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ORIGINAL ARTICLE

Comparison of mandibular first molar mesial root canal morphology using micro-computed tomography and clearing technique

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Abstract

Objective. Micro-computed tomography (MCT) with alternative image reformatting techniques shows complex and detailed root canal anatomy. This study compared two-dimensional (2D) and 3D MCT image reformatting with standard tooth clearing for studying mandibular first molar mesial root canal morphology. ***Materials and methods.*** Extracted human mandibular first molar mesial roots (n = 31) were scanned by MCT (Skyscan 1172). 2D thin-slab minimum intensity projection (TS-MinIP) and 3D volume rendered images were constructed. The same teeth were then processed by clearing and staining. For each root, images obtained from clearing, 2D, 3D and combined 2D and 3D techniques were examined independently by four endodontists and categorized according to Vertucci's classification. Fine anatomical structures such as accessory canals, intercanal communications and loops were also identified. ***Results.*** Agreement among the four techniques for Vertucci's classification was 45.2% (14/31). The most frequent were Vertucci's type IV and then type II, although many had complex configurations that were non-classifiable. Generally, complex canal systems were more clearly visible in MCT images than with standard clearing and staining. Fine anatomical structures such as intercanal communications, accessory canals and loops were mostly detected with a combination of 2D TS-MinIP and 3D volume-rendering MCT images. ***Conclusions.*** Canal configurations and fine anatomic structures were more clearly observed in the combined 2D and 3D MCT images than the clearing technique. The frequency of non-classifiable configurations demonstrated the complexity of mandibular first molar mesial root canal anatomy.

Key Words: *canal configuration, mandibular first molar, minimum intensity projection, tooth clearing, volume rendering*

Introduction

Endodontic success depends on thorough cleaning and shaping and complete obturation of the root canal system [1]. However, anatomical variations and complexities of the canal system pose challenges to endodontic treatment. For instance, mandibular first molar mesial roots have complex canal anatomy that includes intercanal communications and isthmuses [2] and they appear to have lower rates of success with endodontic treatment [3]. Yet, being the first permanent teeth to erupt, mandibular first molars are the most frequently in need of endodontic treatment [4].

Since Weine et al. [5] reported four canal configurations, Vertucci [6] suggested a root canal classification system composed of eight canal configurations by observing 100 teeth *in vitro*. After that, even though non-classifiable configurations not comprised in Vertucci's classification have been reported,

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Vertucci's classification has been used as criteria in many studies including the present study, whether additional types are included or not [2,7–10].

Earlier studies of root canal morphology used the clearing technique as the gold standard for studying root canal anatomy [6–8]. However, the clearing technique is destructive and can distort internal anatomy and create artifacts. These shortcomings have now been overcome by micro-computed tomography (MCT) that provides detailed three-dimensional (3D) visualizations of internal and external root canal anatomy in a non-destructive process [9–14]. Moreover, the MCT analysis can be further enhanced by utilizing various image reformatting techniques to visualize more detailed anatomy.

The MCT imaging technique known as two-dimensional thin-slab minimum intensity projection (2D TS-MinIP) involves projecting rays that are perpendicular to the targets' axis. This enables the detection of low-density structures within a given volume and is widely used in medical research and clinical diagnosis to visualize airways, ducts and vessels of fine diameter [15,16]. Our previous studies showed that 2D TS-MinIP, together with 3D volume-rendered images of MCT, provided the best visualization of detailed canal structures like intercanal communications, loops and accessory canals in maxillary molar mesiobuccal roots [17,18].

The aim of this study is to compare the 2D and 3D image reformatting and combined 2D and 3D techniques in MCT with clearing technique to study detailed root canal morphology in mandibular first molar mesial roots. The null hypothesis is that these four imaging techniques are not different in visualization of the root canal systems.

Materials and methods

Sample preparation

The methods used in this study were based on our prior research [18]. Approval was obtained from the Institutional Review Board (IRB) of Seoul National University Dental Hospital, Seoul, Korea (ERI 12007). Mandibular first molars (n = 69) that had been extracted due to prosthodontic and periodontal reasons from patients (aged 25–57 years) were examined with periapical radiographs. Teeth with only a single canal were excluded, so that mesial roots with two canals and complex configurations could be studied. Teeth with fractured mesial roots and those previously accessed for initiating root canal treatment were also excluded, so that only unmodified and uninstrumented canal anatomy was studied. The selected teeth ($n = 31$) were immersed in 3.5% sodium hypochlorite for 1 h and any remaining organic residue or calculus removed mechanically with an ultrasonic scaler and curette. They were then stored in 0.5% sodium azide solution until use.

