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Evaluation in the danger zone of mandibular molars after root canal preparation using novel CBCT software

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Abstract: This study measured the thickness of cementum/dentin in the danger zone of the mandibular molars after root canal preparation using novel cone-beam computed tomography (CBCT) software. Eighty-four teeth were distributed into four groups: ProTaper Next, BioRace, Reciproc Blue, and WaveOne Gold. E-Vol DX® CBCT software was used to measure initial and final remaining cementum-dentin thicknesses after root canal preparation of the mesial root of mandibular molars at 1 and 3 mm from the furcation. The Kolmogorov-Smirnov test was used to test variable symmetry. The variables were described as mean and standard deviations, compared among the groups using analysis of variance (ANOVA), and within the groups using the Student *t* test. A generalized estimating equation model was used to compare the variation before and after root canal preparation. The level of significance was set at 5%. Differences between mean initial and final thicknesses of the mesiobuccal (MB) and mesiolingual (ML) canals were not statistically significant. The mean initial thickness was 3 mm (0.900 mm ± 0.191), considering that a mean lower than 1 mm (1.035 mm ± 0.184) indicates the danger zone. Although cementum/dentin is thinner at 3 mm from the furcation (0.715±0.186) after root canal preparation, the greatest amount of dentin removed was found at 1 mm (0.734 ± 0.191). The cementum-dentin remaining after preparation was thicker than 0.715 mm in root canals prepared using #35 (WaveOne Gold®) and #40 (ProTaper Next®, BioRace® and Reciproc Blue®) instruments. This confirms the safety of canal preparation in the danger zone using these systems.

Keywords: Root Canal Preparation; Cone-Beam Computed Tomography; Root Canal Therapy.

Introduction

Root canal preparation is indubitably a valuable stage of cleaning and shaping. However, endodontic treatment failure at this stage may be associated with operative errors that could result in ledges, perforation, canal decentralization, and apical transportation in curved canals.[1,2]

Nickel-titanium (NiTi) endodontic instruments are used in continuous rotation or reciprocating motion, and are manufactured with a special metal alloy and file designs that ensure the root canal will be preserved during preparation. Several NiTi instruments have been developed and tested. One recent innovation introduced heat treatment (M-Wire) in the current manufacture of NiTi alloy instruments, together with changes in file microstructure and design. In addition, instruments now have more rhomboid sections, different shapes along their active portion, helix angles and variable tapers. Heat treatment is a process that changes the molecular structure of the alloy to make the system files more flexible and resistant to cyclic fatigue and torsional fractures, and reduce their shape memory,[2-4] particularly WaveOne Gold® and Reciproc Blue® instruments.[5-7] ProTaper Next instruments are manufactured with M-Wire metal alloy, and have an off-centered rectangular cross-section and regressive taper.[3,8] BioRace nickel-titanium rotary instruments are manufactured using electropolishing as a surface treatment for conventional austenitic NiTi, and have a triangular cross-section with alternate cutting blades.[9]

Stainless steel or NiTi endodontic instruments used during canal preparation may change the original path, hence alter tooth morphology.[1] Root perforations are usually caused by excessive instrumentation of a dentin wall, which may seriously compromise the results of root canal treatments. Regions where the dentin is thinner may become more fragile following canal preparation. The distal wall of the mesial root of mandibular molars has an anatomic region termed the danger zone.[10-14]

Several methods have been used to evaluate the amount of dentin remaining in the danger zone of mandibular molars after instrumentation using different techniques.[10-24] Cone-beam computed tomography (CBCT) has been used as a method of investigation in different areas of dentistry, particularly endodontics.[25] Its use is fundamental to preserving the samples in laboratory studies, and providing knowledge about patient age and gender in clinical studies.[25,26]

E-Vol DX® (CDT-Brazil) CBCT software has been recently developed as an important tool to improve CBCT image quality, and provide different filters for specific uses in clinical applications and research. One recently developed filter is used to measure cementum-dentin structures in micrometers.[27] Few studies have evaluated the safety of new endodontic files in removing dentin from the danger zone, using different CBCT software programs to measure these areas. The present study measured the thickness of the remaining cementum-dentin on the distal surface of mesial roots of mandibular molars after root canal preparation with NiTi instruments in continuous rotation and reciprocating motion, using this novel CBCT software.

Methodology

Sample selection

This study included 210 human first and second mandibular molars extracted for different reasons from adult patients seen at the School of Dentistry of the Federal University of Goiás, Brazil. The current laboratory investigation was submitted to the Research Ethics Committee of the Universidade Federal de Goías, Brazil, and its Certificate of Submission for Ethics Review was approved under number 06486919.0.0000.5083. The teeth were stored in 0.2% thymol (Fitofarma, 20442, Goiânia, Brazil).

