A High-resolution Computed Tomographic Study of Changes in Root Canal Isthmus Area by Instrumentation and Root Filling

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Abstract

Introduction: The aim of this study was to obtain a three-dimensional analysis of the isthmus area of the mesiobuccal root canal system in mandibular molars using high-resolution micro–computed tomography (μ-CT) scanning and to measure the amount of debris and root filling material in the isthmus after instrumentation/irrigation and root filling. **Methods:** Mandibular molars with two separated mesial root canals (10 teeth) were scanned by using the Skyscan 1172 μ-CT system (Skyscan, Aartselaar, Belgium) before and after instrumentation and after filling using the Thermafil root filling technique. An isthmus was defined as the ribbon-shaped or thin connecting structure between two root canals after instrumentation. The characteristics of the isthmuses were quantitatively monitored during the whole treatment. The images were segmented and quantified. The surface area of the isthmus, volume of debris after rotary instrumentation, and volume of the filled space in the isthmus after obturation were evaluated. **Results:** Of the seven mesial roots, two had isthmus/anastomoses somewhere along its length in the apical 5 mm, and five had an isthmus that was continuous all the way from the coronal part to the apical part. The average percentage of isthmus surface area and isthmus volume after instrumentation was 21.4% and 9.4% of the whole root canal system, respectively. About 35.2% of the isthmus volume was filled with apparent hard tissue debris after instrumentation/irrigation. The average percentage of volume of filling material in the isthmus areas was significantly lower (57.5%) than in the main root canals (98.5%, $p < 0.001$). **Conclusions:** A considerable amount of dentin debris is produced and packed into the isthmus area during rotary instrumentation of mesial canals of lower molars despite continuous irrigation during and after instrumentation. The debris may partly prevent penetration of the filling material and sealer into the isthmus area. *(J Endod 2011;37:223–227)*

Key Words

Debris, isthmus, instrumentation, micro–computed tomography scanning, root filling

The goal of endodontic therapy is the removal of all vital or necrotic tissue, microorganisms, and microbial byproducts from the root canal system. Although this may be achieved through chemomechanical debridement (1), it is difficult to predictably reach this goal (2–4) because of the intricate nature of root canal anatomy (5–7). Isthmuses, fins, webs, and other irregularities within the root canal often harbor tissue, microbes, and debris after instrumentation (8).

Instrumentation of the root canal system must always be supported by an irrigation capable of removing pulp tissue remnants and other loose material. The efficacy of an irrigation delivery system is dependent not only on its ability to deliver the irrigant to the apical and noninstrumented regions of the canal space and to create a current strong enough to carry the debris away from the canal systems (9–13) but also on the ability of the irrigating solutions to dissolve both organic and inorganic matter. The effervescence created by conventional irrigation with syringes has been used to remove debris from the root canal although without tissue dissolving ability of the solutions this alone is not effective. Irrigation also is essential for eliminating or reducing the number of bacteria in an infected root canal. There has been increased interest in recent years in the effect of instrumentation and irrigation on the isthmus area. Comparative studies with traditional needle irrigation and irrigation facilitated by ultrasound indicated that complete soft-tissue removal requires effective ultrasonic agitation of sodium hypochlorite (14).

High-resolution micro–computed tomography (μ-CT) scanning is an emerging technology with several promising applications in different fields of dentistry. In endodontic research, μ-CT technology is frequently used for the study of root canal anatomy and for the assessment of changes in root canal morphology by instrumentation (7, 15–21). The quality of root canal obturation has also been investigated by μ-CT (22–26), and a few studies have focused on filling of the isthmus area by obturation. Recently, Paqué et al (8) showed that dentin debris is formed and packed into the isthmus area during rotary instrumentation. However, no irrigation was used in this study, leaving the question of the role of irrigation in debris retention unanswered. The aim of this study was therefore (1) to examine if dentin debris is packed into the isthmus area of mesial roots of lower molars during rotary instrumentation and vigorous irrigation using high-resolution μ-CT scanning; (2) to measure the amount of dentin debris, if any, in the isthmus after instrumentation/irrigation; and (3) to measure the percentage of the volume of the isthmus area filled by gutta-percha coated solid core thermoplastic root filling.

