3-dimensional Analysis of Regenerative Endodontic Treatment Outcome

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Abstract

Introduction: A growing body of evidence supports the regeneration potential of dental tissues after regenerative endodontic treatment (RET). Nevertheless, a standard method for the evaluation of RET outcome is lacking. The aim of this study was to develop a standardized quantitative method for RET outcome analysis based on cone-beam computed tomographic (CBCT) volumetric measurements. **Methods:** Five human teeth embedded in mandibular bone samples were scanned using both an Accutomo 170 CBCT machine (Morita, Kyoto, Japan) and a SkyScan 1174 micro-computed tomographic (μ CT) system (SkyScan, Antwerp, Belgium). For subsequent clinical application, clinical data and low-dose CBCT scans (preoperatively and follow-up) from 5 immature permanent teeth treated with RET were retrieved. *In vitro* and clinical 3-dimensional image data sets were imported into a dedicated software tool. Two segmentation steps were applied to extract the teeth of interest from the surrounding tissue (livewire) and to separate tooth hard tissue and root canal space (level set methods). *In vitro* and clinical volumetric measurements were assessed separately for differences using Wilcoxon matched pairs test. Pearson correlation analysis and Bland-Altman plots were used to evaluate the relation and agreement between the segmented CBCT and μ CT volumes. **Results:** The results showed no statistical differences and strong agreement between CBCT and μ CT volumetric measurements. Volumetric comparison of the root hard tissue showed significant hard tissue formation. (The mean volume of newly formed hard tissue was $27.9 [\pm 10.5] \text{ mm}^3$ [$P < .05$]). **Conclusions:** Analysis of 3-dimensional data for teeth treated with RET offers valuable insights into the treatment outcome and patterns of hard tissue formation. (*J Endod* 2015; ■:1–8)

Key Words

Cone-beam computed tomographic imaging, MeVislab, regenerative endodontic treatment, teeth segmentation

Humans, unlike species such as salamander or newt, lack the ability to naturally regenerate their own tissues. To overcome this limitation, tissue engineering strategies using combinations of biocompatible scaffolds, growth factors, and stem cells to mimic natural morphogenesis are currently in development (1). Among those strategies are regenerative endodontic procedures, which can be defined as biologically based processes designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex (2).

The potential for dental tissue regeneration and continued root development is well documented in studies of replanted and autotransplanted teeth (3–5). There is a growing body of evidence from case reports to suggest that regenerative endodontics may in fact be possible in teeth with pulpal necrosis, apical pathosis, and immature apices (6–9). This treatment procedure has acquired many names such as pulp revitalization, root canal revascularization, and regenerative endodontic treatment (RET) (2, 9–11). The outcomes of these case reports have been evaluated clinically and radiographically in 2 dimensions. The most frequent pretreatment diagnosis of these cases was of pulpal necrosis with or without apical periodontitis. Postoperative recalls showed regression of clinical symptoms in addition to radiographic evidence of hard tissue formation and apical closure (6–10, 12). However, the application of nonstandardized 2-dimensional (2D) radiographs to evaluate an increase in root length and dentin thickness should be interpreted with caution because a slight change in the angulation at the preoperative or recall appointment might produce inconsistent images and inaccurate interpretations (12).

Taking into consideration the limitations of 2D imaging, 3-dimensional (3D) imaging using cone-beam computed tomographic (CBCT) imaging has proven itself as a valuable tool in dentistry (13–15) as long as radiation doses are controlled at reasonably low levels (16). Moreover, volumetric measurements from CBCT data have been reported and validated in several studies *in vitro* and *in vivo* (13–15, 17–20). The overall aim of this study was to develop a standardized quantitative method for the evaluation and analysis of the outcome of RET based on CBCT volumetric measurements.