Reconstruction of 2D TS-MinIP and 3D volume-rendered images

Each tooth (n = 31) was scanned by MCT (SkyScan 1172, SkyScan b.v.b.a., Aartselaar, Belgium), with a voxel dimension of 15.91 μm using 100 kV, 100 μA, a 0.5 mm thick aluminum filter and 0.5° increments through 180° of rotation. Each image was included to reconstruct a cross-sectional dataset of the mesial root by using NRecon (Version 1.6.4.7; Skyscan NV, Kontich, Belgium) on a personal computer (Intel Core i5 3.40-GHz CPU, Windows 7, Santa Clara, CA). This dataset was used to create 2D TS-MinIP and 3D volume rendered images of each mesial root canal system by using OnDemand3D software (Cybermed Inc., Seoul, Korea), as previously outlined [17]. The stacks of image slices of the tooth were positioned so that the root apex faced upwards. The slab thickness depended on the diameter of the canals and they ranged from 0.5–1.0 mm.

Using this dataset, OnDemand3D software was used to transmit a virtual ray perpendicular to the curved slab in the mesiodistal direction and the smallest grey value was recorded to obtain the MinIP image. Similarly, the OnDemand3D volume rendering tool was used with thresholding and manual volume segmentation to create 3D images. Additionally, segmented volumes of canal structure were represented by an opaque red color and the external morphology of the mesial root was rendered transparent so as to enhance the visualization of fine anatomical structures.

Clearing and staining technique

After access preparation, a #10 K-file was inserted into the mesial root canals through their apical foramina. The distal root was resected with a high speed diamond bur. The teeth were then placed in 5% sodium hypochlorite for 2 h to dissolve pulp tissue and organic residue followed by rinsing under running tap water for 4 h to remove the sodium hypochlorite and dissolved debris. The opening into the pulp chamber from the distal root resection was sealed with flowable resin (Denfil flow, Varicom Co. Ltd., Seoul, Korea). The mesial root canals were injected with india ink (Higgins Fountain India Ink, Tampa, FL) using a 27-gauge needle on a disposable syringe (Korea Vaccine Co., Seoul, Korea) placed through the pulp chamber. Negative pressure from a suction tip was applied to the apical foramina. Additional india ink was injected into the pulp chamber and the access opening was sealed with utility wax. Then the teeth were decalcified in 5% nitric acid (Sigma 438073, St. Louis, MO) for 3 days with a change of new solution after 12 h. The decalcified teeth were washed under running tap water for 2 h. Finally the decalcified teeth were dehydrated gradually in ascending concentrations of ethanol (70% for 12 h, 90% for 6 h and 100% for 6 h) and placed in methyl salicylate solution (Sigma M6752, St. Louis, MO) for 24 h to clear and harden the specimens. The cleared and stained mesial roots were photographed under a dissecting microscope (5× magnification, Olympus, Tokyo, Japan).

Classification of canal configurations and fine structures

For each mesial root (n = 31), the images from clearing, 2D, 3D and combined 2D and 3D techniques were prepared independently. For the combined 2D and 3D technique, the two types of images were observed concurrently. Each root canal system was carefully examined and categorized independently by four endodontists using the Vertucci classification systems [6] as follows:

- Vertucci type I. One canal extends from the pulp chamber to the apex.
- Vertucci type II. Two separate canals leave the pulp chamber and join short of the apex to form one canal.
- Vertucci type III. One canal leaves the pulp chamber, divides into two within the root and then merges to exit as one canal.
- Vertucci type IV. Two separate and distinct canals extend from the pulp chamber to the apex.
- Vertucci type V. One canal leaves the pulp chamber and divides short of the apex into two separate and distinct canals with separate apical foramina.
- Vertucci type VI. Two separate canals leave the pulp chamber, merge in the body of the root, then divide short of the apex and exit as two distinct canals.
- Vertucci type VII. One canal leaves the pulp chamber, divides and then merges within the body of the root and finally divides into two distinct canals short of the apex.
- Vertucci type VIII. Three separate and distinct canals extend from the pulp chamber to the apex.

The descriptions of the main canal, as well as accessory canals, intercanal communications and loops were based on the terminology provided by Vertucci [1]. Those configurations that did not fit into Vertucci's [6] classification system were categorized as non-classifiable.

