The isthmuses of the mesial root of mandibular molars: a micro-computed tomographic study

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Abstract

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Aim To investigate the prevalence of root canal isthmuses in the apical 5 mm of the mesial root of mandibular molars by means of micro-computed tomography (MCT) and to describe the morphology of the isthmuses.

Methodology Twenty extracted mandibular first molars that had fully formed roots were selected. The mesial roots were sectioned from the distal roots and subjected to MCT. The number of sections showing isthmuses in each of the apical 5 mm of the root canals was recorded. In each one of the apical 5 mm of the 20 roots examined, 40 sections were observed, giving a total of 800 sections observed in each group. Data were analysed as a contingency table using the chi-square statistic to test the null hypothesis that location of the sections in each of the apical 5 mm and presence of the isthmus were independent. **Results** Isthmuses were found to be present at all levels with prevalence figures between 17.25 and 50.25%. The chi-square test indicated a significant difference in the distribution of isthmuses with section (P = 0.001). It was found that sections in the first millimetre from the apex had fewer isthmuses than expected and that sections in the third millimetre from the apex had more isthmuses than expected under the null hypothesis. Calcifications were found to be present in most isthmuses, occasionally lateral canals originated from the central part of the isthmuses.

Conclusions Isthmuses were present in the vast majority of roots observed. The third millimetre from the apex showed more isthmuses than expected. The results of clinical and surgical endodontic procedures performed in the mesial root of mandibular molars may be affected by this aspect of the root canal anatomy.

Keywords: anatomy, endodontics, isthmus, microcomputed tomography.

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Introduction

An isthmus has been defined as a narrow, ribbonshaped communication between two root canals that contains pulp tissue (Weller *et al.* 1995). Isthmuses may be poorly accessible to root canal instruments, act as bacterial reservoirs and may reduce the success rate of surgical and nonsurgical endodontic procedures (Teixeira *et al.* 2003). Isthmuses have been found to be present in all types of root in which two canals are normally found, including the mesial roots of maxillary and mandibular molars, the distal root of mandibular molars, the maxillary and mandibular first and second premolars and mandibular incisors. The prevalence of isthmuses in the mesial root of mandibular molars has been observed in previous studies (Skidmore & Bjørndal 1971, Cambruzzi & Marshall 1983, Vertucci 1984, Hsu & Kim 1997) in which observations were performed using different methods and at varying distances from the apex (Table 1). Two studies reported that the number of isthmuses increased in an apicocoronal direction in the last 5 mm of the root of

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 Table 1
 Prevalences of isthmuses in the

 mesial roots of mandibular molars
 reported in previous studies

Author	Method used	lsthmuses (%)	Distance from apex	Number of roots
Skidmore & Bjørndal (1971)	Plastic casts	6	Coronal third	45
		18	Middle third	
		60	Apical third	
Cambruzzi & Marshall (1983)	Not specified	60	3 mm	108
Vertucci (1984)	Dissecting	12	Cervical third	100
	microscope	75	Middle third	
		13	Apical third	
Hsu &Kim (1997)	Surgical microscope	6	1 mm	Not
		30	2 mm	reported
		64	3 mm	
		80	4 mm	
		80	5 mm	
Teixeira <i>et al</i> . (2003)	Stereomicroscope	9	1 mm	50
		48	2 mm	
		44	3 mm	
		54	4 mm	
		58	5 mm	

mandibular molars but neither included any statistical analysis (Hsu & Kim 1997, Teixeira *et al.* 2003). If a simple visual inspection of sectioned or cleared teeth is used (Cambruzzi & Marshall 1983, Vertucci 1984) magnification may not be sufficient to detect the presence of isthmuses. Even if stereomicroscopes (Teixeira *et al.* 2003) or surgical microscopes (Hsu & Kim 1997) are used, the sectioning procedures may result in the deposition of smear layer that can obliterate the thin isthmuses. It can be concluded that observations performed with all these techniques may misinterpret the number and also give a poor description of the morphology of isthmuses detected.

Micro-computed tomography (MCT) is a radiographic technique that blends the concepts of thin layer radiography (tomography) with computed image synthesis. The applicability of X-ray CT in endodontics was first investigated in 1990 (Tachibana & Matsumoto 1990), when it was shown that image reconstruction of teeth was feasible, but the available spatial resolution of 0.6 mm, which produced three-dimensional images of external and internal tooth anatomy, was not fine enough to allow a detailed structural analysis. The MCT instruments, operating at resolution of 1-200 µm are able to measure enamel thickness in teeth (Spoor et al. 1993) and volumes of teeth (Nielsen et al. 1995) accurately and reproducibly (Rhodes et al. 1999, 2000). High-resolution MCT was used in several studies to evaluate and compare instrumentation techniques (Gambill et al. 1996, Peters et al. 2000, 2001, 2003, Rhodes et al. 2000, Bergmans et al. 2001, 2002, 2003, Gluskin et al. 2001). The MCT technique requires virtually no sample preparation and can image teeth in their integrity, without necessitating sectioning that would cause loss of root material or artefact (Bjørndal *et al.* 1999, Rhodes *et al.* 1999). The technique has not been previously used to detect isthmuses.

