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Micro-CT analyses of apical enlargement and molar root canal complexity

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Abstract

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Aim To compare the effectiveness of two rotary hybrid instrumentation techniques with focus on apical enlargement in molar teeth and to quantify and visualize spatial details of instrumentation efficacy in root canals of different complexity.

Methodology Maxillary and mandibular molar teeth were scanned using X-ray microcomputed tomography. Root canals were prepared using either a GT/Profile protocol or a RaCe/NiTi protocol. Variables used for evaluation were the following: distance between root canal surfaces before and after preparation (distance after preparation, DAP), percentage of root canal area remaining unprepared and increase in canal volume after preparation. Root canals were classified according to size and complexity, and consequences of unprepared portions of narrow root canals and intraradicular connections/isthmuses were included in the analyses. One- and two-way ANOVA were used in the statistical analyses.

Results No difference was found between the two techniques: $DAP_{apical\text{-}third}$ ($P = 0.590$), area $unprepared_{apical\text{-}third}$ ($P = 0.126$) and volume $increase_{apical\text{-}third}$ ($P = 0.821$). Unprepared root canal area became larger in relation to root canal size and complexity, irrespective of the technique used. Percentage of root canal area remaining unprepared was significantly lower in small root canals and complex systems compared to large root canals. The isthmus area *per se* contributed with a mean of 17.6%, and with a mean of 25.7%, when a narrow root canal remained unprepared.

Conclusions The addition of isthmuses did not significantly alter the ratio of instrumented to unprepared areas at total root canal level. Distal and palatal root canals had the highest level of unprepared area irrespective of the two instrumentation techniques examined.

Keywords: apical enlargement, instrumentation, intraradicular connection, isthmus, micro-CT, root canal.

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Introduction

Biomechanical instrumentation of the root canal system during root canal treatment is a major element of preventing or eliminating apical periodontitis (Ørstavik & Pitt Ford 2007). Various combinations of instrumentation systems are used both in the general dental practice (GDP) environment and within the endodontic community, and hybrid techniques are used in clinical trials (Kvist *et al.* 2004). In the apical stop preparation, instruments of increasing tip size are used, and thereby the size of the apical preparation increases. Numerous studies have shown that this approach significantly reduces the number of bacteria in the root canal (Ørstavik *et al.* 1991, Sjögren *et al.* 1997, Dalton *et al.* 1998, Shuping *et al.* 2000, Card *et al.* 2002).

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Previous micro-CT studies have produced data on the effectiveness of various NiTi instruments. A crucial methodological element in these studies is to use standardized material, e.g. specific selected distal roots (Paqué *et al.* 2010) or mesial roots of mandibular molars (Bergmans *et al.* 2002, 2003, Endal *et al.* 2011). Obviously, these experimental models do not reflect the clinical situation in a GDP environment. Another standardization approach has been to use preselected maxillary molars with comparable root canal volumes before instrumentation (Peters *et al.* 2000, 2001, 2003, Paqué *et al.* 2009a). But again, the studies were not aimed to mimic a clinical situation as instrumentation of the second mesiobuccal (mb_2) root canal was purposely avoided in maxillary molars.

Molars have become the teeth most frequently involved in root canal treatment within the GDP environment (Kirkevang *et al.* 2001, Bjørndal *et al.* 2006). Therefore, it is of interest to evaluate attempts to instrument the entire molar root canal system. The complexity of root canals in molars using three-dimensional (3D) methods has been described recently (Markvart *et al.* 2011). A high frequency of intraradicular connections or isthmuses was observed in the mesiobuccal root of maxillary molars and in the mesial root of mandibular molars.

Studies have shown that a relatively large part of the root canal area in molars remains unprepared after instrumentation (Peters *et al.* 2001, 2003, Hübscher *et al.* 2003, Paqué *et al.* 2010). Hence, it is relevant to evaluate the relative contribution of an isthmus when studying the ratio of instrumented to unprepared areas after canal preparation in molars. It is hypothesized that the inclusion of isthmus alters this ratio.

The aims in this study were to compare the effectiveness of one hybrid instrumentation technique and one standard instrumentation technique, both with focus on apical enlargement, in molar teeth as performed in a simulated general practice environment, and to visualize spatial details of instrumentation efficacy in root canals of different complexities.