The inclusion criteria for the mandibular molars, according to CBCT scans, were: intact root structure; complete root formation; no history of orthodontic or endodontic treatment; slightly (r > 8 mm) or moderately (r > 4 mm and r < 8 mm) curved root canals; and canals at least 20 mm long. The exclusion criteria were: obturated or obliterated root canals; calcifications; internal or external root resorptions; root fractures; and incomplete root formation.

CBCT scans of the selected teeth were used to define inclusion and exclusion, as well as internal tooth morphology. Eighty-four teeth were included in the study.

CBCT image acquisition and measurements

The teeth were fixed to a double layer of utility wax (Lysanda, São Paulo, Brazil) measuring 7 cm in diameter, and their buccal surfaces was positioned toward the same side. Initial CBCT images were acquired using a high-resolution 13-bit PreXion 3D Elite scanner (PreXion Inc., San Mateo, USA). The scanner parameters were as follows: isotropic voxel size of 0.146 mm, FOV 81 mm high x 56 mm diameter, 37-s exposure, 512 exposures per capture, X-ray output of 90 kVp, 16 bits, 4 mA current, focal spot of 0.20 mm x 0.20 mm, and total beam filtration of > 2.5 mm eq. Al.

The DICOM files were analyzed using e-Vol DX software (CDT Software, Bauru, Brazil) installed in a desktop computer equipped with Windows 10 (Microsoft Corporation, Redmond, WA, USA), an i7 - 8750 processor, 4.1 GHz (Intel Corporation, Santa Clara, CA, USA), an 8 GB NVDIA GTX 1070 graphics card (NVIDIA Corporation, Santa Clara, USA), and a PreXion3D Image Analysis System (TeraRecon, Foster City, USA) running on the same desktop computer.

Root canal preparation

The samples were secured in a repeatable position with a bench vise, and all the procedures were conducted by the same operator, a specialist in endodontics with 15 years of clinical experience. The crowns were flared using a high-speed handpiece, a #1014 round diamond bur (FG Dentsply/Maillefer, Ballaigues, Switzerland), and an Endo-Z bur (FG Dentsply/Maillefer), under refrigeration. Afterwards, the mesial root canals were explored using stainless steel #10 and #15 K-files (Dentsply/Maillefer, Ballaigues, Switzerland). The coronal third was prepared using the instruments of the corresponding system, according to group distribution. The working length (WL) was established at 1 mm short of the apical foramen, determined using a #15 K-file (Dentsply/Maillefer, Ballaigues, Switzerland).

The mesial roots of mandibular molars were prepared using a NiTi system in continuous rotation or reciprocating motion. The instruments were replaced after preparing three teeth each.

ProTaper Next Group (n = 21)

The teeth in the ProTaper Next group were prepared using instruments from the ProTaper Next® (Dentsply/Maillefer, Ballaigues, Switzerland) system, according to the manufacturer's instructions, at 300 rpm and 2.5 N/cm^2 torque. The sequence used was X1 (#17.04), X2 (#25.06), X3 (#30.07), and X4 (#40.06) variable tapers.

Root canals were irrigated with 2.5% sodium hypochlorite (Fitofarma, Lt. 20442, Goiânia, Brazil), applied with an Ultradent 5mL tip and a NaviTip irrigation tip (Ultradent, South Jordan, USA), 0.30 mm in diameter, placed 2 mm short of the WL. Each canal was irrigated with 30 mL of irrigant at each instrument change during root canal preparation. Canal preparation was complete when the last instrument reached the WL in free rotation. Patency was checked with a #15 K-file (Dentsply/Maillefer, Switzerland). Next, the root canals were dried with paper points and irrigated with 5 mL of 17% EDTA (Biodinâmica Química e Farmacêutica, Ibiporã, Brazil), which was left in the canal for 3 minutes to remove the smear layer. A last irrigation was performed with 5 mL of distilled water, after which the canals were dried again with absorbent paper points.

BioRace Group (n = 21)

The root canals in the BioRace group were prepared using BioRace® (FKG Dentaire, La Chaux-de-Fonds, Switzerland) instruments, according to the manufacturer's instructions, at 600 rpm and 1.5 N torque, and an X-Smart Plus (Dentsply Maillefer, Ballaigues, Switzerland) motor. The coronal and middle thirds were prepared using BR0 (#25.08) and BR1 (#15.05) instruments. The apical third was prepared according to the following sequence: BR2 (#25/0.04), BR3 (#25.06), BR4 (#35.04) and BR5 (#40.04), and irrigation followed the same procedure as that described above.