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Materials and Methods

Root Specimens

Ten extracted mandibular molars were externally cleaned with pumice and then stored in 0.001% sodium hypochlorite (NaOCl) before use. The pulp chambers and mesial root canals were accessed conventionally. Size 10 K-type files were inserted through both the mesiobuccal and mesiolingual canals 1 mm beyond the apical foramen (two separated canals all the way to the apex) to establish apical patency. The working length was established 1 mm shorter than the length of the root. The tooth specimens were embedded in acryl, which was then polymerized. Scanning of the embedded teeth was performed with the Skyscan 1172 μ-CT system (Skyscan, Aartselaar, Belgium).

The canals were prepared with six ProTaper Universal rotary instruments (Dentsply Tulsa Dental Specialties, OK) in the sequence recommended by the manufacturer (S1, S2, F1-F4). Apical enlargement of the root canal was carried out to size F4 (#40, 06 taper); 5% NaOCl was used as the first irrigating solution delivered with a 30-G Max-i-Probe needle (Dentsply-Rinn, Elgin, IL) placed to 1 mm short of working length. Each canal was filled with irrigant during instrumentation. Two milliliters of 5% NaOCl was used to irrigate the canal between each instrument. After completed instrumentation, the canal was irrigated with 10 mL 5% NaOCl for 5 minutes followed by 17% EDTA (5 mL) for 3 min. The tooth specimens were scanned for the second time with the μ-CT.

Root Filling

All canals (14 canals) were fitted with Thermafil ProTaper point (Dentsply Maillefer, Ballaigues, Switzerland) size F4. A Thermaprep oven (Dentsply, Maillefer) was used to soften the gutta-percha on the Thermafil points as recommended by the manufacturer. A thin layer of sealer (AH Plus, Tulsa Dental) was applied to the canal walls with a size 35 paper point (taper .02; Maillefer, Ballaigues, Switzerland) before filling with a size F4 Thermafil point following the manufacturer's instructions. Excess coronal gutta-percha and the plastic handle were removed with a Thermacut bur without water cooling, and the gutta-percha was vertically condensed with root canal pluggers (model LM 41-42 XSi; LM-Dental, Naantali, Finland). Access cavities were sealed with intermediate restorative material (Dentsply Caulk, Milford, DE) and the teeth were stored at 37°C in 100% humidity until being scanned for the third time by the μ-CT scanner.

μ-CT Measurements and Evaluations

A commercially available high-resolution Skyscan 1172 μ-CT system was used to scan the specimens before and after instrumentation and after obturation. The x-ray tube was operated at 75 kV and 100 mA (0.5 mm Al filter), and the scanning was performed by 360° rotation around the vertical axis and with rotation step of 0.3°. The cross-sectional pixel size and intersection distance were 11 μm. Subsequently, the cross-section image was reconstructed from the projection image with beam-hardening compensation of 45%. The cross-section images were segmented, registered, visualized, and quantified by using image software from Mevislab (Germany) (available from www.mevislab.de/) (27). The isthmus was defined as the ribbon-shaped or thin structure between the two mesial root canals after preparation. Accordingly, the isthmus was segmented with Mevislab software and the volume and the surface area of the isthmus were calculated. In addition, the following parameters were measured: the volume of debris and the volume of the filled space (by Thermafil and sealer) in the isthmus after instrumentation and obturation, respectively. Three teeth were excluded because of technical errors in scanning (because the samples mounted on stubs were slightly moved during scanning process).