Materials and methods

Radiographs were acquired of a batch of freshly extracted molars to select intact molars with varying sizes of pulp cavities. The teeth were the same as previously used (Markvart *et al.* 2011). The 11 maxillary and seven mandibular molar teeth were prepared using either a hybrid technique consisting of SystemGT™ and Profile instruments (Dentsply Maillefer, Ballaigues, Switzerland) or a RaCe® protocol (FKG Dentaire, La Chaux-de-fonds, Switzerland) including NiTi-K-Flex hand instruments (Dentsply Maillefer). The teeth were divided in two similar groups with respect to tooth type (maxillary or mandibular) and size of the root canal curvature of the mesiobuccal root as described by Schneider (1971). Type of instrumentation technique was then randomly assigned for each of the two groups. The roots were embedded in Epofix Kit (Struers A/S, Copenhagen, Denmark).

Details of the micro-CT scanning procedure, segmentation and surface modelling have been described previously (Markvart *et al.* 2011). The teeth were scanned using a μCT40 microtomography scanner (SCANCO Medical AG, Brüttisellen, Switzerland), producing an isotropic voxel size of 30.7 μm. The objects were semi-automatically segmented, and polygonal surface models were produced from each tooth (micro-CT image volume) using the software *Landmarker* (version 2.0.3) (Darvann 2008).

Scanning and segmentation procedures

Identical scanner settings were used for the scanning of the teeth before and after instrumentation. The scanning precision was determined and described in Markvart *et al.* (2011). 3D surface models were created from the scans using the Marching Cubes algorithm (Lorensen & Cline 1987) before and after instrumentation employing the same intensity threshold, and the two surface models were subsequently spatially registered to each other using the Iterated Closest Point algorithm (Zhang 1994).

Instruments and preparation techniques

Access preparation

Access cavities were prepared. The root canals were then initially instrumented with C+ files sizes 8-15 (Dentsply Maillefer) followed by Flexofiles sizes 15-20 (Dentsply Maillefer).

Rotary NiTi instrumentation

Teeth allocated for instrumentation with SystemGT Series 20 combined with Profile taper .04 were treated as previously described (Kvist *et al.* 2004). The root canals were sequentially enlarged using first SystemGT size 20, .10 taper to size 20, .04 taper and subsequently Profile size 25, .04 taper to size 50, .04 taper. Teeth allocated for instrumentation with RaCe.02 taper instruments including NiTi-flex hand instruments were used according to the manufacturer's instructions and with a speed of 600 rpm. The NiTi-flex instruments were used after the RaCe size 40, .02 taper instruments. Irrespective of treatment technique, the root canals were prepared to size 40 with the exception of palatal and distal root canals, which were prepared to size 50.

After coronal preparation, a radiograph was taken of each tooth to verify the preparation length 1 mm from the radiological apex. New instruments were used for the preparation of each tooth. All root canals were instrumented without separating the crown, the roots were not visible, but were embedded in resin and the operators were neither aware of the actual 3D canal morphology nor any other 3D information prior to the instrumentation. All root canals were aimed to be negotiated and prepared. However, if a root canal remained unprepared, the consequences of this were accepted without the root being excluded from the study. In the course of root canal preparation, each canal was irrigated with 9 mL 2.5% NaOCl, using a 3-mL Monoject syringe and 0.4 × 32 mm Latex-free Endodontic Needle (Tyco Healthcare Group LP, Mansfield, MA, USA); the irrigation was performed between every change of instruments.

Micro-CT measurement

The following variables were defined in terms of quantities that could be assessed in the 3D surface models and used for the evaluation of the instrumentation: (i) distance (μm) between the root canal surfaces before and after preparation [distance after preparation (DAP)], (ii) percentage of root canal area remaining unprepared and (iii) increase in volume of the root canals before and after preparation.

Whilst the first variable provided a local measure at every point location on the 3D surfaces as well as a global measure (by averaging the local values), the last two variables provided global measures (for the total root canal or part of the root canal). The global assessments were performed in three parts of equal length: the apical, middle and coronal thirds of the root canal, respectively.

Root canals were classified according to size and anatomical complexity, sectioning the material into *small* separated root canals, *large* separated root canals and *complex* nonseparated root canals with isthmus. When an isthmus was present, the relative volume and area of isthmus were assessed as well.