Reciproc Blue Group (n = 21)

In the Reciproc Blue group, the R25 file (#25/variable taper), followed by the R40 file (#40/variable taper) from the Reciproc Blue® (VDW, Munich, Germany) system were used with an X-Smart Plus (Dentsply Maillefer, Ballaigues, Switzerland) motor, according to the manufacturer's instructions for setting speed and torque, in the Reciproc ALL mode. Slight apical pressure was applied during instrumentation. After short consecutive movements of penetration and removal, the file was removed from the canal and cleaned using a piece of sterile gauze. These procedures were repeated until the file reached the original WL, after which irrigation was performed following the same procedure as that described above.

WaveOne Gold Group (n = 21)

Preparation in the WaveOne Gold group used the Small (#20/variable taper), Primary (#25/ variable taper) and the Medium (#35/ variable taper) files of the WaveOne Gold® (Dentsply/Maillefer, Ballaigues, Switzerland) system, according to the manufacturer's instructions for adjusting speed and torque, and the WaveOne Gold® mode. Slight apical pressure was applied during sample preparation. After short consecutive movements of penetration and removal, the instrument was removed from the canal and cleaned using a piece of sterile gauze. These procedures were repeated until the file reached the original WL, after which irrigation was performed following the same procedure as that described above.

Measurement of cementum-dentin thickness

The cementum-dentin thickness on the distal wall of the mesial root of the mandibular molars was measured before (T1) and after (T2) root canal preparation using CBCT images. Root thirds were assessed at 1 and 3 mm below furcation. Their thickness was measured by first separating each sample from the others using the crop tool, and then aligning the samples with the three anatomical planes – axial, coronal and sagittal. The mesial canals were aligned axially, and the sagittal and coronal planes were used to keep the long axis of the sample parallel to the ground, to correct the parallax error.

The diameter of the cementum-dentin thickness on the CBCT images was measured using a specific filter from the novel e-Vol DX CBCT software.[25] The method used to perform the measurements has recently been described by Bueno et al.[27]. First, the correct positions to be measured were established by defining a point on the edge of the anatomical structure, and then adjusting the intermediate position on the grayscale of the CBCT image. Thin, 0.10-mm slices were obtained from the 3D slices reconstructed using the measurement filter, and the edge of the anatomic surface was defined on the axial plane. Positions in 3D mode were replicated in multiplanar CBCT image reconstruction, and the correct position was defined using a positioning guide. Three-dimensional density was adjusted so that it would have the same dimension as the 2D image, and the dimensions were then calibrated until the 3D and 2D modes coincided. Afterwards, the intermediate position was checked using the grayscale on the CBCT scans. After one side was completed, the guide was moved to the other side, and the same guidelines were followed. The position of the marking was defined on the other edge using the 2D mode as a reference. Next, the measurements were taken on the two edges of the root canal.

This method was used to obtain the linear measurement of the cementum-dentin thickness on the distal walls of the mesial root canals of the mandibular molars, at T1 and T2, in the four groups (ProTaper Next, BioRace, Reciproc Blue, and WaveOne Gold) at 1 and 3 mm from the furcation. Measurements were recorded using an Excel spreadsheet, and later exported to SPSS 20.0 for statistical analysis.

All imaging studies were evaluated by two observers, a radiologist and an endodontist, each with 15 years of experience, and previously calibrated using 10% of the sample. When differences occurred in their evaluations, the image was examined by a third observer, for the purpose of arriving at an ultimate consensus.

Statistical analysis

The Kolmogorov-Smirnov test was used to test variable symmetry. Variables were described as means and standard deviations, and were compared among the groups using analysis of variance (ANOVA), and within the groups using the Student *t* test for paired samples. A generalized estimating equation model was used to compare the variation before and after preparation in the different groups. The level of significance was set at 5% (SPSS software version 20, Chicago, USA).

Results

Tables 1 and 2 summarize the results. Table 1 shows the mean cementum-dentin thickness of the root canal at T1 in each group, third and root. There were no statistically significant differences among the groups in regard to mean thickness of the mesiobuccal (MB) and mesiolingual (ML) canals at 1 mm ($p = 0.135$ and $p = 0.167$) and 3 mm ($p = 0.304$ and $p = 0.618$) from the furcation.

Table 2 shows the mean thicknesses at T2 in each group, third and root. There were no statistically significant differences among the groups in regard to mean thickness of the MB and ML canals at 1 mm ($p = 0.268$ and $p = 0.179$) and 3 mm ($p = 0.601$ and $p = 0.600$) from the furcation.

Figures 1 and 2 show values of cementum-dentin thickness of mesial roots at 1 and 3 mm from the furcation at T1 and T2, according to the different instrument systems under analysis. Figures 3 and 4 use CBCT scans and 3D reconstructions to illustrate the methodology and filter of e-Vol DX software used in the study. An alternative to checking the border references to obtain the measurements was created using a color map, which better characterizes the dental structures according to their density (Figure 4C).