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Materials and Methods

In Vitro Validation (CBCT Imaging Versus Micro-computed Tomographic Imaging)

Image Acquisition and Export. Five human teeth in bone samples from the left and right mandible (1 incisor, 2 canines, 1 single-rooted premolar, and 1 molar) were scanned using a high-resolution CBCT machine (80 μm) (3D Accuitomo 170; Morita, Kyoto, Japan). Each sample was placed in a sponge block to prevent any movement during the scanning process. Scanning parameters were fixed at 360° rotation, 30.8-second exposure time, 80- μm voxel size, high resolution, and a field of view of 60 $\times$ 60 mm. Images were acquired under the exposure condition of 90.0 kV (x-ray tube voltage) and 5.0 mA (x-ray tube electric current). All data sets were exported using the Digital Imaging and Communications in Medicine (DICOM) file format with an isotropic voxel size of 80 μm , a slice interval and thickness of 80 μm and 160 μm , and a slice interval and thickness of 160 μm .

The samples were subsequently scanned with the SkyScan 1174 micro-computed tomographic (μCT) system (SkyScan, Antwerp, Belgium). The μCT parameters were 30- μm voxel size, 50 kVp, 800 mA, 1-mm aluminum filter, angular rotation step of 0.8°, 360° scanning, 450 projections, and an exposure time of 5 seconds with a total scan duration of 1 hour 31.6 minutes. All data sets were imported using the DICOM file format with a voxel size of 30 μm .

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Study Sample. This case series included 5 immature permanent teeth with infected necrotic pulps and apical periodontitis/abscess from 4 patients who attended the Pediatric Dental Clinic of the University Hospital of the KU Leuven, Leuven, Belgium. Table 1 presents the demographic details of the subjects, signs and/or symptoms, and pulpal and periapical status of involved teeth. The age of the patients ranged in age from 9.9–14.8 years at the start of treatment. The patients presented with dental trauma (1 maxillary incisor), dens evaginatus (3 maxillary incisors), and pulp necrosis after autotransplantation (1 premolar). Radiographically, all teeth showed evidence of periapical lesions caused by root canal infection; 1 tooth showed early signs of external cervical root resorption (premolar). All teeth had an immature apex, either blunderbuss or in the form of a wide canal with parallel walls and an open apex. After clinical and radiographic examination, a vitality pulp test was performed on the affected tooth and a control (contralateral but unaffected) tooth. The patients and their parents were informed of the treatment plan (for RET) and the potential risks before giving their consent to the treatment.

RET Technique

All teeth were anesthetized using mepivacaine 3% without epinephrine (Scandonest; Septodont, Saint Maur des Fosses, France) to avoid compromising the blood supply and then isolated with a rubber dam. The access cavity was prepared with a #2 high-speed access bur (round-end cutting tapered diamond bur) (Maillefer, Dentsply, Switzerland) and copious water spray. The canals were gently rinsed with sodium hypochlorite (NaOCl) 2.5% solution without instrumenta-

tion. The canal was then dried by aspiration (without air blow) and application of sterile paper points (Henry Schein, Melville, NY). A biantibiotic mixture of ciprofloxacin and metronidazole was placed in the root canal and left for 10–14 days. The access cavity was sealed with resin-modified glass ionomer cement (Fuji VII; GC, Tokyo, Japan). In the next visit, under an endodontic microscope, the root canal was re-entered and irrigated with a copious amount of 2.5% NaOCl to remove the antibiotic dressing material and all debris. The root canal dentin was conditioned using EDTA 17% (21). The canal was then dried with sterile paper points and was confirmed to have no exudate. A size #10 K-file was introduced into the root canal until vital tissue was felt, and this instrument was used to irritate the tissue to create some bleeding into the root canal (Fig. 1A). The bleeding was allowed to reach a level of 3–4 mm below the cemento-enamel junction (Fig. 1B). Then, an absorbable collagen barrier (CollaPlug; Zimmer Dental, Carlsbad, CA) was placed to create a barrier to apply a dense layer of 4 mm white mineral trioxide aggregate (ProRoot MTA; Dentsply Tulsa Dental Products, Tulsa, OK) until the cemento-enamel junction (Fig. 1C–E). The tooth was then temporarily sealed using a moist sterile paper point to provide the water needed for mineral trioxide aggregate to fully set and resin-modified glass ionomer cement (Fuji VII). After 7–14 days, the temporary filling and moist paper point were removed, and the hardness of mineral trioxide aggregate was inspected. The final coronal seal was established using adhesive composite restoration, and the patient was scheduled for a recall appointment (Fig. 1F–J).