Interobserver agreement amongst the four endodontists for each of the four techniques was assessed independently using Fleiss kappa [19], which is statistically significant when the coefficient is greater than 0.7. Disagreements between observers were discussed to reach consensus. When all four images of a root canal system were categorized as having the same configuration, the techniques were deemed to be in agreement on the classification. Additionally, the incidence and location of accessory canals, intercanal communications and loops that were detected by the four techniques were compared.

Results

Classification of canal configurations

For the classification of canal configurations there was substantial interobserver agreement between the four endodontists. Their Fleiss kappa coefficients were 0.736, 0.705, 0.712 and 0.712 for the clearing, 2D, 3D and combined 2D and 3D techniques, respectively. All four techniques resulted in agreement on the Vertucci's classification for 45.2% (14/31) of the roots. The agreement on their classification when it included non-classifiable configurations increased to 74.2% (23/31). Regardless of the technique, non-classifiable configurations were the most frequent in these mesial roots that had multiple canals, followed by Vertucci's type IV and type II (Table I). Sixteen (51.6%) mesial roots on MCT images had 10 non-classifiable configuration types that are not included in Vertucci's classification. These were types 1-3-1, 2-1-2-3, 2-1-3-2, 2-3, 2-3-2, 2-3-5, 3-2, 3-2-1, 3-4-3 and 3-4-3-4. Among these, six configurations (types 1-3-1, 2-1-2-3, 2-1-3-2, 2-3-5, 3-4-3 and 3-4-3-4) are the first to be reported in mandibular first molar mesial roots. Some representative examples are shown in Figure 1. This frequency of non-

Table I. Types and incidence of canal configurations of 31 mesial roots classified according to the Vertucci classification.

		Type of canal configuration								
		Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII	TypeVIII	NC
Type of images	Tooth clearing	0 (0%)	5 (16.1%)	1 (3.2%)	10 (32.3%)	2 (6.5%)	1 (3.2%)	0 (0%)	1 (3.2%)	11 (35.5%)
	2D MinIP	0 (0%)	4 (12.9%)	1 (3.2%)	6 (19.4%)	2 (6.5%)	2 (6.5%)	0 (0%)	0 (0%)	16 (51.6%)
	3D	0 (0%)	4 (12.9%)	1 (3.2%)	6 (19.4%)	2 (6.5%)	2 (6.5%)	0 (0%)	0 (0%)	16 (51.6%)
	2D MinIP + 3D	0 (0%)	4 (12.9%)	1 (3.2%)	6 (19.4%)	2 (6.5%)	2 (6.5%)	0 (0%)	0 (0%)	16 (51.6%)

NC, Non-classifiable.

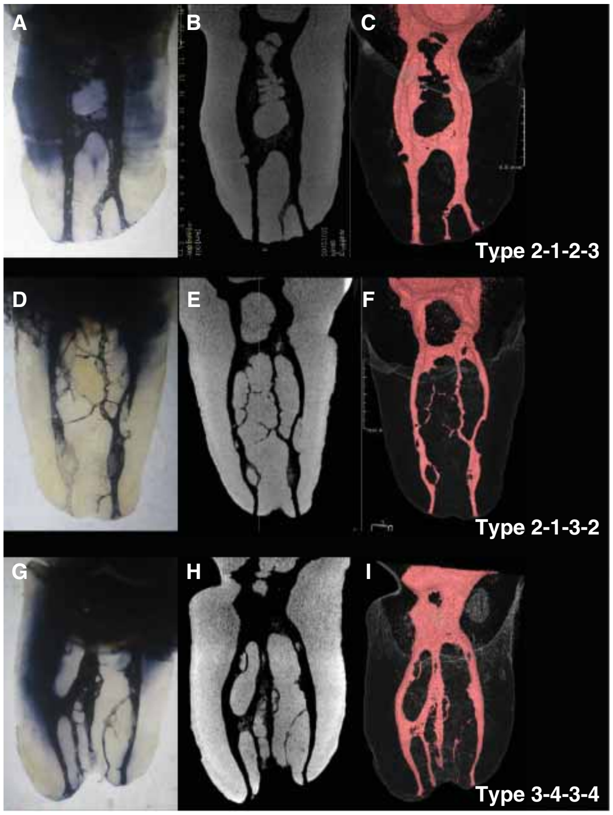

Figure 1. Representative images of some examples of new non-classifiable configuration types that were not included by Vertucci classification (A, D, G; clearing image, B, E, H; 2D TS-MinIP image, C, F, I; 3D volume rendered image).

classifiable configurations was due to the complexity of mandibular first molar mesial root canal anatomy.