Discussion

The mean cementum-dentin thickness was greater than 0.670 mm at 1 and 3 mm from the furcation after preparation of the mesial roots of the mandibular molars using ProTaper Next®, BioRace®, Reciproc Blue®, and WaveOne Gold® NiTi instruments #35 to #40. The last instrument had a taper ranging between 0.04 and 0.06. The mean cementum-dentin thickness in the danger zone of the mandibular molars, on the distal wall of the mesial root, ranged from 0.953 to 1.099 mm at 1 mm from furcation, and 0.840 to 0.975 mm at 3 mm. At T2, the thickness of the remaining cementum-dentin ranged from 0.670 to 0.816 mm at 1 mm from the furcation, and 0.680 to 0.757 mm at 3 mm (Table 2). A comparison among the canal preparation systems and evaluation of the different thirds showed no influence of these variables on the root canals (Figures 1 and 2). These results indicate that the measurement filter available on e-Vol DX CBCT software has the potential to measure anatomic structures in micrometers.

The selection of an endodontic instrument or a treatment protocol, more especially, should take into account the complex anatomy of each tooth and its pathological conditions.[1] The mesial root of mandibular molars has a thin dentin layer on the distal wall close to the furcation, and its

Table 1. Mean and standard deviations of cementum-dentin thickness (mm) in each group at 1 and 3 mm from the furcation in MB and ML canals at T1.

Variable	ProTaper Next	BioRace	Reciproc Blue	WaveOne Gold	p-value
	X ± sd	X ± sd	X ± sd	X ± sd	
1 mm					
MB	1.062 ± 0.174	0.972 ± 0.131	1.099 ± 0.194	1.025 ± 0.211	0.135
ML	1.071 ± 0.193	0.953 ± 0.152	1.045 ± 0.160	1.052 ± 0.220	0.167
3 mm					
MB	0.880 ± 0.160	0.883 ± 0.120	0.975 ± 0.177	0.907 ± 0.251	0.304
ML	0.916 ± 0.224	0.840 ± 0.148	0.900 ± 0.178	0.899 ± 0.234	0.618

sd: standard deviation; p value according to ANOVA; MB – mesiobuccal canal. ML: mesiolingual canal.

Table 2. Mean and standard deviations of cementum-dentin thickness (mm) in each group at 1 and 3 mm from the furcation in MB and ML canals at T2.

Variable	ProTaper Next	BioRace	Reciproc Blue	WaveOne Gold	p-value
	X ± sd	X ± sd	X ± sd	X ± sd	
1 mm					
MB	0.748 ± 0.162	0.670 ± 0.148	0.781 ± 0.231	0.711 ± 0.204	0.268
ML	0.816 ± 0.187	0.690 ± 0.200	0.724 ± 0.191	0.730 ± 0.182	0.179
3 mm					
MB	0.682 ± 0.192	0.715 ± 0.116	0.745 ± 0.169	0.745 ± 0.203	0.601
ML	0.704 ± 0.219	0.688 ± 0.161	0.680 ± 0.210	0.757 ± 0.210	0.600

sd: standard deviation; p value according to ANOVA; MB – mesiobuccal canal. ML: mesiolingual canal.

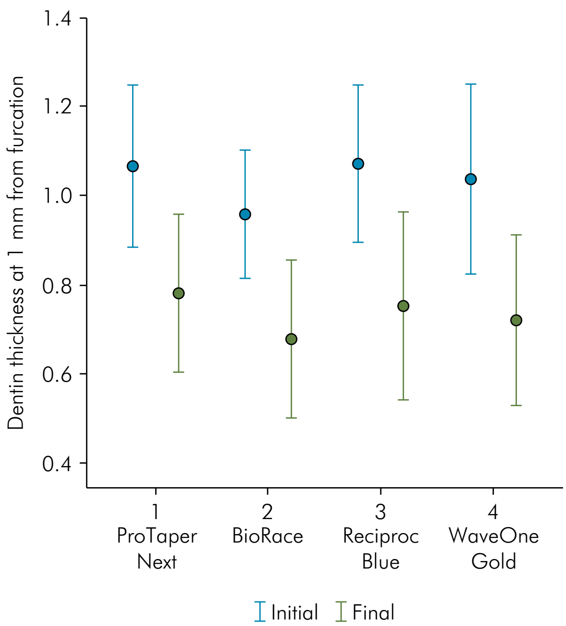

Figure 1. Dentin thickness (mm) before and after preparation of mesial roots at 1 mm from furcation, according to the file systems under analysis.

