ORIGINAL RESEARCH

Micro-computed tomography (micro-CT) evaluation of root canal morphology in immature maxillary third molars

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Abstract

The endodontic treatment of immature permanent teeth with necrotic pulp is a significant clinical challenge. The success of regenerative endodontic procedure is highly dependent on disinfection of the root canal and an accurate anatomical knowledge of the root canal. The aim of this study was to use micro-computed tomography (micro-CT) analysis to investigate the configuration of root canals in the upper permanent third maxillary molars with incomplete root development in their coronal, apical and middle third portions. Thirty immature third permanent maxillary molars were scanned using a micro-CT system. Then, we measured the diameters and areas of the root canal in the coronal, middle and apical third of the roots. The ratio between the long and short diameter of each root canal was then calculated and the canals were divided into several groups: round, oval, long oval, flat and irregular. The round configuration was not observed in the distobuccal and mesiobuccal roots in any of their anatomical regions. Oval and long oval canals predominated in the distobuccal root. The greatest variations were observed in the mesiobuccal root, with the ribbon-shaped canal predominating in the middle region and an irregular shape in the apical region. In the coronal region of the palatal canal, the round configuration predominated; in the middle third, we observed an almost equivalent distribution between round and oval configurations; apically, the oval shape predominated. In conclusion, we observed significant complexity and variation in the morphology and configuration of root canals in immature permanent molars, thus generating additional obstacles for the success of regenerative endodontics.

Keywords
Immature permanent teeth; Canal anatomy; Regenerative endodontics; Apexification

1. Introduction

The first permanent molars in childhood are the teeth that are most often affected by carious lesions. In some cases, these teeth are also affected by molar-incisive hypomineralization and other forms of dysplasia. These teeth often necessitate endodontic treatment soon after the eruption and prior to the completion of root development [1–3]. The endodontic treatment of immature permanent teeth with necrotic pulp represents a significant challenge for the clinician and also generates a notable public health problem [4, 5]. Several factors contribute to the difficulties associated with such cases, including limited cooperation from patients, the peculiarities associated with the disinfection and filling of such root canals, and the increased risk of root fracture [6]. Severe destruction of a clinical crown or the early loss of these teeth in young patients can lead to further problems, including occlusion, mastication or bone development [7]. Severe destruction of a clinical crown or the early loss of these teeth in young patients can lead to further problems, including occlusion, mastication or bone development [7]. Early extraction can create an unbalanced occlusion due to the distances between adjacent teeth, super-eruption of non-occluding teeth from the opposite dental arch, and displacement of the midline with associated disturbances in the psychological and social development of the child [5, 7]. Regardless of our potential ability to perform an apexification procedure, the long-term prognosis of such teeth is questionable due to the thin root walls which are incompletely formed [8]. For such cases, regenerative endodontic procedures represent an appropriate form of treatment because these techniques allow for the completion of root development with root elongation, an increase in the thickness of the dentinal wall, and a narrowing of the apical foramen [9, 10]. Endodontic procedures have the potential to save fragile immature permanent teeth over the long term by providing better root resistance, reduced fracture risk and a reduced clinical treatment time. These factors benefit both the patient and the clinician [11].

The success of the regenerative endodontic procedure depends on disinfection of the root canal, the migration of mesenchymal stem cells, the creation of a good coronal barrier and hermetic restoration [12]. An excellent knowledge of root anatomy is also an important factor when performing...
endodontic procedures. This is because root morphology can vary depending on age, population, ethnicity and the choice of research method [13]. Research has shown that persistent root canal infection is the primary reason for treatment failure [14]. Irrigants are known to play crucial role in cleaning and disinfecting root canals whereas instrumentation is considered as a means of providing access to treat the complex root canal system [15]. Isthmuses, curvatures, lateral canals and the oval shape of these canals can render their disinfection a significant challenge; this is because it is difficult to distribute irrigating solutions adequately [16, 17]. The maxillary first molars are the second most common teeth after the mandibular first molars to be treated endodontically [18]. In the specialized literature, there is a significant lack of studies relating to the morphology of root canals in the immature permanent teeth. In the present study, we aimed to use micro-computed tomography (micro-CT) analysis to investigate the configuration of root canals in the third permanent maxillary molars with incomplete root development in their coronal, apical and middle third regions. We considered that the accuracy of our study would be optimal if focussed on the immature first maxillary permanent molars. It is important to note that preservation of the immature first permanent molars is more cost-effective than the early loss of these teeth and that this procedure prevents the occurrence of severe orthodontic consequences [19, 20]. Furthermore, despite their anatomical variations, most maxillary third molars exhibit root anatomy and morphology that are similar to the first molar with three separated roots, and are often extracted for orthodontic reasons [21, 22].