Identification of fine structures

Fine anatomical structures such as accessory canals, intercanal communications and loops were identified by clearing (13, 60 and 10, respectively) by 2D TS-MinIP (20, 70 and 13), by 3D MCT (20, 49 and 13) and by the combined 2D and 3D MCT technique (24, 71 and 17). Accessory canals were most frequently detected in the apical third (23/24 in combined technique) and intercanal communications were observed in most roots (25/31 in combined technique). Fine anatomical structures such as the intercanal communications and loops that were located in the coronal and middle third were more clearly observed by 2D TS-MinIP MCT than by clearing (Figures 2A–C). On the contrary, lateral (accessory) canals were more clearly observed in 3D images than 2D TS-MinIP (Figures 2E–G).

Discussion

Until recently, the clearing technique served as the gold standard for studying root canal anatomy *in vitro* [1,6–8]. Now MCT is becoming a standard method

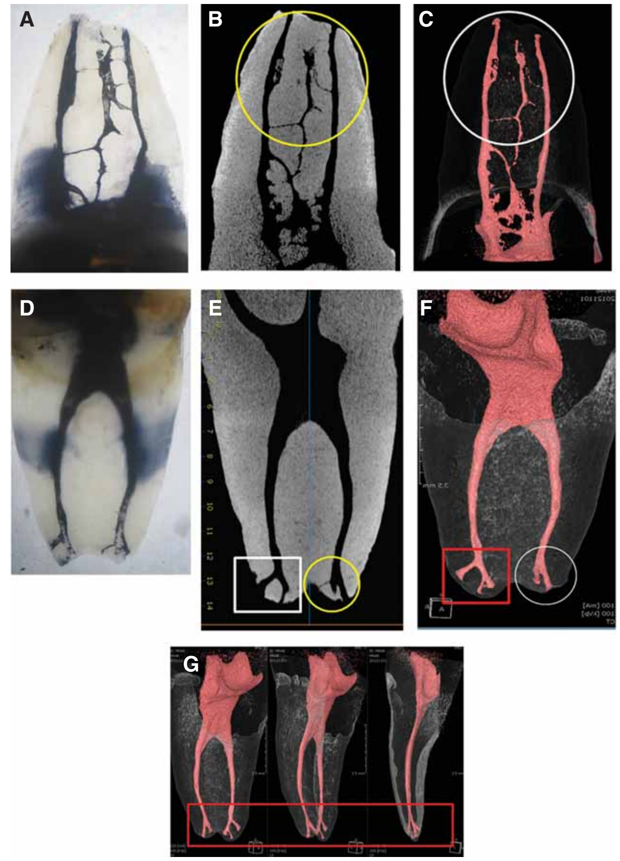

Figure 2. Comparison of three different images of a same sample (A, D; clearing image, B, E; 2D TS-MinIP image, C, F, G; 3D volume rendered image). Generally, the 2D TS-MinIP image (B, E; yellow circles) shows a more clear view of fine canal structures such as intercanal communications, accessory canals and loops than the 3D image (C, F; white circles). In a clearing specimen, the ink of the apical end could be washed out throughout the clearing process (D; apical area). However, a lateral (accessory) canal that is perpendicular to the main root canal is shown more clearly in a 3D volume rendered image (F, G; red rectangular) than 2D MinIP image (E; white rectangular).

that overcomes limitations in the clearing technique [9–14]. Furthermore, our recent study showed that complex canal configurations and fine anatomical structures are more readily observed by MCT, when using a combination of 2D and 3D image reformatting techniques [18]. Those findings based on maxillary first molar mesiobuccal roots have now been verified in this investigation of mandibular first molar mesial root canal anatomy.

Once again we found that MCT afforded much greater visualization of fine anatomical structures. This was likely due to its greater capacity to distinguish between empty air space and the surrounding dentin through different scales of grey [12,13]. Furthermore, the use of different image reformatting techniques with MCT, such as 2D TS-MinIP and 3D volume rendering, provided better visualization of detailed anatomy. Indeed, the 2D and 3D MCT images alone provided the same canal configurations, but, when combined, they identified much more fine anatomical structures. Similarly, other studies found that MCT clearly visualizes complex anatomical structures, including the pulp chamber, dentin thickness and canal dimensions [10].

