthest pixels in the root canal, while the minimum diameter is the longest chord that can be drawn through the root canal in a direction orthogonal to the long diameter[26].

### Statistical analysis

After using SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) software to test the normality and homogeneity of variance using the Shapiro-Wilk test, the data in accordance with normal distribution were compared by one-way ANOVA, the data with differences between groups were compared by Tukey HSD test. The paired t test was used to compare pre- and post- preparation parameters within groups.  $P \leq 0.05$  means the difference is statistically significant.

## Results

As shown in Table 1, no significant difference was found in centering ability among three different instrument groups in the lingual canals ( $P > 0.05$ ). As comparing the degree of canal transportation (Table 2), we found that the buccal-lingual and mesial-distal apical transportation from apical 1 mm in M3 group was significantly greater than that in the HCM and XPS group ( $P < 0.05$ ), but there was no significant difference between HCM and XPS group ( $P > 0.05$ ). Compared with the XPS group, the buccal-lingual and mesial-distal apical transportation from the apical 2 mm in the M3 group was significantly increased ( $P < 0.05$ ). The buccal-lingual apical transportation from the apical 3 mm in M3 group was significantly higher than that in HCM group ( $P < 0.05$ ). There was no statistical difference between the other groups ( $P < 0.05$ ).

For 3D changes of the root canal shape, the mean increases in the canal volume showed no significant difference among three different preparation instruments ( $P > 0.05$ ). HCM group and XPS group left less unprepared canal walls than the M3 group in the lingual canals ( $P < 0.05$ ) (Table 3).

In the 2D analysis, major diameter and perimeter after preparation was lower in the HCM group than in the XPS group for the lingual canals ( $P < 0.05$ ) while minor diameter after preparation was lower in the M3 group than in the HCM group ( $P < 0.05$ ). Roundness after instrumentation showed great difference among three types of instruments. In general, HCM group and XPS group showed better quality of instrumentation than the M3 group for the lingual canals, except M3 performed best in roundness of the canals ( $P < 0.05$ ) (Table 4).

Table 1. Centering ability of M3, HyFlex CM and XP-Endo Shaper in the lingual canals of mandibular first premolars (mm,  $n = 12$  for each group)

| Root Level           | M3                         | HCM                        | XPS                        |
|----------------------|----------------------------|----------------------------|----------------------------|
| <b>R1</b>            | 0.168 ± 0.122 <sup>a</sup> | 0.145 ± 0.122 <sup>a</sup> | 0.135 ± 0.152 <sup>a</sup> |
| <b>R bifurcation</b> | 0.105 ± 0.083 <sup>a</sup> | 0.139 ± 0.108 <sup>a</sup> | 0.104 ± 0.088 <sup>a</sup> |
| <b>R midpoint</b>    | 0.181 ± 0.155 <sup>a</sup> | 0.160 ± 0.148 <sup>a</sup> | 0.141 ± 0.138 <sup>a</sup> |

R1: 1 mm from the apical foramen; R bifurcation: root canal bifurcation; R midpoint: the midpoint of both R1 and R bifurcation. Axis center ratio of each group. Different superscript letters in the same line indicate statistical significant difference between groups ( $P < 0.05$ ).

Table.2 Apical transportation of the lingual canals in mandibular first premolars instrumented by M3, HyFlex CM and XP-Endo Shaper (mm,  $n = 12$  for each group)

| Apical levels | Buccal-lingual           |                           |                           | Mesial-distal            |                           |                          |
|---------------|--------------------------|---------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
|               | M3                       | HCM                       | XPS                       | M3                       | HCM                       | XPS                      |
| 1 mm          | 0.088±0.033 <sup>a</sup> | 0.045±0.044 <sup>b</sup>  | 0.033±0.025 <sup>b</sup>  | 0.062±0.032 <sup>a</sup> | 0.033±0.022 <sup>b</sup>  | 0.037±0.040 <sup>b</sup> |
| 2 mm          | 0.086±0.050 <sup>a</sup> | 0.054±0.027 <sup>ab</sup> | 0.041±0.030 <sup>b</sup>  | 0.058±0.035 <sup>a</sup> | 0.038±0.036 <sup>ab</sup> | 0.030±0.020 <sup>b</sup> |
| 3 mm          | 0.081±0.052 <sup>a</sup> | 0.041±0.018 <sup>b</sup>  | 0.049±0.025 <sup>ab</sup> | 0.051±0.042 <sup>a</sup> | 0.041±0.025 <sup>a</sup>  | 0.046±0.032 <sup>a</sup> |
| 5 mm          | 0.080±0.054 <sup>a</sup> | 0.054±0.050 <sup>a</sup>  | 0.047±0.025 <sup>a</sup>  | 0.059±0.036 <sup>a</sup> | 0.054±0.036 <sup>a</sup>  | 0.044±0.034 <sup>a</sup> |