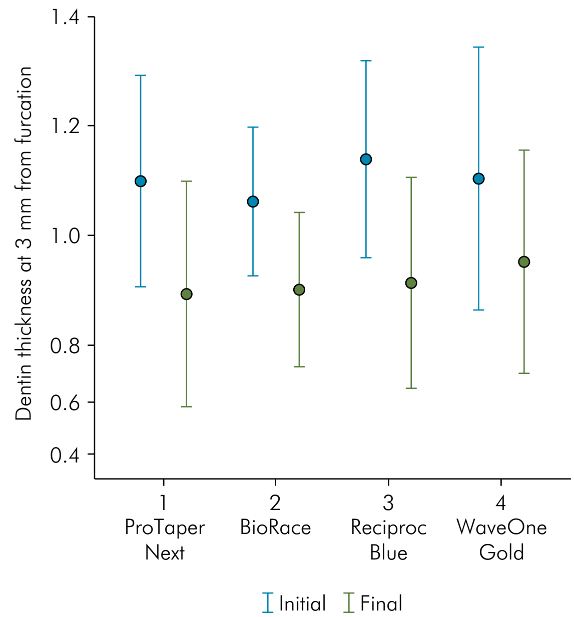

Figure 2. Dentin thickness (mm) before and after preparation of mesial roots at 3 mm from furcation, according to the file systems under analysis.

characteristics are difficult to define on a periapical radiograph.[10,13] Abou-Rass et al.[10] recommended using an anti-curvature filing to prepare a curved root canal. Our study used a controlled method of preparing the areas of thick dentin, and those classified as safe zones. A controlled preparation can minimize the amount of dentin removed from areas where this layer is thin, called danger zones. These zones are vulnerable to root perforations, and may be weakened by preparation.

Other studies[10,11,13-19,21,23,28] have evaluated thicknesses at 1 and 5 mm from furcation on the distal wall of the mesial root of mandibular molars. Pinto et al.[21] measured the dentin thickness in the danger zone of mesial roots of mandibular molars using CBCT images. The thinnest dentin in the safe zone was found at 4 mm from the canal opening, at a mean of 1.03 mm; in contrast, the thinnest point was at 3 mm below the canal orifice, at a mean of 0.81 mm. The mean distance from the pulp chamber to the furcation was 2.23 mm. The mean thickness in the danger zone of the mesial roots of the mandibular molars was < 1.0 mm. In addition to these important anatomic details, it should be pointed out that mandibular first molars often have to be treated endodontically.[29]

This study chose positions of 1 and 3 mm from the furcation as sites for analysis, because these are areas where the thin walls of the mesial root of mandibular molars are more vulnerable.[13,14] In both MB and ML canals, the safe thickness values for preparations using various instrument systems, such as ProTaper Next®, BioRace®, Reciproc Blue® and WaveOne Gold®, were not significantly different. Zhou et al.[24] measured the thinnest distal dentin in the danger zone of the mesial roots of mandibular first molars in a Chinese population using CBCT images. In MB and ML canals, it was at about 3–4 mm from the furcation. There were no differences between MB and ML canals, but the thinnest dentin values of MB and ML canals were thicker in men than in women, except at 1 and 3 mm of ML canals. The distal dentin in MB and ML canals became thicker with age in both men and women, at each site. The thinnest distal dentin values at all sites were significantly different between long and short teeth in both men and women, and the short teeth had the lowest mean values. The thinnest distal dentin values of the mesial roots of the mandibular first molars were closely correlated with root length and patient age and sex.