Therefore, to rigorously compare MCT, it was essential that we optimized the clearing technique. Accordingly, this study included a refinement in the staining technique that increased the visualization of anatomical structures. Unlike our previous study [18], the mesial root canals were stained prior to clearing, so as to enhance ink penetration of fine structures. Since methyl salicylate has low water solubility, its penetration may be reduced in canals that become more hydrophobic after decalcification. Therefore, the method of staining before clearing that was used in this study was expected to increase the visualization of anatomical structures.

Indeed, there was an increase in agreement on the classification of canal configurations in this study (45.2%), compared to our original findings (33.3%) [18]. Additionally, the clearing technique was no longer the least sensitive method for identifying fine anatomical structures [18]. As we had previously reported, the combined 2D and 3D technique was the most effective at identifying fine structures, followed by the 2D alone. However, unlike our previous study, clearing now appeared to be a little more effective than 3D alone. A few images from the clearing technique showed more precise anatomical structures than the corresponding images from 3D MCT. Furthermore, in some roots, intercanal communications that were located within the middle and apical sections were more readily visible by clearing than by 3D MCT.

Yet, despite these refinements, the clearing technique was found to have numerous shortcomings. We found that some of the teeth became too fragile to handle after they were decalcified. In fact, teeth that had been processed through multiple procedures would ultimately disintegrate from mechanical or chemical stimuli. Additionally, the india ink would sometimes fail to penetrate canals, which was likely due to its strong affinity to proteins retained within their lumen [20]. Of course, ultimately we found that the greatest disadvantage was that the pungent odor irritated our eyes and throats and the toxicity of methyl salicylate remained a major concern [21].

Since Vertucci [6] suggested a classification that consists of only eight canal configurations, there have been several reports of non-classifiable canal types. Gulabivala et al. [7,8] reported four additional canal configurations (types 2-1-2-1, 2-3, 3-1 and 3-2) in Burmese mandibular first molar mesial roots and one (type 3-4) in Thai mandibular first molars. Sert et al. [22] and Peiris et al. [23] reported one (type 1-2-3-2) and two (types 1-2-3 and 3-1-2) new canal configurations in Turkish and Sri Lankan mandibular first molar mesial roots, respectively. Al-Qudah and Awawdeh [24] also reported additional configurations (types 2-3-1, 2-3-2, 3-2-1 and 3-2-3) in Jordanian mandibular first molar mesial roots. Chen et al. [25] reported a new configuration (type 2-3-1-2) in Taiwanese mandibular first molars. Likewise, the present study found 10 non-classifiable canal configurations that are not included in Vertucci's classification. Among these, six canal configurations (types 1-3-1, 2-1-2-3, 2-1-3-2, 2-3-5, 3-4-3 and 3-4-3-4) have not previously been reported in mandibular first molar mesial roots. The frequency of non-classifiable configurations demonstrated the complexity of mandibular first molar mesial root canal anatomy. Furthermore, it showed that the present classification system has limitations in encompassing the variety of root canal configurations. Therefore, the development of a systematic classification that embraces these complex canal configurations could advance the field.

However, the high frequency of complex and non-classifiable configurations reported in this study may have been due to the non-random process for selecting the teeth. Mesial roots with at least two radiographically visible canals were intentionally chosen so as to compare the different techniques for studying canal morphology. Furthermore, these teeth had been extracted for prosthodontic and periodontal reasons from older patients, in whom dentin accumulation within the canals may have contributed to the complexity of the configurations seen [26]. Therefore, these limitations along with the sample size mean that the high frequency of non-classifiable canal configurations may not be as prevalent in the general population.

Nevertheless, except non-classifiable, through all of these techniques we found that the most common mesial root canal configuration was Vertucci's type IV, followed by type II, which is consistent with previous studies of mandibular first molar anatomy [2,7–10]. The accessory canals were found mostly in the apical third (95.8%), which is also consistent with prior findings [9,10]. Additionally, the high incidence (80.6%) of intercanal communications in these mandibular first molar mesial roots has also been reported recently [2,10,14].

In conclusion, under the limitation of the present study, canal configuration and fine anatomic structures were more clearly observed in the combined 2D and 3D MCT images than the clearing technique. The high incidence of non-classifiable configuration showed the complexity of mandibular first molar mesial root canal anatomy.

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