Different superscript letters in the same line indicate statistical significant difference between groups ( $P < 0.05$ ). The value with superscript containing two different letters and the other two values have no significant difference ( $P > 0.05$ ).

Table.3 Changes of volume and surface area of the lingual canals in mandibular first premolars after root canal preparation (apical 2/3,  $n = 12$  for each group)

| -                               | Lingual canal                   |                                 |                                 | Δ, mean increase. Different superscript letters in the same line indicate statistical significant difference between groups ( $P < 0.05$ ). The value with superscript containing two different letters and the other two values have no significant difference ( $P > 0.05$ ). Bold values in the same column indicate statistical difference within groups (paired $t$ test, $P < 0.05$ ). |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--|
|                                 | M3                              | HCM                             | XPS                             |  |
| Type of instrument              |                                 |                                 |                                 |  |
| Volume (mm <sup>3</sup> )       |                                 |                                 |                                 |  |
| Before                          | <b>0.72 ± 0.28<sup>a</sup></b>  | <b>0.64 ± 0.23<sup>a</sup></b>  | <b>0.75 ± 0.38<sup>a</sup></b>  |  |
| After                           | <b>1.45 ± 0.23<sup>a</sup></b>  | <b>1.52 ± 0.42<sup>a</sup></b>  | <b>1.60 ± 0.48<sup>a</sup></b>  |  |
| Δ                               | 0.73 ± 0.29 <sup>a</sup>        | 0.88 ± 0.37 <sup>a</sup>        | 0.85 ± 0.36 <sup>a</sup>        |  |
| Surface area (mm <sup>2</sup> ) |                                 |                                 |                                 |  |
| Before                          | <b>8.08 ± 3.60<sup>a</sup></b>  | <b>8.76 ± 3.53<sup>a</sup></b>  | <b>10.90 ± 7.29<sup>a</sup></b> |  |
| After                           | <b>12.07 ± 3.05<sup>a</sup></b> | <b>14.75 ± 4.54<sup>a</sup></b> | <b>3.90 ± 6.93<sup>a</sup></b>  |  |
| Δ                               | 3.99 ± 2.16 <sup>ab</sup>       | 5.99 ± 3.66 <sup>a</sup>        | 3.00 ± 1.78 <sup>b</sup>        |  |
| Untouched surfaces (%)          | 33.81 ± 10.94 <sup>a</sup>      | 19.14 ± 5.49 <sup>b</sup>       | 24.05 ± 5.61 <sup>b</sup>       |  |

difference between groups ( $P < 0.05$ ). The value with superscript containing two different letters and the other two values have no significant difference ( $P > 0.05$ ). Bold values in the same column indicate statistical difference within groups (paired  $t$  test,  $P < 0.05$ ).

## Discussion

Lingual canals in MFPs are not rare according to reports on various ethnic populations [27-30], and even the cone-beam computed tomography can detect its presence, it is still a great challenge in properly managing it during root canal