The objectives of this investigation were to count the number of isthmuses detected by MCT in transverse serial sections of the apical 5 mm of the mesial roots of mandibular molars and to describe the morphology of the isthmuses observed in the same sections. The null hypothesis was that location of the sections in each of the apical 5 mm and isthmus presence were independent.

Materials and methods

Twenty mandibular first molars were selected that had mesial roots with fully formed apices, similar root length and angle of curvature and two separate apical foramina in the mesial root. The teeth had been stored in 1% formalin solution following extraction.

The mesial roots were separated from the distal roots with longitudinal saw cuts. Each root was then embedded in epoxy resin in a clear plastic cuvette, up to the cemento-enamel junction. The mesial roots were X-rayed, using a high-resolution CT scanner (G.E. nondestructive Testing Lab, Cincinnati, OH, USA). The CT scanner consisted of four hardware subsystems: a radiation source, a radiation detector system, a mechanical manipulator, and a computer with display. The X-ray source was set at 100 kV to achieve a focal spot of 6 μ m. The sample was positioned between the source and the detector to achieve ×4 magnification.

A dual field image intensifier was coupled to a CCD video camera (Dalsa 1M15; Dalsa, Waterloo, Ontario, Canada). Two-dimensional individual images with a pixel size of 12.5 μ m \times 12.5 μ m, and a slice thickness of 25.0 µm were obtained. In each one of the apical 5 mm of the 20 roots examined, 40 sections were observed, giving a total of 800 sections observed in each group. The acquired data were transferred to the workstation for observation. The number of sections showing isthmuses in each of the apical 5 mm of the root canals was recorded by two examiners; an agreement was reached between the two examiners regarding the sections that had received a different evaluation in a first instance. Data were analysed as a contingency table using the chi-square statistic to test the null hypothesis that location of the sections in each of the apical 5 mm and isthmus presence were independent. Significance was predetermined at P = 0.05and analysis was carried out using Stata version 8.2. (Stata Corp., College Station, TX, USA).

Results

Of the 20 roots observed 17 had isthmuses in one or more of the apical 5 mm. Only four of the 17 roots with isthmuses had an isthmus that was continuous from its coronal beginning to its apical end, the other 13 roots showed a pattern of sections with and without isthmuses. The results are summarized in Table 2. The percentage of sections showing isthmuses ranged from 17.25 to 50.25% in the apical 5 mm of the root canals. The chi-square test indicated a significant difference in the distribution of isthmuses with section (P = 0.001). Adjusted residuals were used to identify which cells of the contingency table indicated a lack of fit to the null hypothesis. Adjusted residuals are defined as:

$$\operatorname{ar} = \frac{n_{ij} - \mu_{ij}}{\sqrt{\mu_{ij}(1 - p_{i+})(1 - p_{+j})}}$$

where n_{ij} and μ_{ij} are the observed and expected frequencies, and p_{i+} and p_{+i} are the row and marginal

Table 2 Number of sections showing isthmuses at each levelin the apical 5 mm of the mesial root of the mandibular firstmolar

Level from apex	Presence of isthmus	%
1	138	17.25
2	294	36.75
3	402	50.25
4	264	33
5	278	34.75

proportions. If the null hypothesis, row and column independence, is true each adjusted residual has a large-sample standard normal distribution. An adjusted residual that exceeds about 2 or 3 in absolute value indicates a lack of fit of to the null hypothesis for that cell (Agresti 1996).

It was found that sections in the first millimetre from the apex had fewer isthmuses than expected and that sections in the third millimetre from the apex had more isthmuses than expected. The morphology of the isthmuses varied between teeth and within the same tooth (Figs 1 and 2). Calcifications of various dimensions were found to be present in most of the isthmuses (Figs 1 and 2) and occasionally lateral canals originated from the central part of the isthmuses (Fig. 1). Some isthmuses were connected to the main canals by branches encompassing calcified areas (Fig. 3), while



Figure 1 Isthmus in the second millimetre from the apex with calcification (C) and a lateral canal (L). Bar = 1 mm.



Figure 2 Thin is thmus in the fourth millimetre from the apex with calcification (C). Bar = 1 mm.

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Figure 3 Isthmus connecting the main canals with small branches (B). Bar = 1 mm.



Figure 4 Thin is thmus showing small branches (B) that connect different segments of the is thmus. Bar = 1 mm.

small branches connecting different parts of the isthmuses were also observed (Fig. 4).