The debris in the isthmus after instrumentation was identified and calculated as follows: voxels with black color were identified as soft tissue, liquid, or air (black color) in the preoperative scan; in the second scan, voxels that had changed from black to opaque were assumed to be dentin debris. The unfilled space of the isthmus after filling was identified and calculated as follows: voxels in the isthmus that were identified as soft tissue, liquid, or air (black color) in the preoperative scan and that were not occupied with debris or filling material (bright, opaque color) in the postobturation scan were considered to be the unfilled space of the isthmus. Three-dimensional images of the three stages were generated for visualization. Data were analyzed using a nonparametric test (Mann-Whitney rank sum test).

Results

Of the seven mesial roots, two had isthmus or anastomoses somewhere in the apical 5 mm of the root, and five had an isthmus that was continuous all the way from the coronal part to the apical part (Figs. 1 and 2). The percentage of isthmus surface area and volume were 21.4% (12%-27%) and 9.4% (3%-15%) of the whole root canal system after instrumentation, respectively (Table 1); 35.2% (13%-56%) of the volume of the isthmus was filled with apparent hard tissue debris after instrumentation/irrigation. The average percentage of the volume of filling material in the isthmus areas was significantly lower (57.5%: 45%-68%) than in the main root canals (98.5%: 97%-99%) ($p < 0.001$).

Discussion

The present research used a model that allows for the assessment of changes of the root canal isthmus area caused by instrumentation/irrigation and root filling. In recent years, the resolution of μ-CT has improved considerably from 81 μm (18) and values between 34 and 68 μm (7, 20, 23) to 25 μm (28). At present, axial scanning steps of 14 μm are possible (25, 29). The SkyScan 1172 system could provide nondestructive three-dimensional microscopy of the internal structures of small objects with high spatial resolution and unprecedented speed. The advantage of such a high resolution is a greater accuracy of the rendered images. The main morphologic characteristic of isthmuses in molars is the presence of a fin, web, or ribbon connecting the main root canals. In the present study, the isthmus was defined as a narrow, often ribbon-shaped communication between two root canals after instrumentation. Instrumentation created a round bulge in the main canal, leaving unprepared area(s) between the two mesial canals. Therefore, the consistent definition of boundaries of the isthmus area from μ-CT images was possible after instrumentation. A limitation in the present and similar studies is the fact that only hard-tissue debris and filling material can be viewed; the remaining soft tissue is invisible. This is because μ-CT scanning is based on radiographic images. Theoretically, it could be possible to obtain indirect evidence of the presence of residual organic/soft tissue in the root canal (eg, a thin line between an opaque root filling material and mineralized dentin might be an indication of soft-tissue debris [or predentin] that remains in the canal space after instrumentation/irrigation and filling).

To our knowledge, this is the first study in which accumulation of dentin debris in the isthmus area during rotary instrumentation/irrigation and its relationship to subsequent penetration of filling material have been quantitatively monitored in human teeth. The only previous micro-CT study in which packing of dentin debris by rotary instruments into isthmus and other noninstrumented areas was examined was done without irrigation (8). The main goals of these two studies are different; in the previous study, the goal obviously was to answer a baseline question: do rotary NiTi instruments create debris when cutting dentin and to