| Type of instrument      | Lingual canal                 |                              |                               |
|-------------------------|-------------------------------|------------------------------|-------------------------------|
|                         | M3                            | HCM                          | XPS                           |
| Areas(mm <sup>2</sup> ) |                               |                              |                               |
| Before                  | <b>0.04±0.01<sup>a</sup></b>  | <b>0.04±0.02<sup>a</sup></b> | <b>0.06±0.02<sup>a</sup></b>  |
| After                   | <b>0.17±0.04<sup>a</sup></b>  | <b>0.18±0.06<sup>a</sup></b> | <b>0.22±0.03<sup>a</sup></b>  |
| Δ                       | 0.13±0.03 <sup>a</sup>        | 0.14±0.04 <sup>a</sup>       | 0.16±0.02 <sup>a</sup>        |
| Perimeter (mm)          |                               |                              |                               |
| Before                  | <b>0.83±0.29<sup>a</sup></b>  | <b>0.78±0.37<sup>a</sup></b> | <b>1.13±0.41<sup>a</sup></b>  |
| After                   | <b>1.93±0.19<sup>a</sup></b>  | <b>1.90±0.29<sup>a</sup></b> | <b>2.05±0.48<sup>b</sup></b>  |
| Δ                       | 1.09±0.28 <sup>a</sup>        | 1.12±0.30 <sup>a</sup>       | 0.92±0.49 <sup>a</sup>        |
| Major diameter (mm)     |                               |                              |                               |
| Before                  | <b>0.47±0.16<sup>a</sup></b>  | <b>0.41±0.11<sup>a</sup></b> | <b>0.47±0.12<sup>a</sup></b>  |
| After                   | <b>0.64±0.15<sup>ab</sup></b> | <b>0.67±0.24<sup>a</sup></b> | <b>0.69±0.12<sup>b</sup></b>  |
| Δ                       | 0.17±0.11 <sup>a</sup>        | 0.26±0.15 <sup>a</sup>       | 0.23±0.04 <sup>b</sup>        |
| Minor diameter (mm)     |                               |                              |                               |
| Before                  | <b>0.17±0.03<sup>a</sup></b>  | <b>0.18±0.04<sup>a</sup></b> | <b>0.17±0.03<sup>a</sup></b>  |
| After                   | <b>0.49±0.03<sup>a</sup></b>  | <b>0.53±0.05<sup>b</sup></b> | <b>0.55±0.05<sup>ab</sup></b> |
| Δ                       | 0.32±0.05 <sup>a</sup>        | 0.35±0.06 <sup>a</sup>       | 0.38±0.05 <sup>a</sup>        |
| Roundness               |                               |                              |                               |
| Before                  | <b>0.55±0.18<sup>a</sup></b>  | <b>0.51±0.18<sup>a</sup></b> | <b>0.54±0.15<sup>a</sup></b>  |
| After                   | <b>0.75±0.05<sup>a</sup></b>  | <b>0.77±0.27<sup>b</sup></b> | <b>0.74±0.15<sup>c</sup></b>  |
| Δ                       | 0.20±0.14 <sup>a</sup>        | 0.26±0.23 <sup>a</sup>       | 0.20±0.12 <sup>a</sup>        |

therapy[13]. In a retrospective study, the outcomes of treatment was poor in mandibular premolars with complex apical anatomies during a 12-month follow-up[31]. In the X-ray evaluation of 26 infected teeth, only 50% (13 out of total 26 teeth) of the teeth healed completely. Based these studies, it is obvious that successfully treating such canals has enormous challenges for clinician.

Considering that a deep radicular groove in MFPs always corresponds to a thin dentin wall at the groove bottom[10], the concept of coronal flaring was not followed in this study. We selected three types of Ni-Ti instruments which can be precurved to enter the lingual root canals. During the preliminary experiments, it was found that Protaper Next (Dentsply Maillefer, Ballaigues, Switzerland) was difficult to enter lingual canals due to its unmaintained pre-curved property. A recent study demonstrated that Protaper Next may produce more changes in root canal geometry as well as greater transportation and a less remaining dentin thickness in the lingual canals toward the RG compared to XPS[32]. The current study was the first to compare the shaping ability of three pre-curved Ni-Ti instruments (M3, HCM, XPS) and the ability to retain the original shape of root canals. Since uneven removal of root canal dentin is inevitable for instrumentation of a severely curved canal, and procedural errors and incidence were especially apt to occur at the site of root canal bifurcation, the priority should be

put on the protection of the dangerous zone. The lingual canals of MFPs are characterized by small, curved and complex shape and Hyflex CM performed well in such root canals due to the increased flexibility, and this can be attributed to the thermal pre-treatment of the CM alloy during manufacturing, which makes the alloy more ductile and thereby reduces the restoring forces[33]. XPS is made of MaxWire alloy and it will transform into austenite at 35°C in the root canal[34]. It can adapt to the expansion and shortening of the root canal morphology and that is why XPS has better centering ability. The softness of XPS solves the difficulty of fine bending of root canals, and its unique mode of movement and austenitic phase can adapt to the complex root canal morphology, which can be prepared to enough areas while maintaining the original shape of root canals[35]. Less apical transportation attributes to the proportion of microleakage after root canal filling, which increased significantly when the apical transportation was greater than 0.3mm[36]. The reason why M3 performed best in roundness of lingual canals may be the minor diameter after preparation in M3 group was lower while there is no significance in major diameter among the different instruments. According to the analysis of the root canal dentine thickness of double-root canal mandibular premolars, it was found