2. Materials and methods

In this study, we investigated 30 immature permanent maxillary third molars that were extracted for orthodontic reasons. The inclusion criteria were as follows: (1) maxillary third molars without fractures and/or defects and/or cracks on the root surfaces; (2) maxillary third molars with a wide-open apical foramen and an almost complete root length, and (3) maxillary third molars that were macroscopically similar to the maxillary permanent first molars and included mesiobuccal (MB), distobuccal (DB) and palatal (P) roots.

Following extraction, the teeth were cleaned with gauze soaked in hydrogen peroxide and stored in a 10% solution of formalin. Specimens were then scanned using a micro-CT system SkyScan Desktop X-ray Microtomograph (Bruker, Billerica, MA, USA) with an X-ray tube voltage of 100 kV, a current magnitude of 100 µA, and a 0.55 mm copper filter. We used a conical beam with a single voxel size of 12 µm. On transverse sections, the first image with completely visible walls was considered as the apex of the root canal, and the first section with enamel was considered as the neck (the enamel-cementum border). The length of the root canal was defined as the distance from the orifice of the canal to the apical foramen, after which the root was divided into three equal regions (apical, middle and coronal). The diameters and areas of the root canals in each third of the roots were measured using CTAn software (v. 1.18.8.0, Bruker, Billerica, MA, USA), preloaded on the micro-CT system. Each parameter was measured three times, and a mean value was calculated. The shape of the root canals was determined based on established criteria [23]. The ratio between the long and short diameter of the root canal was then calculated. A score of 1 indicated that a canal was round; a score of up to 2 indicated that a canal was oval; a score between 2 and 4 indicated that a canal was long oval, a score of more than 4 indicated that a canal was ribbon-shaped (flat), and a score exceeding 5 indicated an irregular shape.

3. Statistical analysis

The difference between MB, DB and P roots at the same radicular level were analysed using a non-parametric Mann-Whitney U test because assumption of normality could not be met (Shapiro-Wilk test, \( p < 0.05 \)). The significance level was set at \( p = 0.05 \). Statistical analysis was conducted with a statistics computer software SPSS v.19.0 (SPSS Inc., Chicago, IL, USA).

4. Results

The mean values of long and short diameters of MB, DB and P root at three cross-sectional levels are shown in Table 1. The results showed that there was significant difference between the three roots at each level (\( p < 0.05 \)).

Table 2 shows the results for long/short diameter ratio at coronal, middle and apical level. There was statistically significant difference (\( p < 0.05 \)) at all levels.

Our analysis showed that the round configuration was not observed in the DB and MB roots in any of their component regions. In the MB canal, the ratio between the long and short diameter of the root canal progressively increased from the coronal to apical regions. Coronally, irregular root canals were dominant (36.67%). In the middle third region, the root canals were flat while in the apical region, root canals generally exhibited an irregular shape (46.67%). Oval and long oval canals predominated in the DB root. The greatest variations in the individual parts of the canal are observed in the MB roots. The ribbon-shaped canal predominated in the middle region while an irregular shape was dominant in the apical region. The ratio between the long and short diameters in the apical region exceeded 5. In the palatal root, the coronal configuration of the canal was round; in the middle third, an almost equivalent distribution was observed between the round and oval configurations. Apically, the oval shape dominated (73.33%). No other canal configurations were observed in these roots root (Table 3). There was statistically significant difference (\( p < 0.05 \)) in the distribution of all configurations or root canal at all cross-sectional levels except of long oval canals at coronal level between MB and DB root (\( p > 0.05 \)).