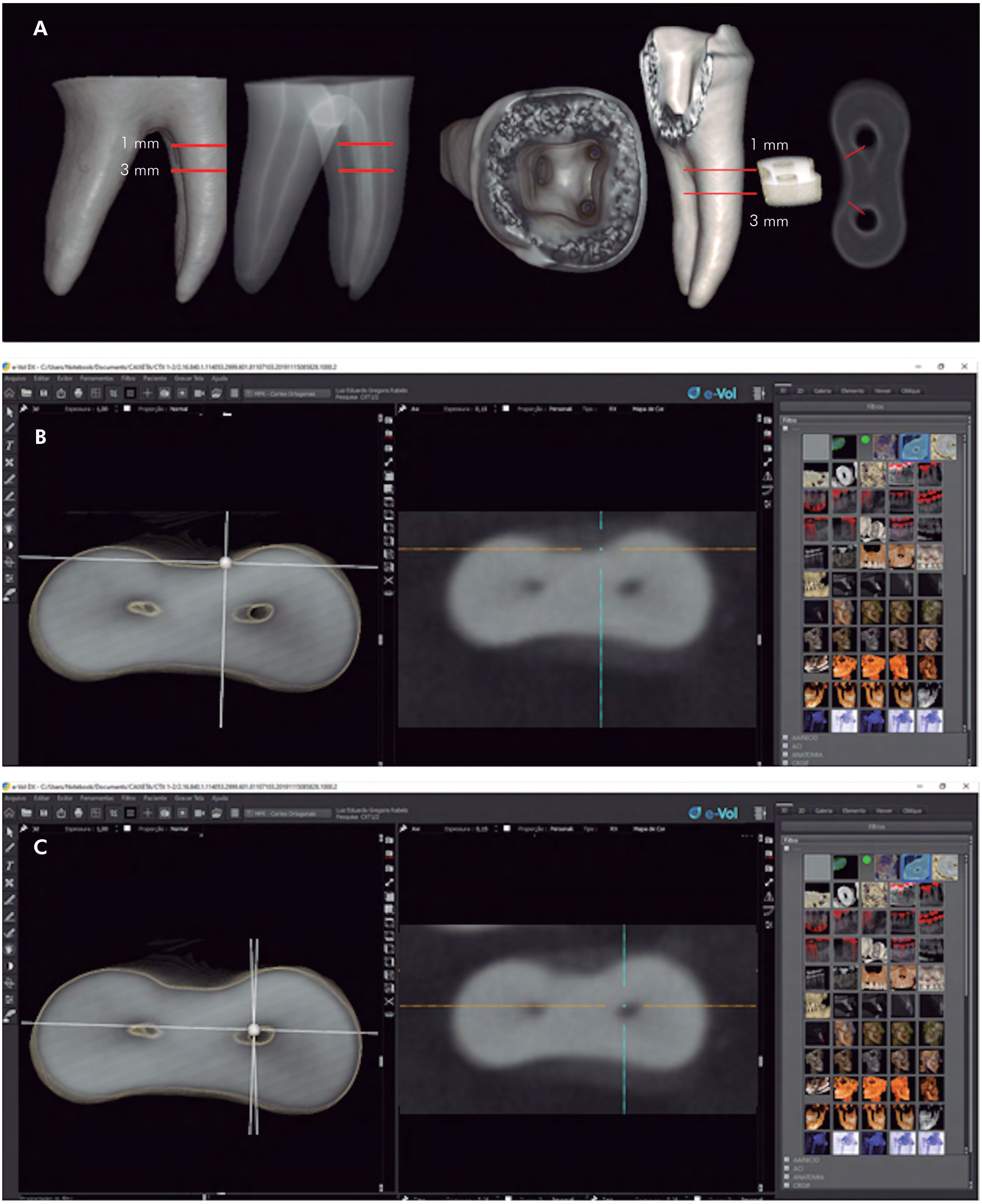

Figure 3. (A) Illustration with CBCT scans and 3D reconstructions of areas measured at 1 and 3 mm below the furcation. (B, C) The diameter of the cementum-dentin thickness areas on the CBCT images was measured using a specific filter of the novel e-Vol DX software. To measure the areas of the danger zone on the CBCT scans, the measurement filter of the software allows synchronization between multiplanar CBCT image and 3D mode, with precise delimitation of the edges to be measured.