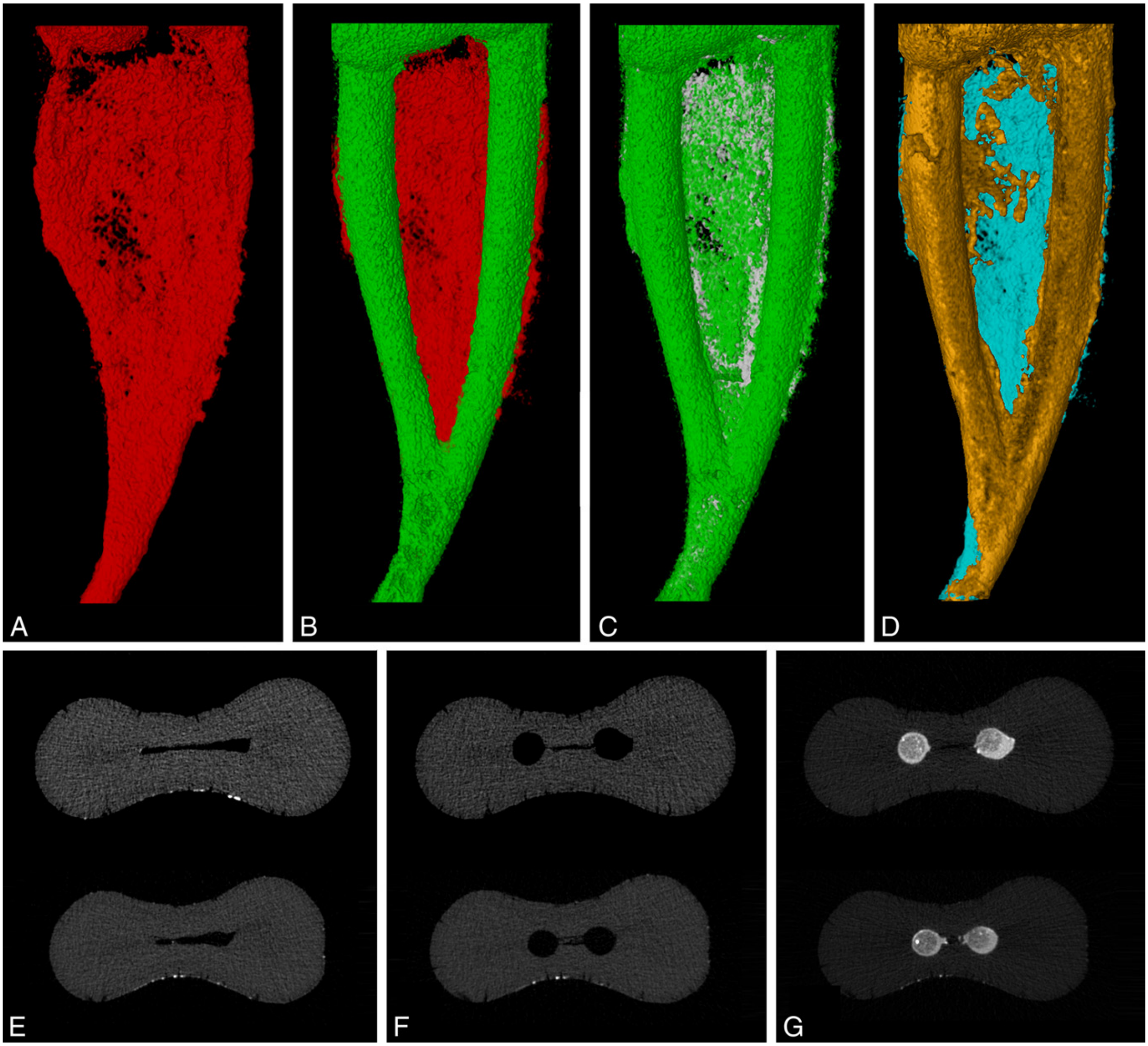

Figure 1. Three-dimensional reconstruction μ-CT scans of the mesial root canal system of the mandibular molar under investigation. (*A*) The initial canal configuration with the complex isthmus area from its coronal part to the apical part. (*B*) Corresponding three-dimensional μ-CT reconstruction after instrumentation. Prepared canal areas are indicated in green, and untouched areas indicated in red. (*C*) Superimposition of the apparent accumulated hard-tissue debris areas indicates with silver color. The canal space and empty space in isthmus after instrumentation are indicated in green. (*D*) Root filling material is indicated in brown, and the nonfilling material area on the ribbon-shaped isthmus is indicated in blue, which includes debris and void. (*E-G*) A cross-section of mesial root at the (*top*) middle part and (*bottom*) apical part: (*E*) before instrumentation, (*F*) after instrumentation, and (*G*) after obturation.