that the mean thickness of the mesial and distal dentin wall was less than that of the buccal and lingual canal wall[10]. Since the mesial wall was the thinnest among four sides, strip perforation was the most likely to occur after overzealous instrumentation [37]. Therefore, in the process of root canal preparation, we should prevent the over-preparation of the mesial lateral wall of the lingual canal, and the thinnest dentin thickness after the preparation of these three instruments was above 0.5 mm, so these three instruments are safe and reliable in this respect.

According to Slowey[38], mandibular premolars may be the most difficult teeth to be endodontically treated. Complications such as instrument separation and strip perforation may occur during the treatment of MFPs, whereas, none of the 42 samples failed in this study, profiting from the rational designs of instruments and the proper operation procedure. The description above indicates that the common characteristic of these three instruments is that they are all very flexible and have great flexural fatigue resistance. Besides, proper operation procedure plays an important role in avoiding the occurrence of complications. Violence is forbidden in the process completely and the lingual canals should be prepared gently. Once resistance occurred, a #10K file was used to confirm patency and then we reuse the previous file was repeated to ensure it can reach work length smoothly. Adequate rinse is also an essential step. The tip of the instrument should be precurved in order to enter the lingual canal and the increased risk of fatigue fracture resulting from plastic deformation ought to arouse our attention. Brand-new instrument and the control of preparation time contribute to solve the problem. The use of an instrument for a prolonged period of time not only increases the risk of fractures[39], but also causes extra dentine loss[40, 41]. Mesial wall of lingual canal ought to be protected consciously, so it requires us to control the time of preparation.

However, distinctions exist between the experiment and the clinic. Because of the limitation of vision and operation, it is much more difficult to prepare MFPs with two canals in vivo than in vitro. Meanwhile, to obtain the anatomy of canals of MFPs through micro-CT before canal preparation would help us better manipulate the files. Clinical situation is complicated and varies at any time, so the experience of operator may also influence the success rate of treatment.

## Conclusions

Preparation of the lingual canal in mandibular first premolar was more challenging than that of the main buccal canal. Appropriate Nickel-Titanium instruments and operation procedure can help us avoid complications when treating such canals. All the three nickel-titanium instruments are safe for canal preparation, and generally, the HyFlex CM and XP-endo Shaper performed better than the M3.

## Declarations

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### Availability of data and materials

Data are available upon request by coauthors and reviewers.

### Ethics approval and consent to participate

This experimental study was approved by the Ethics Committee of the Affiliated Stomatological Hospital of Nanjing Medical University (PJ-2017-054-001). Written informed consent from parents and/or patients was obtained. During 2017-2020, a total of 125 extracted MFPs with radicular developmental grooves were collected from Department of Oral and Maxillofacial Surgery, Affiliated Stomatological Hospital of Nanjing Medical University due to irreparable dental caries, trauma, periodontitis and orthodontics.

### **Consent to publish**

Not applicable.

### **Competing interests**

The authors declare that there is no competing interest.

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### **Contributions**

Jin Li is Principal Investigator and developed research protocol, led the research team in sample collection, performed data management and report writing. Yunqin Wang and Yaqian Lu contributed to collecting and preparing the sample teeth. They found three appropriate instruments for such teeth with peculiar structure by analyzing morphologic data. Yongchun Gu, Yuhua Xiong, Nan Geng, Yixin Cai and Yingying Ji took part in experimental design and data management.