The area of the root canal was then calculated. In the middle third, an almost equivalent distribution was observed between the round and oval configurations. Apically, the oval shape dominated (73.33%). No other canal configurations were observed in these roots root (Table 3). There was statistically significant difference (\( p < 0.05 \)) in the distribution of all configurations or root canal at all cross-sectional levels except of long oval canals at coronal level between MB and DB root (\( p > 0.05 \)).

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Fig. 1 shows micro-CT sections acquired from the different root areas with superimposed contours of the root canal showing small and large lumen diameters, respectively.
### Table 1. Long and short diameter values at apical, middle and coronal cross-sectional levels for MB, DB and P root.

<table>
<thead>
<tr>
<th></th>
<th>Coronal (mm)</th>
<th>Middle (mm)</th>
<th>Apical (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long diameter</td>
<td>Short diameter</td>
<td>Long diameter</td>
</tr>
<tr>
<td>Mesiobuccal</td>
<td>2.98 ± 0.36</td>
<td>0.61 ± 0.09</td>
<td>2.72 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>(2.43–3.46)</td>
<td>(0.47–0.75)</td>
<td>(2.17–3.17)</td>
</tr>
<tr>
<td>Distobuccal</td>
<td>1.46 ± 0.36</td>
<td>0.72 ± 0.08</td>
<td>1.31 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>(0.69–2.11)</td>
<td>(0.54–0.87)</td>
<td>(0.79–1.88)</td>
</tr>
<tr>
<td>Palatal</td>
<td>1.75 ± 0.18</td>
<td>1.57 ± 0.09</td>
<td>1.61 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>(1.49–2.04)</td>
<td>(1.44–1.71)</td>
<td>(1.36–1.89)</td>
</tr>
</tbody>
</table>

Statistically significant difference ($p < 0.05$; Mann-Whitney U Test) between the three roots at all levels.

### Table 2. Long/short diameter ratio at coronal, middle and apical levels for MB, DB and P root.

<table>
<thead>
<tr>
<th></th>
<th>Coronal (mm)</th>
<th>Middle (mm)</th>
<th>Apical (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesiobuccal</td>
<td>5.04 ± 1.14</td>
<td>5.51 ± 1.22</td>
<td>4.94 ± 1.98</td>
</tr>
<tr>
<td>Distobuccal</td>
<td>2.05 ± 0.71</td>
<td>2.05 ± 0.70</td>
<td>2.22 ± 0.83</td>
</tr>
<tr>
<td>Palatal</td>
<td>1.04 ± 0.08</td>
<td>1.12 ± 0.10</td>
<td>1.29 ± 0.15</td>
</tr>
</tbody>
</table>

Statistically significant difference ($p < 0.05$; Mann-Whitney U Test) between the three roots at all levels.

### Table 3. Configuration of root canals of the maxillary molars in the coronal, middle and apical regions.

<table>
<thead>
<tr>
<th></th>
<th>Round</th>
<th>Oval</th>
<th>Long oval</th>
<th>Ribbon-shaped (flat)</th>
<th>Irregular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesiobuccal (MB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>0</td>
<td>10.00%</td>
<td>33.33%</td>
<td>20.00%</td>
<td>36.67%</td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
<td>0</td>
<td>6.67%</td>
<td>66.67%</td>
<td>26.66%</td>
</tr>
<tr>
<td>Apical</td>
<td>0</td>
<td>6.67%</td>
<td>13.33%</td>
<td>30.00%</td>
<td>46.67%</td>
</tr>
<tr>
<td>Distobuccal (DB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>0</td>
<td>66.67%</td>
<td>33.33%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
<td>73.33%</td>
<td>26.67%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apical</td>
<td>0</td>
<td>50.00%</td>
<td>36.67%</td>
<td>13.33%</td>
<td>0</td>
</tr>
<tr>
<td>Palatal (P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>100.00%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>56.67%</td>
<td>43.33%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apical</td>
<td>26.67%</td>
<td>73.33%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Statistically significant difference ($p < 0.05$; chi-square test) between the three roots at all levels except in the distribution of long oval canals at coronal level between MB and DB root.