Distance after preparation

For each surface element (triangle) on the original surface, the Euclidian distance from the geometrical midpoint of the triangle to the closest location on the surface after preparation was determined. The spatial distribution of this local measure was visualized by colour mapping the original surface according to the distance (μm), providing insight into the relationship between anatomy and efficacy of preparation. A spatial mean of the distances was calculated as a global value of DAP in the entire examined root canal, as well as divided in apical, middle and coronal parts of the root canal. A slice-by-slice assessment was applied, including the apical stop as the reference point and the slice beneath the furcation wall. The area between the apical stop and the furcation wall defined the examined part of the root canal, as described by Paqué *et al.* (2010). Thus, the colour mapping approach visualized the distance between the paired root canal surfaces before and after instrumentation. The purple colour displayed regions with no instrumentation (DAP = 0), whereas the red colour represented a DAP of more than 260 μm (Fig. 1).

Quantitative assessment of volume before and after preparation

Volumes of spatially registered root canals before and after preparation, respectively, were calculated from the surface models. The volume was calculated as the sum of the volume of all elements in the interior of the surface model (Cohen-Or & Kaufman 1995). The volume increase was calculated as follows:

$$\text{Volume increase (\%)} = \frac{\text{volume after} - \text{volume before}}{\text{volume before}} \times 100$$

Percentage of remaining unprepared surface area

Spatially registered surface models of the roots, before and after preparation, were compared to evaluate the amount of remaining unprepared surface area. This variable was calculated by using the distances between the surface of the root canals before and after preparation that had been determined at every surface point. The definition of the variable was based on the notion that those locations where the two surfaces were coinciding (i.e. closer to each other than a predefined distance limit E) represent remaining unprepared parts of the root canal. The limit E was set to the error of the method (precision) determined in a previous study (Markvart *et al.* 2011), where the scanning and segmentation precision (mean ± SD) was 6.7 ± 10.1

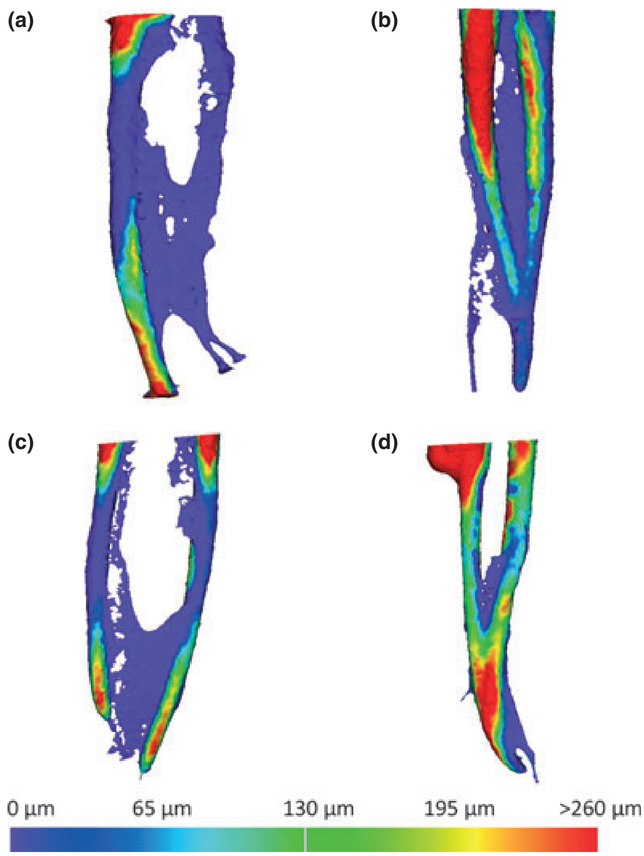

Figure 1 Examples displaying isthmuses in mesiobuccal roots from maxillary molars (a, b) and in mesial roots from mandibular molars (c, d). Root canal instruments crossed an incomplete separation structure leaving the apical parts of the second mesiobuccal (mb_2) and the mandibular mesiobuccal root canal uninstrumented (b, d). In (a), the mb_2 was not negotiated. The colour scale visualizes distance after preparation (DAP) and ranges from 0 µm (purple) to ≥260 µm (red).

and 8.6 ± 8.5 µm, respectively. The percentage of remaining unprepared surface area was defined as:

$$\text{Unprepared surface area (\%)} = \frac{\text{area unprepared}}{\text{area before preparation}} \times 100$$

instrumentation according to the surface models coloured in terms of DAP. Tools from *Landmarker* were applied to the surface models, and an indirect calculation of the volume and surface area of the isthmus region according to the following equations was made:

$$\mathrm{RV_{isthmus}} = \frac{\text{volume after prep. total} - \text{volume after prep. without isthmus}}{\text{volume before prep.}} \times 100$$

Area unprepared was calculated using custom software developed in Interactive Data Language (version 7.1; ITT Visual Information Solutions, Boulder, CO, USA).