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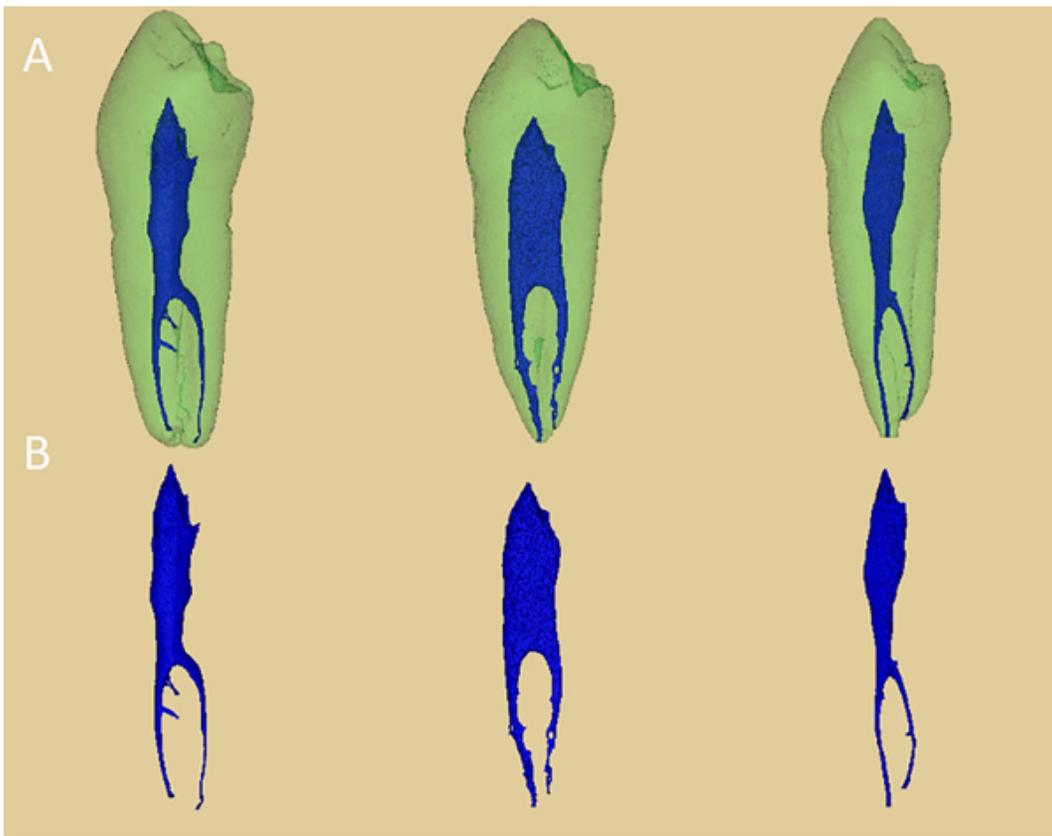
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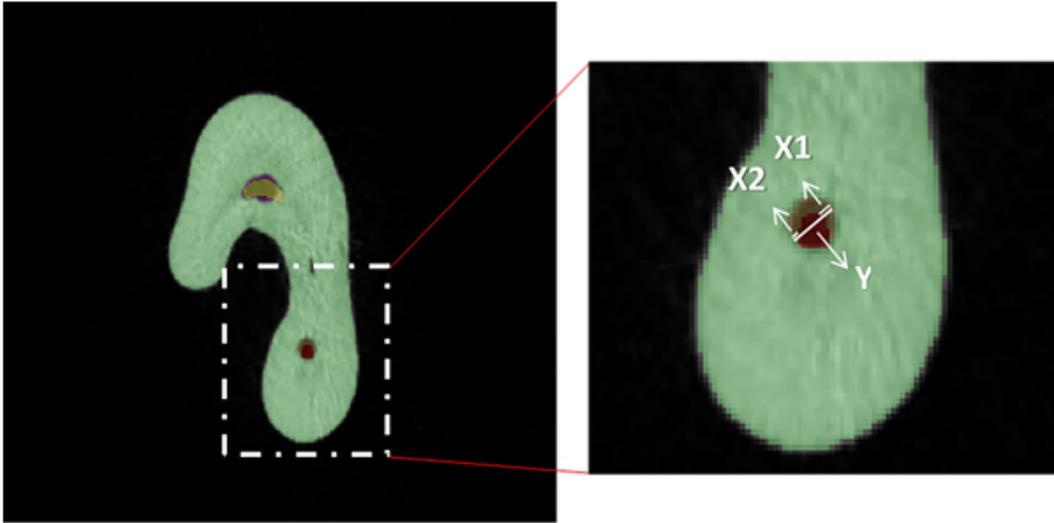
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## Figures