discover what happens to this debris in the first place. In order to clarify this basic question, irrigation would have been a confounding factor. In the present study, a step further was taken, and copious amounts of irrigating solutions (NaOCl and EDTA) were used during and after instrumentation before the second scanning was performed. Finally, a thermoplastic root filling method, Thermafil, with recognized, excellent capability to penetrate laterally during compaction into the fine details of the root canal system (ie, lateral canals, fins, and isthmuses) was used to examine the spatial relationship of the original isthmus space and space occupied by the mineral debris and the root filling material.

It has been recommended that once canal preparation is completed, the canal should be flushed by means of a small-diameter irrigation needle with a safety tip (side vented), and the tip should be placed in the apical third of the canal (12, 13, 30, 31). The results of the present study show that even copious irrigation during and after instrumentation with solutions dissolving both organic and inorganic matter was not able to prevent or remove the debris packed into the isthmus area between the main root canals. Recently, computational fluid dynamics model (31) recently showed that the fastest flow was found in the turbulent boundary, whereas the minimum velocity was observed on the wall in all root canal irrigations. Fluid stagnation makes adequate irrigant replacement difficult, resulting in gross debris retention especially in apical third area. Hence, it is perhaps not surprising that in the present study irrigation was ineffective in removing debris from the isthmus using syringe irrigation. Despite the current progress in endodontic treatment, cleaning the isthmus with