Assessments with and without isthmus

The contribution of the isthmus to the total volume and surface area was determined as the relative volume ($RV_{isthmus}$) and relative surface area ($RSA_{isthmus}$) of the isthmus between the root canals. It is difficult to measure the size of the isthmus region as the border between the root canals, and the isthmus is often diffuse. The size of the isthmus region was defined after

$$\mathrm{RSA_{isthmus}} = \left(\frac{\text{SA total} - \text{SA without isthmus}}{\text{SA total}}\right) \times 100$$

Statistical methods

The statistical analyses were carried out using SAS 9.1.3 (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA). Mean DAP ± SD, unprepared area, volume before treatment, volume after treatment and volume increase were analysed in the examined part of the root canal as well as in apical-, middle- and coronal thirds, with one- and two-way ANOVA.

Table 1 Mean degree of canal curvature before treatment

	GT+Profile	RaCe/NiTi	P*
Moderate (n)	16.84 ± 3.10 (19)	15.53 ± 6.78 (15)	0.184
Severe (n)	33.63 ± 8.94 (8)	29.63 ± 5.42 (8)	0.346

*ANOVA.

Interactions between variables were tested; instrumentation technique and root canal complexity. Data were transformed logarithmically when the standard residuals were not normally distributed. A level of $P < 0.05$ was considered significant.

Results

There was a similar distribution of moderate and severe root canal curvatures in the treatment groups, and there were no straight root canals amongst the objects (Table 1). In the GT/Profile group, there were three mandibular and six maxillary molar teeth. In the RaCe/NiTi group, there were four mandibular and five maxillary molar teeth. Three instruments fractured in two teeth making further analyses in these canals impossible, and therefore they were excluded. Seven mb_2 were left untreated because of lack of negotiation; however, these were included in the analyses. No significant difference was found in the root canal volumes between the two treatment groups before instrumentation (Table 2). After instrumentation with GT/Profile versus RaCe/NiTi, no significant difference was observed in $DAP_{total\text{-}canal}$ ($P = 0.152$), area unprepared$_{total\text{-}canal}$ ($P = 0.058$) or volume increase$_{total\text{-}canal}$ ($P = 0.475$). Similarly, no difference was found between the two techniques in the apical third with respect to DAP ($P = 0.590$), area unprepared ($P = 0.126$) and volume increase ($P = 0.821$). Irrespective of the two instrumentations, the distal root canals of mandibular molars and palatal root canals of maxillary molars were always less prepared as visualized by the anatomical ranking (Table 3). The material was categorized according to size and anatomical complexity. The *small root canal group* included separated mesiobuccal and distobuccal root canals from maxillary molars as well as separated mesial root canals from mandibular molars. The *large root canal group* included separated palatal root canals from maxillary molars and separated distal root canals from mandibular molars. The *complex root canal group* included nonseparated root canals from maxillary and mandibular mesial molar roots with the presence of one or more isthmuses. Interactions between the three groups and instrumentation techniques were tested, and no significant effect was found. $DAP_{total\text{-}canal}$ and volume increase$_{total\text{-}canal}$ were significantly larger in the *small* and *complex root canal groups* than in the *large root canal group*. Similarly, area unprepared$_{total\text{-}canal}$ was significantly lower in the *small root canal group* (30.9%) and the *complex root canal group* (36.4%) than in the *large root canal group* (57.5%) (Table 4). At total root canal level, the inclusion of the isthmus region did not alter the ratio of unprepared area to volume increase as illustrated between the *small* and *complex root canal groups* (Table 4). The intracanal analyses showed significant differences only between *small* and *large* root canals. The isthmus area *per se* contributed with a mean of 17.6%, and 25.7% when mb_2 remained unprepared in the *complex root canal group* (Table 5). The mean relative volume of the isthmus region was 20.8% of the volume of the root before instrumentation (Table 5). The mean relative volume of the isthmus region when the mb_2 was left uninstrumented was 49.5% of the total volume before instrumentation (Table 5). Qualitative comparisons of isthmus regions before and after instrumentation showed a minor reduction in the isthmus volume, when superimposing two surface models. In the material, the consequences of leaving a narrow root canal (mb_2) uninstrumented are illustrated in a maxillary mesiobuccal root component (Fig. 1a). In this particular root, 49% of the total surface area was left unprepared, corresponding to 58% of the original volume. In the mesial root of a mandibular molar containing isthmus (Fig. 1c), 48% of the total surface area was left unprepared, corresponding to 25% of the original volume. In two scenarios, the root canal instruments had entered the isthmus region, leaving the apical parts unprepared. This occurred in maxillary and mandibular molars irrespective of instrumentation protocol (Fig. 1b,d).