### Table 4. Root canal areas in different regions.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± standard deviation (mm$^2$)</th>
<th>Minimal value (mm$^2$)</th>
<th>Maximal value (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area mesial canal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>1.53 ± 0.30</td>
<td>1.17</td>
<td>1.97</td>
</tr>
<tr>
<td>Middle</td>
<td>1.13 ± 0.19</td>
<td>0.81</td>
<td>1.47</td>
</tr>
<tr>
<td>Apical</td>
<td>1.49 ± 0.09</td>
<td>1.38</td>
<td>1.64</td>
</tr>
<tr>
<td>Area distal canal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>0.60 ± 0.24</td>
<td>0.26</td>
<td>0.92</td>
</tr>
<tr>
<td>Middle</td>
<td>0.55 ± 0.23</td>
<td>0.22</td>
<td>0.90</td>
</tr>
<tr>
<td>Apical</td>
<td>0.67 ± 0.30</td>
<td>0.24</td>
<td>1.23</td>
</tr>
<tr>
<td>Area palatal canal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronal</td>
<td>2.12 ± 0.20</td>
<td>1.77</td>
<td>2.38</td>
</tr>
<tr>
<td>Middle</td>
<td>1.88 ± 0.32</td>
<td>1.32</td>
<td>2.25</td>
</tr>
<tr>
<td>Apical</td>
<td>2.33 ± 0.57</td>
<td>1.38</td>
<td>2.93</td>
</tr>
</tbody>
</table>

Statistically significant difference ($p < 0.05$; Mann-Whitney U Test) between the three roots at all levels.
Reconstructed three-dimensional (3D) micro-CT image is shown on Fig. 2. All teeth exhibited three roots with single root canal in each root.

**5. Discussion**

In this study, we investigated the configuration of root canals in immature permanent teeth. The configuration of the root canal with complete root development has been extensively reviewed in the existing literature. Root canals can exhibit several different shapes in cross-section: round, oval, long-oval, ribbon-shaped or irregular. In a previous study, Jou et al. defined the oval canal as a canal with a maximum diameter of up to twice its minimum diameter; for the long oval, the maximum size was 2- to 4-fold its short diameter. A previous study reported the frequency of these configurations: 45% in the apical third of the root canals of teeth with complete root development, 50% in the middle, and 56% at the coronal level. In the present study, we demonstrated that permanent third maxillary molars with incomplete root development exhibit substantial variation in the configuration of the mesial- and distobuccal roots, with long oval, flat and irregular shapes predominating. In the palatal roots, the canal configuration changed gradually from round to oval in a coronal to apical direction. All the examined teeth exhibited three roots with single root canal in each root probably because root canal variations occur in the later stages of root maturation. The highly diverse nature of canal anatomy, along with the different canal shapes and configurations we observed in the present study, highlight the importance of good chemo-mechanical canal preparation in any single-endodontic treatment involving teeth with incomplete root development. Bacteria and debris hidden in unprepared areas can be the source of persistent infection and lead to the failure of root canal therapy. Thus, the problem of cleaning and disinfecting root canals remains one of the most significant clinical challenges due to anatomical complexities. A round configuration in the apical third predominates in all canals in the permanent maxillary molars with complete root development. The flat configuration was the second most common configuration in mesiobuccal canals; this was followed by the long oval configuration. The results of the present study, involving teeth with incomplete root development in the same canals, showed that the apically region is dominated by a flat configuration, followed by the long oval configuration. Our data also showed that with the progression of root canal maturation, there is a change in their configuration that makes chemical and mechanical treatment more predictable in teeth with complete root development than in immature ones. Insufficient and incomplete chemo-
mechanical treatment caused by anatomical features may lead to residual bacterial infection; this may also maintain periapical inflammation [31].