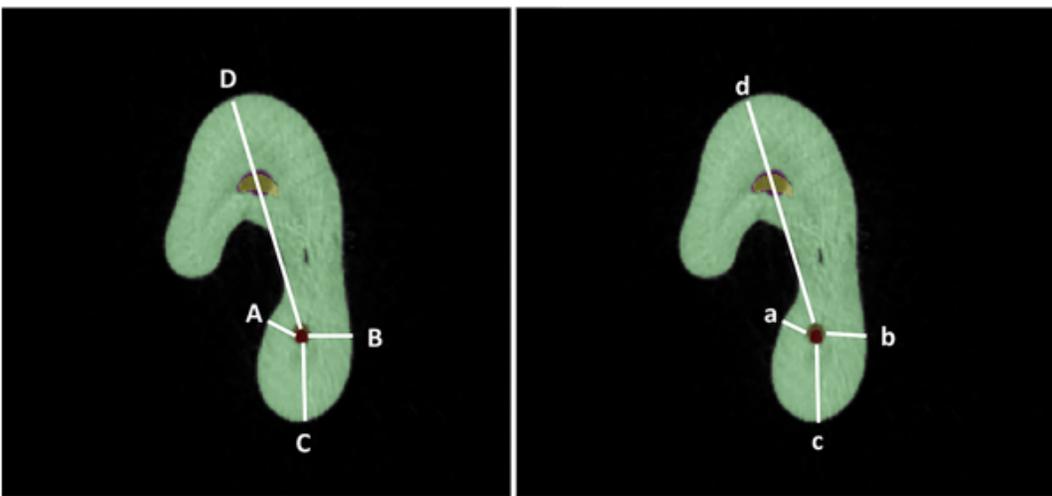
**Figure 1**

Representative micro-CT 3D images of three mandibular first premolars with a Vertucci's type V canal. A: Tooth models, B: Root canal models.