Table 2 Mean volume before treatment

Mean volume before treatment (mm^3)	GT/Profile (n = 27)	RaCe/Niti (n = 23)	
	Mean	Mean	P*
Total canal	3.73 ± 2.71	4.10 ± 2.13	0.326
Apical third	0.54 ± 0.39	0.66 ± 0.40	0.294
Middle third	1.22 ± 0.90	1.33 ± 0.70	0.277
Coronal third	1.99 ± 1.55	2.13 ± 1.18	0.423

*ANOVA.

Discussion

One hybrid and one standard instrumentation technique in maxillary and mandibular molar root canals

Table 3 Mean DAP, unprepared area and volume increase in the two instrumentation techniques with respect to total root canal and thirds

	GT/Profile (n = 20)			RaCe/NiTi (n = 18)			
	Mean	Min	Max	Mean	Min	Max	P*
DAP (μm)							
Total canal	101 ± 38	37	188	85 ± 38	35	149	0.152
Apical third	76 ± 28	39	132	74 ± 38	24	183	0.590
Middle third	74 ± 34	22	170	63 ± 33	29	133	0.298
Coronal third	151 ± 77	32	331	115 ± 63	37	228	0.161
Ranking of DAP total related to anatomy	$d<p<mb\ root_{max}<db<ml<mb_{man}<mroot_{man}<mb_1$			$d<p<mb_{man}<m\ root_{man}<ml<mb_1<db<mb\ root_{max}$			
Unprepared area (%)[a]							
Total canal	38.00	9.68	73.38	48.62	16.30	73.11	0.058
Apical third	39.00	3.18	74.78	49.98	11.47	100	0.126
Middle third	45.06	4.83	84.76	58.45	25.24	89.20	0.059
Coronal third	31.98	0.89	72.99	40.50	3.19	74.52	0.206
Ranking of unprepared area total related to anatomy	$ml<mb_{man}<mb_1<db<m\ root_{man}<mbroot_{max}<p<d$			$ml<m\ root_{man}<mb_{man}<db<mb\ root_{max}<d<p$			
Volume increase (%)							
Total canal	94.88	13.33	254.48	83.67	10.77	323.87	0.475
Apical third	101.63	3.22	330.96	96.09	6.25	405.09	0.821
Middle third	70.52	4.94	280.50	56.69	7.62	148.70	0.860
Coronal third	116.23	10.28	307.12	113.13	7.05	645.89	0.398
Ranking of volume increase total related to anatomy	$d<p<mb\ root_{max}<db<ml<mb_{man}<mb_1<m\ root_{man}$			$d<p<mb_{man}<ml<mb_1<db<mb\ root_{max}<m\ root_{man}$			

*ANOVA.
[a]GT: n = 21; RaCe: n = 19.
d, distal; p, palatal; mb_1, first mesiobuccal root canal maxillary; mb $root_{max}$, mesiobuccal root maxillary; mb $root_{man}$, mesiobuccal root mandibular; m $root_{man}$, mesial root mandibular; ml, mesiolingual; mb_{man}, mesiobuccal root canal mandibular; db, distobuccal; DAP, distance after preparation.