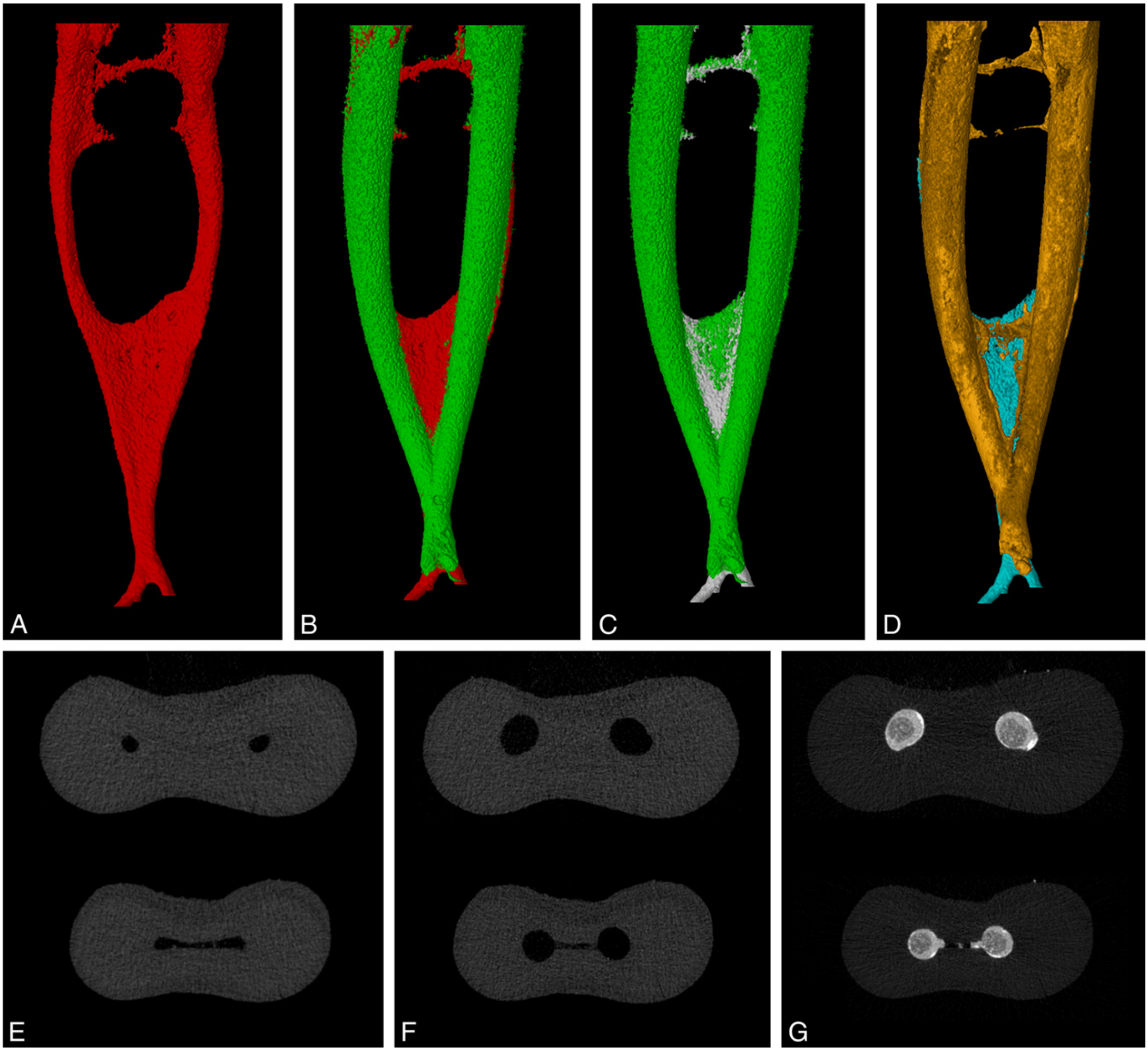

Figure 2. Three-dimensional reconstruction μ-CT scans of the mesial root canal system of another mandibular molar examined. Legends to images *A* through *G* as in Figure 1.

nonsurgical methods is still problematic (32). Studies using a new type of ultrasonic device showed that although the addition of 1 minute of ultrasonically activated irrigation after hand/rotary instrumentation significantly increased the isthmus cleanliness values, complete debridement of the isthmuses was not always achieved (33, 34). However, in these studies, the emphasis was on soft-tissue retention and analysis was based on histological sections. Despite obvious difficulties to predictably obtain complete cleanliness, the management of the isthmus area is considered a potentially important factor that may have an impact on the long-term prognosis of nonsurgical endodontic treatment or periapical surgery. A recent case report (35) showed that a complex, variable, multispecies biofilm was present at the entire length of the isthmus in which the tooth had been initially treated 10 years earlier and then retreated 2 years ago. Both gram-positive and gram-negative organisms were detected surviving in an extremely harsh and nutrient-deficient environment that had existed for more than a decade after root canal treatment. Further work using various methods, including μ-CT, is required to examine the effect of contemporary irrigant delivery and agitation on cleaning the isthmus and anastomoses between root canals.

TABLE 1. The Relative Proportions (expressed as volume %) of Isthmus, Dentin Debris, and Root Filling Material after Instrumentation and Root Filling

	N	Isthmus volume of the entire root canal systems	Amount of debris in isthmus	Root filling volume in isthmus/entire isthmus volume	Root filling material main root canals
%	7	9.4 ± 3.5	35.2 ± 13.1	57.5 ± 10.6*	98.5 ± 1.6*

*There was significant between root filling material in isthmus versus main root canal area ($p < 0.001$).

The incidence of canal isthmuses varies depending on the type of tooth (36), root level (6), and age (37). The incidence of canal isthmuses in first molars ranges from 54% to 89% in mesial roots of mandibular first molars (36, 37). Mannocci et al (38) reported that the prevalence of isthmuses ranged from 17% to 50% in the apical 5 mm of the mesial root of mandibular first molars, with the highest prevalence at the 3-mm level. A recent study (37) showed that the highest prevalence of isthmus is 4 to 6 mm from the apex in mandibular first molars using μ-CT scanning. Despite the low number of teeth examined in the present study, these findings are consistent with our results.