Mechanical instrumentation has two purposes. The first purpose is to widen the diameter of the root canal to improve the penetration of irrigants in the apical zone; this procedure is not required for teeth with incomplete root development due to the wide root canal and thin dentinal walls. The second goal is direct debridement and the removal of infected dentin and biofilm from the inner root walls as these structures may be resistant to chemical disinfection [32]. An analysis of failed endodontic regenerative cases previously identified a direct relationship between treatment failure and persistent endodontic infection [14, 33]. Complete disinfection by the elimination of microorganisms from the root canal in immature teeth is difficult because of the limitations imposed by mechanical instrumentation, which may lead to further weakening of the roots [34]. In addition, residual infection in the root canal can modify the differentiation of stem cells and disturb their migration and the release of transforming growth factor beta 1 (TGF-β1) from the dentin, thus making tissue regeneration more difficult [35–37]. When using instruments with a diameter smaller than the diameter of the root canal, it is likely that an increased area of the canal wall will remain uncleaned [38]. Furthermore, in canals with oval, flat or irregular morphology, much larger areas will remain uncleaned [39]. Micro-CT studies have shown that in round canals, between 10% and 50% of their total area remains unprepared [40]. When using instrumentation on the root canal system, approximately 30% of the endodontic space remains unprepared [41, 42]. Such spaces may contain residual pulp or bacterial biofilm in infected canals, thus exerting adverse effects on treatment outcomes [43]. According to some authors, when using popular mechanical preparation methods, between 20% and 40% of the canal surface remains cleaned [44]; according to other authors, 35% or more of the canal surface remains uncleaned [41]. According to recent studies, canal preparation with file number 25 or 40 can lead to more than 20% of the canal surface of a mandibular molar exhibiting residual tissue following instrumentation on a mesiolingual root; furthermore, approximately 35% of the canal wall of premolars remains intact [43]. It has been reported that 30%–60% of root canals contain microbes after preparation, and that residual tissue can serve as a source of nutrients, thus leading to persistent secondary infection [43, 45]. Large areas of the canal wall, particularly in ribbon-shaped and oval canals, cannot be cleaned mechanically; this means that microorganisms residing in these untouched areas may survive and cause further infection [46]. Irrigants are necessary adjuncts that enhance the antimicrobial effects of mechanical cleansing. Irrigation is an essential aspect of the chemomechanical preparation of root canals in revascularizing immature teeth, and aims to remove microbes, dentin debris and pulp tissue [47]. Sodium hypochlorite is the most commonly used irrigant due to its excellent chemical properties and has the potential to dissolve organic tissue, act effectively against a broad range of microorganisms, and neutralize their products [48]. Clinicians need to avoid the extrusion of irrigant into the periapical region. An open apex is considered a significant factor that can promote the extrusion of irrigants [47]. In this study, we demonstrated that irregular, long oval and oval canal configurations predominate in the apical zone (Table 3); these configurations can promote the extrusion of irrigants during the chemical cleaning of these canals and lead to severe clinical complications. There are various canal configuration types in the different zones of root canals. We observed the most notable canal variations in the MB-root and the DB (Table 3). There is a high risk of incomplete elimination of the biofilm in the root canals which in turn may lead to higher risk of endodontic failure. Thus, in immature permanent teeth, it is fundamental to remove necrotic masses and microorganisms from the canal by applying appropriate irrigation [49].

The purpose of this study was to describe the canal morphology of immature third maxillary molars. Some limitations should be mentioned most important one being the small sample size used in micro-CT analysis. Additionally, only maxillary teeth were studied. Including immature permanent mandibular molars would improve the understanding of root canal morphological characteristics in immature teeth.

6. Conclusions

Collectively, root canal morphology of the immature third maxillary molars showed a wide variation in cross-sectional canal shape. An advanced knowledge of root canal anatomy is essential for correct diagnosis, successful treatment, and a positive prognosis. We observed significant complexity in root canal morphology and configuration in immature permanent molars. These factors can create additional obstacles if we are to achieve successful regenerative endodontic treatment. Such teeth should be approached with special care in case they require regenerative endodontic therapy.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

KH—design and implement the research, to perform data extraction, and synthesize the results, to write the manuscript. RG—design and implement the research, to perform data extraction, and synthesize the results, to write the manuscript. NG—design and implement the research to write and revise the final manuscript. LA—implement the research and to revise the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All parents gave written informed consent for using their children’s teeth for research purposes, and all molars were irreversibly anonymized immediately after extraction. Approval from the Ethics Committee of the Medical University of Sofia was also obtained (No 1598/20.05.2022).
The authors declare no conflict of interest.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES