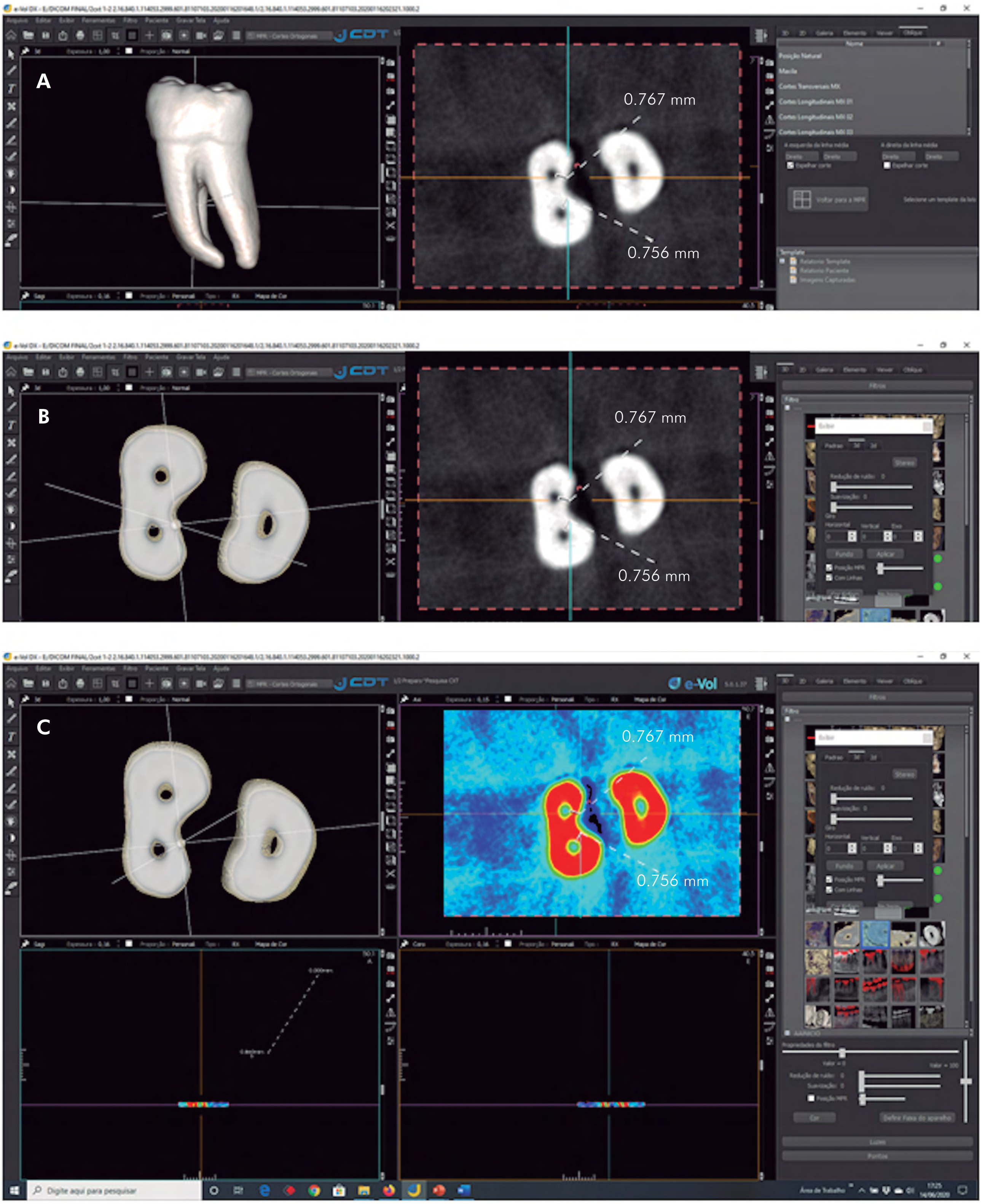

Figure 4. (A) Illustration showing 3D mode adjusted so that it would have the same dimension as the 2D image. (B) The dimensions were calibrated until 3D and 2D modes coincided. After one side was completed, the guide was moved to the other side, and the same guidelines were followed. The position of the marking was defined on the other edge using the 2D mode as a reference. (C) A color map was used to check the densities of the cementum-dentin structure.

Several studies[10,11,13-24,28] compared the dentin removal in the danger zone of mandibular molars. They used different instrumentation techniques and evaluation methods, such as histometry, periapical radiographs, micro-CT and CBCT. Studies that measured dentin thickness in CBCT images[15-17,20] used different sizes of Gates-Glidden cervical preflaring files to undertake the measurements. Our results confirm the importance of taking care to avoid excessive instrumentation, in order to reduce possible lateral perforations in thin areas.

The present study used a specific filter from e-Vol DX® CBCT software to make micrometric measurements, as recently described by Bueno et al.[27] This method essentially and adequately defines the position of the markings on the edges of the images using clear positioning guides. In addition to replicating thin, 0.10-mm slices, positions in 3D mode should also be replicated in multiplanar reconstruction, in order to ensure accurate definition of these positions, according to the positioning guides. The calibration of dimensions should be adjusted to a point that coincides in 3D and 2D modes, and the 2D mode should then be used as a reference.

Lack of standardization complicated the comparison of our findings with those published in previous studies that used CBCT to measure dentin thickness in the danger zone of mandibular molars.[15-17,19,20] Some of the discrepancies may be explained by the size of the files used for canal preparation, the exact distance of the sites analyzed from the furcation, the methods used to undertake the measurements, and the units of reference. The method used in the present study was based on a standard model for the correct use of e-Vol DX CBCT software,[25,27] which was operated by a radiologist previously trained to work with this software. Several steps were taken to ensure the correct acquisition of images using a high-resolution CBCT scanner and e-Vol software for image analysis. Important factors were noise correction, parallax effect correction, navigation sequence, interpretation, and mastery of the measurement technique. This software produces high-resolution images enabled by several features, including sub-millimetric voxel size; multiplanar dynamic image navigation; tools to change volume parameters, such as slice thickness and slice intervals; imaging filters for data correction; brightness and contrast manipulation; and 196-dpi capture screen resolution, with a 384-dpi option, in contrast to the 96-dpi resolution of similar applications.[25] Estrela et al.[30] compared the sizes of intraradicular posts on CBCT, e-Vol DX software and digital micrometer images. The diameters measured using the software and the micrometer alternatives were not statistically different from each other. Their results confirmed the reliability and potential of the blooming artifact reduction (BAR) filter of the software, which may be used in clinical studies without risking loss of any samples, unlike the losses incurred with comparable methodologies.