Complete elimination of microbes from the root canal system is regarded as the prerequisite for optimal long-term prognosis of endodontic treatment. Instrumentation is supposed to create an effective channel for the delivery of irrigating solutions and root filling material. Irrigants play a key role in disinfection of the canal space, and the root filling continues bacterial killing and is supposed to prevent reinfection. Dentin debris formed during instrumentation and soft-tissue remnants, both remaining in the isthmus area and canal anastomoses, prevent root filling material to fully occupy this space and may allow bacteria/biofilm survival. Nutrient deprivation or exhaustion stimulates a starvation response, which allows bacterial long-term persistence in a nongrowing but culturable state (39). Bacteria in the multispecies anaerobic biofilm can be returned to normal physiological state by reestablishing their access to nutrients while still in biofilm (40). Modern understanding of the complexities of the root canal anatomy has made it easier to comprehend that bacteria in such biofilms may well have or later establish a communication with periradicular tissues and compromise healing. With further improvement in μ-CT hardware and software, it might be possible in the future to directly determine the amount of biofilm in peripheral parts of the root canal system before and after endodontic treatment (17).

Within the limitations of this *in vitro* study, isthmuses and canal anastomoses had not been completely cleaned and obturated. The accumulation of dentin debris, despite much irrigation, into these areas may play an important role in their incomplete cleaning and filling. Further research is necessary to determine optimal ways to obtain better cleanliness in all parts of the root canal system.