were analysed. No difference between the two instrumentation techniques was found in relation to volume increase, unprepared area and DAP. It was perhaps expected that the use of instruments with less aggressive instrument designs would have been reflected with significantly smaller values of DAP, but this was not the case within the present set-up. Distance after preparation was a new variable applied to provide an estimate of the amount of removal of dentine in three regions of the root: apical, middle and coronal. In contrast, Bergmans *et al.* (2002, 2003) analysed the net removal of dentine at different levels in the mesial root of mandibular molars to discuss transportation of different NiTi instruments. DAP was estimated between each triangle in the paired surface models and was displayed as a colour mapping of the surfaces (Fig. 1). The use of colour mapping is a powerful quantitative tool that provides a 3D overview of the spatial distribution of DAP. Colour mapping is also useful for displaying spatial distribution of, e.g., precision of segmentation, instrumentation errors and debris accumulation. To compare the two techniques, the volumes of the root canals were assessed for similarity before instrumentation (Table 2) as carried out by others (Peters *et al.* 2000, 2001, 2003, Paqué *et al.* 2009a). The findings related to the categorized root canal groups were of major interest, as no difference was found between the two preparation techniques (Table 3). All the root canals were categorized into groups as either *small* separate root canals, *large* separate root canals or *complex* nonseparated root canals. Each group consisted of a specific root canal with comparable anatomy. There were differences between the groups in terms of volume increase, unprepared area and DAP, reflecting a higher degree of instrumentation in the *small* separated root canals. In the apical region, there was a significant difference between the *small* and *large* separated root canals for all variables, indicating the greatest effect in the *small* root canals. In this study, the focus was on investigating the entire root canal system of each tooth. In previous studies, the focus has most often been on standardized root canals with simple morphology divided from the entire root canal system (Bergmans *et al.* 2002, 2003), or whereas complex root canals have been avoided (Peters *et al.* 2000, 2001, 2003, Paqué *et al.*

Table 4 Mean DAP, unprepared area and volume increase in simple separate root canals versus large separate root canals and complex nonseparate root canals

	Small (n = 14)			Large (n = 14)			Complex (n = 10[a])		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DAP (μm)									
Total canal	115 ± 36^A	67	188	65 ± 30^B	35	124	98 ± 30^A	47	149
Apical third	97 ± 35^A	48	183	54 ± 19^B	24	90	76 ± 29^{AB}	52	126
Middle third	86 ± 37^A	32	170	48 ± 23^B	22	107	70 ± 28^{AB}	34	122
Coronal third	156 ± 69^A	70	331	93 ± 67^B	32	252	148 ± 51^A	54	220
Ranking of DAP total related to anatomy	mb_{low}<ml<db<mb_1			d<p			mb $root_{low}$<mb $root_{up}$		
Unprepared area (%)[b]									
Total canal	30.9^A	9.7	50.7	57.5^B	23.2	73.4	36.4^A	16.3	48.3
Apical third	26.5^A	3.2	69.6	60.9^B	20.6	100	46.4^B	28.8	60.2
Middle third	35.9^A	4.8	69.9	68.9^B	29.9	89.2	43.0^A	23.1	57.6
Coronal third	29.1^A	0.9	59.5	48.4^B	4.6	74.5	24.5^A	3.2	40.2
Ranking of unprepared area total related to anatomy	mb_2<ml<mb_1<mb_{low}<db			p<d			mb $root_{low}$≤mb $root_{up}$		
Volume increase (%)									
Total canal	114.3^A	48.9	254.5	36.0^B	10.8	83.9	141.5^A	63.2	323.9
Apical third	166.0^A	37.2	405.1	38.1^B	3.2	99.5	81.9^A	37.2	217.7
Middle third	88.5^A	21.7	280.5	26.1^B	4.9	107.9	95.5^A	42.6	148.7
Coronal third	125.4^A	27.3	235.1	44.2^B	7.1	115.0	218.4^A	71.4	645.9
Ranking of volume increase total related to anatomy	mb_{low}<db<ml<mb_1			d<p			mb $root_{up}$<mb $root_{low}$		

Two-way ANOVA was conducted with significant level α = 0.05; significant differences are marked with superscript capital letters. AA and BB nonsignificant, AB and BA significant.
[a]Three was excluded from these calculations because of missing negotiation of mb_2.
[b]Small: n = 15 large: n = 15.
d, distal; p, palatal; mb_1, first mesiobuccal root canal maxillary; mb $root_{max}$, mesiobuccal root maxillary; mb $root_{man}$, mesiobuccal root mandibular; m $root_{man}$, mesial root mandibular; ml, mesiolingual; mb_{man}, mesiobuccal root canal mandibular; db, distobuccal; DAP, distance after preparation.