The canals in the current study were prepared to #35 and #40 instruments, which correspond to an internal root volume of at least 350 to 400 µm, in addition to the 0.04 to 0.06 taper of these files. In this case, instrumentation safely removed the dentin in the danger zone, regardless of the instrument size, the use of continuous rotation or reciprocating motion, the heat treatment or the taper. This is because the thickness of the thin wall was greater than 0.670 µm, considered a safe thickness. Shantiaee et al.[19] evaluated the changes in the danger zone at 2 and 4 mm from the furcation after the preparation of curved canals with WaveOne® instruments under continuous counterclockwise rotation or reciprocating motion, using CBCT images. The efficacy of WaveOne® instruments, in regard to the dentin thickness in the danger zone, was not affected by the different types of instrument motion. The results of the present study corroborate theirs, since we also found no differences in the type of instrument motion used by the different instrument systems, whether continuous rotation (ProTaper Next® and BioRace®) or reciprocating motion (Reciproc Blue® and WaveOne Gold®). Elashiry et al.[31] evaluated the shaping ability of WaveOne Gold®, Reciproc Blue®, HyFlex EDM® and One Shape® instrument systems in MB and ML canals of mandibular molars, using micro-CT. They found no significant differences in the centering ability or transportation of the four systems used for measuring the middle and coronal thirds. WaveOne Gold® had better centering ability and the smallest amount of transportation in the apical third of the root canals. The HyFlex EDM® system produced the greatest volume change in the coronal third of the canal. The performance of endodontic instruments during root canal instrumentation depends on their cross-section, taper and surface heat treatment.

In the present study, the lower mean value of initial cementum-dentin thickness at 3 mm (0.900 mm ± 0.191) versus 1 mm (1.035 mm ± 0.184) suggests that the latter is the danger zone. Although initial cementum-dentin was thinner at 3 mm from the furcation after preparation of root canals, the highest mean cementum-dentin removal was found at 1 mm, where the mean cementum-dentin thickness was 0.734 ± 0.191 mm, and at 3 mm, where it was 0.715 ± 0.186 mm. This may be attributed to the greater taper of instruments closer to the shaft, and hence in greater contact with the region closest to the canal orifice (1 mm from the furcation). The root canals were prepared using instruments up to #35 (WaveOne Gold®) and #40 (ProTaper Next®, BioRace® and Reciproc Blue®). Santana-Júnior et al.[18] studied the effects of apical preparation in the danger zone of mesial canals of mandibular molars using the Mtwo® and Reciproc® instrument systems up to an apical diameter of 0.25 and 0.40 mm. The use of Reciproc® instruments resulted in a canal preparation similar to that of Mtwo® instruments when used in the mesial roots of mandibular molars with two separate curved canals. Preparation with a 0.40-mm file significantly increased the root canal volume in the apical third without significantly reducing dentin thickness in the danger zone, in both file systems. Lim and Stock[12] found that the thinnest area in the danger zone after preparation should be no less than 0.3 mm, so as to afford resistance to forces during the obturation of the root canal. Therefore, the mesial canals of mandibular molars in their study had sufficient resistance to root canal obturation after preparation, considering that their mean values were greater than 0.6 mm. However, Raiden et al.[32] and Junqueira et al.[33] found that there should be at least 1 mm of root thickness around the intraradicular posts to resist vertical fracture. This suggests that the mesial canals of mandibular first molars are not sufficiently resistant to vertical fractures when intraradicular posts are used.

Basic understanding of the thickness of the dentin remaining after the preparation of the molar root of the mandibular molars in the danger zone should reduce root perforations. Evaluation methods have changed along the years, in line with new anatomic studies conducted on the danger zone, and with the alternative use of several different instrument systems and preparation techniques. With the advent CBCT incorporated into the field of endodontics, and the development and application of new software,[25-27,30,34,35] the initial cementum-dentin thickness in the danger zone can be measured, and the findings may be used to improve planning, and the decision of the most appropriate size and taper of instruments to be used in specific clinical conditions. The *in vitro* method developed in the study may provide information not only on the loss of dentin structure in the thin walls of human teeth, but also on future *in vivo* applications. CBCT is a method that can be used to preserve samples and provide knowledge on patient age and sex in clinical studies.

Conclusion

The cementum-dentin thickness remaining in the distal aspect of the mesial roots of mandibular molars was greater than 0.715 mm in root canals prepared using #35 (WaveOne Gold®) and #40 (ProTaper Next®, BioRace® and Reciproc Blue®) instruments, hence ensuring the safety of canal preparation in the danger zone.

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