References

1. Sjögren U, Hagglund B, Sundqvist G, et al. Factors affecting the long-term results of endodontic treatment. J Endod 1990;16:498–504.
2. Shuping GB, Østravik D, Sigurdsson A, et al. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751–5.
3. Card SJ, Sigurdsson A, Østravik D, et al. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002;28:779–83.
4. Fariniuk LF, Baratto-Filho F, da Cruz-Filho AM, et al. Histologic analysis of the cleaning capacity of mechanical endodontic instruments activated by the ENDOflash system. J Endod 2003;29:651–3.
5. Skidmore AE, Bjorndal AM. Root canal morphology of the human mandibular first molar. Oral Surg Oral Med Oral Pathol 1971;32:778–84.
6. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol 1984;58:589–99.
7. Peters OA, Laib A, Rüegsegger P, et al. Three dimensional analysis of root canal geometry using high resolution computed tomography. J Dent Res 2000;79:1405–9.
8. Paqué F, Laib A, Gautschi H, et al. Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. J Endod 2009;35:1044–7.
9. Moser JB, Heuer MA. Forces and efficacy in endodontic irrigation systems. Oral Surg Oral Med Oral Pathol 1982;53:425–8.
10. Chow TW. Mechanical effectiveness of root canal irrigation. J Endod 1983;9:475–9.
11. Sedgley CM, Nagel AC, Hall D, et al. Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging in vitro. Int Endod J 2005;38:97–104.
12. Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a computational fluid dynamics study. Int Endod J 2009;42:144–55.
13. Tay FR, Gu LS, Schoeffel GJ, et al. Effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery. J Endod 2010;36:745–50.
14. Burleson A, Nusstein J, Reader A, et al. The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic, human mandibular molars. J Endod 2007;33:782–7.
15. Peters OA, Laib A, Gohring T, et al. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod 2001;27:1–6.
16. Peters OA, Peters CI, Schünenberger K, et al. ProTaper rotary root canal preparation: effects of canal anatomy on final shape analysed by micro CT. Int Endod J 2003;36:86–92.
17. Peters OA, Boessler C, Paqué F. Root canal preparation with a novel nickel-titanium instrument evaluated with micro-computed tomography: canal surface preparation over time. J Endod 2010;36:1068–72.
18. Rhodes JS, Ford TR, Lynch JA, et al. A comparison of two nickel-titanium instrumentation techniques in teeth using microcomputed tomography. Int Endod J 2000;33:279–85.
19. Bergmans L, Van Cleynenbreugel J, Beullens M, et al. Progressive versus constant tapered shaft design using NiTi rotary instruments. Int Endod J 2003;36:288–95.
20. Gluskin AH, Brown DC, Buchanan LS. A reconstructed computerized tomographic comparison of Ni-Ti rotary GT files versus traditional instruments in canals shaped by novice operators. Int Endodc J 2001;34:476–84.
21. Paqué F, Balmer M, Attin T, et al. Preparation of oval-shaped root canals in mandibular molars using nickel-titanium rotary instruments: a micro-computed tomography study. J Endod 2010;36:703–7.
22. Nielsen RB, Alyassin AM, Peters DD. Microcomputed tomography: an advanced system for detailed endodontic research. J Endod 1995;21:561–8.
23. Dowker SE, Davis GR, Elliott JC. X-ray microtomography: nondestructive threedimensional imaging for in vitro endodontic studies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;83:510–6.
24. Jung M, Lommel D, Klimek J. The imaging of root canal obturation using micro-CT. Int Endod J 2005;38:617–26.
25. Hammad M, Qualtrough A, Silikas N. Evaluation of root canal obturation: a threedimensional in vitro study. J Endod 2009;35:541–4.
26. Mirfendereski M, Roth K, Fan B, et al. Technique acquisition in the use of two thermoplasticized root filling methods by inexperienced dental students: a microcomputed tomography analysis. J Endod 2009;35:1512–7.
27. Gao Y, Peters OA, Wu H, et al. An application framework of three-dimensional reconstruction and measurement for endodontic research. J Endod 2009;35:269–74.
28. Verdonschot N, Fennis WM, Kuijs RH, et al. Generation of 3-D finite element models of restored human teeth using micro-CT techniques. Int J Prosthodont 2001;14:310–5.
29. Hammad M, Qualtrough A, Silikas N. Three-dimensional evaluation of effectiveness of hand and rotary instrumentation for retreatment of canals filled with different materials. J Endod 2008;34:1370–3.
30. Haapasalo M, Shen Y, Qian W, et al. Irrigation in endodontics. Dent Clin North Am 2010;54:291–312.
31. Shen Y, Gao Y, Qian W, et al. Three-dimensional numeric simulation of root canal irrigant flow with different irrigation needles. J Endod 2010;36:884–9.
32. Kim S, Pecora G, Rubinstein RA. The resected root surface and isthmus. In: Kim S, Pecora G, Rubinstein RA, eds. Color Atlas of Microsurgery in Endodontics. Philadelphia: Saunders; 2001:98–104.
33. Gutarts R, Nusstein J, Reader A, et al. In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. J Endod 2005;31:166–70.
34. Burleson A, Nusstein J, Reader A, et al. The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic, human mandibular molars. J Endod 2007;33:782–7.
35. Carr GB, Schwartz RS, Schaudinn C, et al. Ultrastructural examination of failed molar retreatment with secondary apical periodontitis: an examination of endodontic biofilms in an endodontic retreatment failure. J Endod 2009;35:1303–9.
36. von Arx T. Frequency and type of canal isthmuses in first molars detected by endoscopic inspection during periradicular surgery. Int Endod J 2005;38:160–8.
37. Gu L, Wei X, Ling J, et al. A microcomputed tomographic study of canal isthmuses in the mesial root of mandibular first molars in a Chinese population. J Endod 2009;35:353–6.
38. Mannocci F, Peru M, Sherriff M, et al. The isthmuses of the mesial root of mandibular molars: a micro-computed tomographic study. Int Endod J 2005;38:558–63.
39. Oliver JD. The viable but nonculturable state in bacteria. J Microbiol 2005;43:93–100.
40. Shen Y, Stojicic S, Haapasalo M. Bacterial viability in starved and revitalized biofilms: comparison of viability staining and direct culture. J Endod 2010;36:1820–3.