Discussion

The MCT technique used in this study offers several advantages compared with the techniques previously used for the evaluation of isthmus presence. First the technique is totally nondestructive, therefore no cutting-grinding artefact because of specimen preparation impaired the results or caused sections to be lost. Another significant advantage was the high number of sections available for observation. For each millimetre of the root, 40 sections were observed, whereas in all the previous studies only one section per millimetre was possible. Unfortunately MCT is expensive and timeconsuming therefore only 20 roots could be examined. The point resolution of the MCT instrument used in this study was 12.5 μ m, probably explaining why the majority of roots (known to contain isthmuses), failed to demonstrate the structures being perpetuated through contiguous MCT sections as their diameter was below this resolution threshold and thus difficult to detect. The necessary thresholding and filtering procedures for three-dimensional image creation were considered too subjective to allow reliable isthmus morphology reconstruction and the resolution limits also failed to provide reliable volume rendering and data reconstruction.

In previous studies (Hsu & Kim 1997, Teixeira *et al.* 2003) the isthmuses observed were classified as type I if either two or three canals were present with no notable communication, type II if a definite connection was present between the two main canals. Type III was differentiated from type II by the presence of three canals while canals extending to the isthmus area were classified as type IV. Type V was recognized as a true connection or corridor throughout the section. When this classification was applied to the present study it was found to be highly subjective, and many morphological features of the isthmuses observed did not fit. Therefore, it was not used and the observed sections were simply divided into those showing or not showing isthmuses.

The high prevalence of calcifications and isthmuses in the apical 5 mm of the root canals is in accordance with those of previous studies (Cambruzzi & Marshall 1983, Vertucci 1984, Hsu & Kim 1997, Teixeira *et al.* 2003). The similar prevalence validates the use of a small number of teeth.

The authors are aware that the statistics obtained by the observations performed in each section may be questionable, considering that the 40 sections obtained in each millimetre may not be regarded as independent, however, each MCT image was independent of the next, being created from contiguous (not overlapping) 25 µm thick sections - thus avoiding double/over sampling at the section interfaces. Additionally, there was no interdependence between the mesial roots chosen (all were from different patient) and at the available resolution level, any isthmus could appear in one section and not in the next - because of narrowing or spontaneous alteration of direction. Thus, although presence in one section increases the likelihood of isthmus detection in an adjacent image, the use of the chosen statistical test is valid as each imaged volume was a distinct and independent entity. To oversample by counting voxels twice in overlapping slices would

create a lack of independence, but by counting the maximum number of independent sections possible at this resolution, the most accurate prevalence data within the instrumentation's capability was achieved. Given the effect of the resolution limits discussed above, loss of data by separating the sampled sections (and by what amount?) could further confound or distort the data.

The observation that the third millimetre from the apex had more isthmuses than expected has important clinical implications. If the isthmuses are not cleared of bacteria there is a potential for treatment to fail (Nair 2004). The presence of unsuspected isthmuses may also affect the quality of the root canal filling; in a clinical situation it is often impossible to assess the number of apical foramina present in the third apical of the root; whenever an isthmus is found in the apical part of a root, clinicians have been advised to fill one canal to the apex with gutta-percha and sealer and the other one up to the confluence, therefore assuming that only one apical foramen is present (Castellucci 1993). This may result in 2 mm of unfilled root canal space if the isthmus is not present in the apical 2 mm, where it has been shown that the two canals are often separate, particularly in the final millimetre. The high prevalence of isthmuses observed in the sections from 2 to 5 mm from the apex may have clinical implications especially when surgical endodontics is performed on the mesial root of mandibular molars. The resection of the root apex is frequently performed in areas occupied by isthmuses; in view of the high prevalence of isthmuses it should be normal clinical practice to include the isthmus in root-end cavity preparation. The ultrasonic tips that are normally used to prepare surgically the apical part of the root canals are far larger than the thin isthmuses observed in these MCT images. The resolution obtained with surgical microscopes may not permit the recognition of the thin isthmuses and branches, detected in these high resolution $(12.5 \,\mu\text{m})$ MCT observations. Attempts to prepare thin isthmuses with such instruments may result in overpreparation and possibly missing the actual isthmuses. The results of this investigation do not allow the production of a recommendation regarding the surgical approach to a thin isthmus, however it does not seem reasonable to attempt the surgical preparation and filling of isthmuses that are not detectable clinically and radiographically. In recent years intra-oral CT devices have been designed for the observation of small intra-oral objects (Accuitomo; J Morita, Osaka, Japan). The use of high-resolution tomographic images intraoperatively

might help in evaluating the quality of root end-fillings in the mesial roots of mandibular molars, in the light of isthmuses being frequently observed, and more common than realized by most clinicians, unfortunately the resolution of Accuitomo is of about 127 μ m and would not show all isthmuses observed in this study.

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