Table 5 Mean unprepared area and relative volume in roots with isthmus (n = 10)

	Mean	Min	Max
Unprepared area (%)			
Total root with isthmus	37.8	16.3	54.2
Isthmus	17.6	5.1	24.0
Total root with isthmus and uninstrumented mb_2	47.2	43.6	49.3
Isthmus and uninstrumented mb_2	25.7	23.7	28.8
Relative volume (%)			
Isthmus	20.8	12.0	33.0
Isthmus and uninstrumented mb_2	49.5	41.0	58.0

2009a). In the group with *complex* nonseparated root canals, the teeth included inter-radicular connections or isthmuses. The hypothesis that the inclusion of isthmus would alter the ratio of instrumented to unprepared areas after molar instrumentation was not confirmed. Besides the apical region, no significant differences were noted between *small* and *complex* root canals (Table 4). The isthmus region represented half the total unprepared area, and the volume of the isthmus region was 20.8% of the volume of the intact root canal system (Table 5). Endal *et al.* (2011) estimated the relative contribution of surface area and volumes of the isthmus regions in mesial roots after instrumentation of mandibular molars and found similar results. When the mb_2 is left uninstrumented together with the isthmus region, it corresponds to half the volume of the mesiobuccal root canal system before instrumentation. Therefore, the isthmus region could maintain the presence of apical periodontitis when it is related to an uninstrumented root canal, and conversely, the implication of isthmus might be reduced when both root canals are otherwise treated optimally. During the segmentation procedure, a minor reduction in the isthmus volume after instrumentation was noticed. This could reflect the accumulation of debris as described recently by others (Paqué *et al.* 2009b, 2011, Endal *et al.* 2011). Therefore, the measurements of isthmus may partly include the contribution of debris accumulation. Root canal instruments could cross the isthmus region and enter the neighbouring root canal (Fig. 1c,d). If these cases had been encountered in a real clinical situation, it might have been concluded that they were a result of a Vertucci Type II configuration, whilst in fact being a Type IV (Vertucci 2005). Under clinical circumstances, if such a large amount of unprepared area remained, it will be difficult to prevent or eliminate infection. A method to estimate the amount of hard tissue removal (DAP) was demonstrated. The colour mapping indicated the spatial location of successful removal of potentially infected dentine. The mean apical DAP in the *small* root canals was ~100 μm, which is significantly different from the mean DAP at ~60 μm in the *large* root canals. Studies indicate that microorganisms invade to a distance of 200 μm or more (Chirnside 1961, Love *et al.* 1997). Even though the focus was on instrumentation protocols involving apical enlargement, it is clear that therapeutic DAP – values beyond 200 μm – was not achieved. Moreover, when microorganisms invade the dentinal tubules, they may be protected from chemical exposure to irrigation or intracanal dressings (Ørstavik & Haapasalo 1990). Therefore, the reduction in viable bacteria is a combination of chemical and mechanical removal of infected soft/hard tissue (Byström *et al.* 1985, Shuping *et al.* 2000, Card *et al.* 2002, Walters *et al.* 2002, Kvist *et al.* 2004). Irrespectively of the use of a well-accepted biomechanical instrumentation protocol, the relative importance of the degree of unprepared area as well as microorganisms left beyond the DAP border still remains unclear. However, it should be remembered that classical studies providing clinical outcome data in the range of 90% or more did not particularly focus on these variables (Kerekes & Tronstad 1979, Sjögren *et al.* 1990, de Chevigny *et al.* 2008). The results indicate that *large* root canals are more difficult to instrument than *small* and even *complex* systems. A recent study emphasized the difficult instrumentation aspects in large root canals, such as the distal root of the mandibular molar (Paqué *et al.* 2010).

Conclusion

There was no significant difference between the two instrumentations techniques with respect to DAP, unprepared area or volume increase, although the instrument designs differed markedly. The inclusion and evaluation of the isthmus did not significantly alter the ratio of instrumented to unprepared areas at total root canal level. Distal and palatal root canals had the highest level of unprepared areas. Future micro-CT and clinical studies are needed to investigate various biomechanical instrumentation protocols and their actual impact on the biofilm harbouring the necrotic and infected root canal.

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