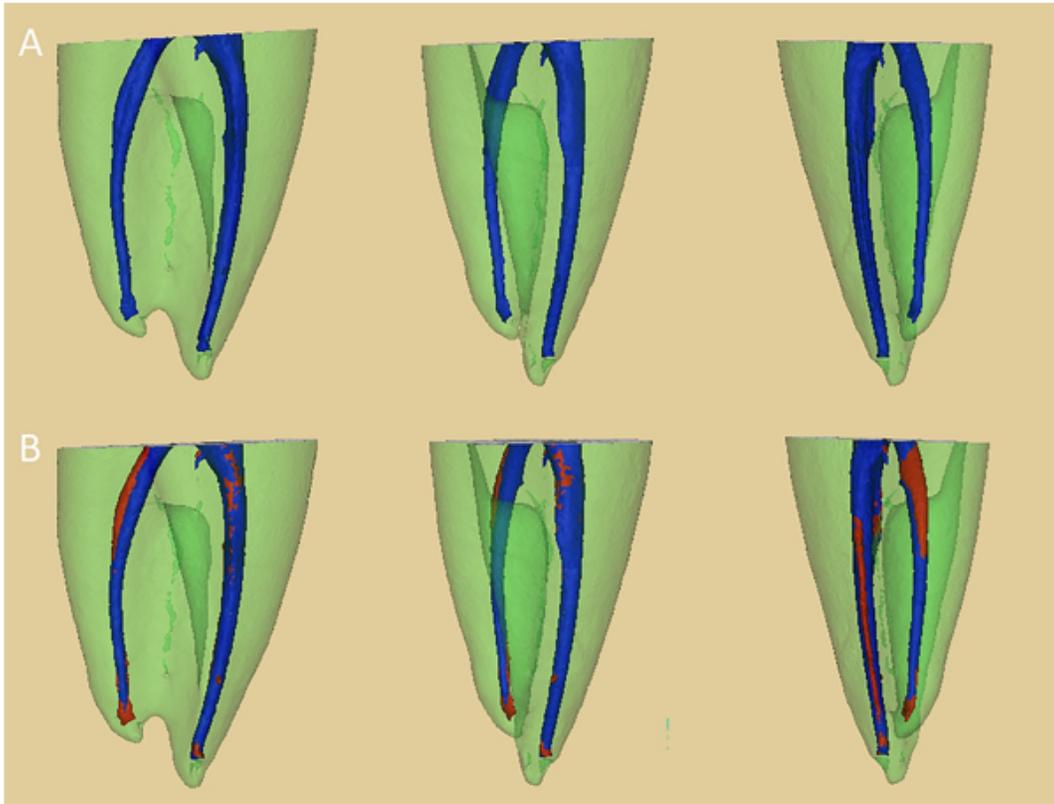
**Figure 2**

Measurement of the centering ability of Hyflex CM in the cross-sectional micro-CT image Y: the maximum diameter of root canal after preparation; X1: the maximum distance between the root canal walls before and after preparation in the direction of maximum diameter; X2: the maximum distance between the walls of root canals before and after preparation in the direction of maximum diameter and in the opposite direction of X1.



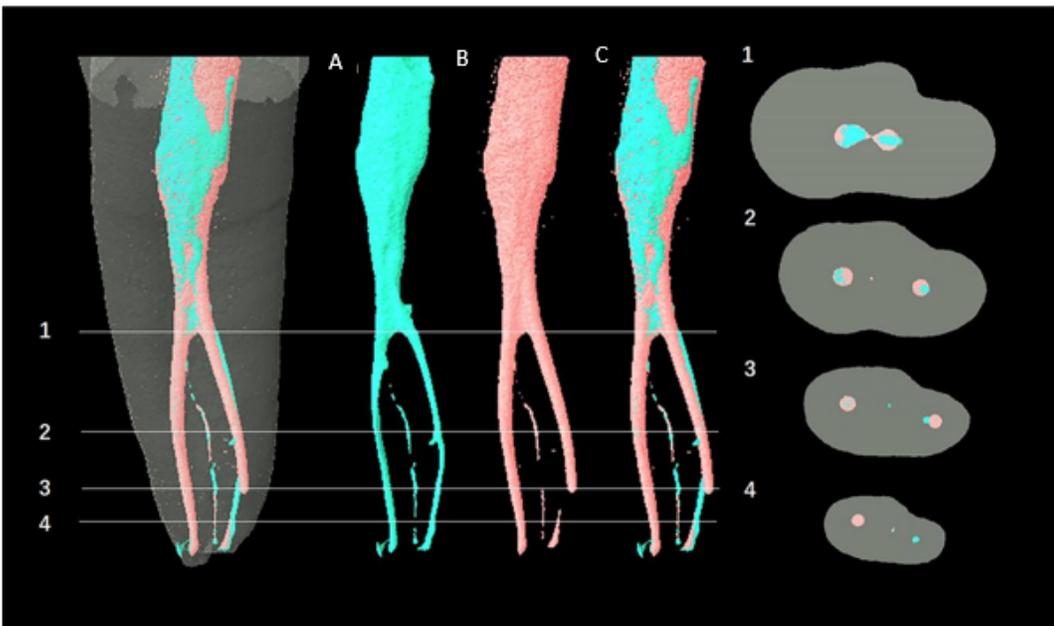
**Figure 3**

Measurement of canal transportation after root canal instrumentation by Hyflex CM in cross-sectional micro-CT images A: the minimum distance between the mesial edge of the root and the medial wall of the uninstrumented canal; B: the minimum distance between the distal edge of the root and the medial wall of the uninstrumented canal; C: the maximum distance between the lingual edge of the root and the medial wall of the lingual canal; D: the maximum distance between the buccal edge of the root and the medial wall of the buccal root canal before preparation. a: the minimum distance between the mesial edge of the root and the medial wall of the root canal after preparation; b: the minimum distance between the distal edge of the root and the medial wall of the root canal after preparation; c: the maximum distance between the lingual edge of the root and the medial wall of the lingual canal after preparation; d: the maximum distance between the buccal edge of the root and the medial wall of the root canal after preparation.



**Figure 4**

Representative micro-CT 3D images of tooth before (red) and after (blue) instrumentation by Hyflex CM. A: Anatomy before treatment. B: Anatomy of the flat canal after treatment. Red represents areas unaffected by the procedure.



**Figure 5**

Micro-CT images of the morphological changes in the root canal system instrumented by Hyflex CM A: original root canal model; B: root canal model after preparation; C: superimposed model before and after preparation; 1: bifurcation plane; 2: midpoint plane of plane 1,4; 3: lowest plane before root canal blockage; 4: apical 1mm plane