Image Acquisition and Export. CBCT images were obtained based on clinical justification by the treating endodontist as part of the treatment protocol (preoperatively and at >12 months follow-up) because of the severity of the lesions and in the framework of the insurance case follow-up. Images were obtained with the Accuitomo 170 using an 80 $\times$ 80 mm field of view. Images were acquired under the exposure condition of 90.0 kV (x-ray tube voltage) and 5.0 mA (x-ray tube electric current). The exposure time was 17.5 seconds. All data sets were exported using the DICOM file format with a voxel size of 160 μm and a slice interval of 160 μm (1 voxel).

Segmentation Strategy. All scanned samples (CBCT and μCT) and full CBCT scans (case series study) were imported into MeVisLab (MeVis Research, Bremen, Germany), a highly developed free framework system that provides an easy-to-learn modular visual programming interface with a comprehensive suite of image processing and visualization tools (<http://www.mevislab.de/>). For the clinical data, the region of interest including the tooth treated with RET (pre- and postoperative) and the contralateral negative control tooth were saved as a new image in DICOM format to reduce computation time. All region of interest images were normalized using an intensity windowing filter and then a median filter to suppress any noise and decrease confounding variables between the images.

Two segmentation steps were applied to extract the teeth of interest from the surrounding tissue (bone) and to separate tooth hard tissue (enamel and dentine) and root canal space. The first step is a dedicated tool developed in MeVisLab that applies interactive livewire boundary extraction to extract the tooth of interest from the surrounding tissue.

TABLE 1. Demographic Details of the Subjects, Signs and/or Symptoms, and Pulpal and Periapical Status of Involved Teeth

Case number	Age	Sex	Tooth number	Signs/symptoms	Pulpal status	Periapical lesion
1	9.9	F	Maxillary right central incisor	Swelling and pain	Necrosis	+
2	8.9	F	Maxillary right central incisor	Swelling and pain	Necrosis	+
3	14.8	F	Mandibular right first premolar	No	Necrosis	+
4	11.8	F	Maxillary right lateral incisor	Swelling and pain	Necrosis	+
5	12.2	F	Maxillary left lateral incisor	Swelling and pain	Necrosis	+

F, female.

Figure 1. RET procedure: (A and B) the induction of bleeding inside the canal, (C and D) the placement of the CollaPlug to act as a barrier for MTA condensation, and (E) MTA placement. (F and G) At the second visit, the hardness of MTA is controlled, and the coronal seal is established using glass ionomer and adhesive composite resin. (H) A preoperative radiograph. (I) A periapical radiograph directly after the second visit. (J) A periapical radiograph 3 months after RET.

Livewire allows for a semi-interactive segmentation of structures with prominent edge image features. Internally, the module generates a graph representation of the image to work on; the graph's nodes represent image pixels, and edges connect neighboring pixels. The edges are weighted on the basis of the cost function (image gradient magnitude). If starting and ending points are defined on such a graph, the shortest path (minimal cost path) is computed using dynamic programming (F^* algorithm) (22, 23). The second step applies semiautomatic user-guided 3D active contour segmentation using level set methods (23, 24), available in the ITK-SNAP tool kit (www.itksnap.org) (24). In the first step, a probability map was computed by applying a 1-sided smooth threshold once for hard tissue and another for the root canal space. In the second step, segmentation was initialized by placing 1 or more spherical seeds in the region of interest (hard tissue or canal). The last step was specifying the weights of the various terms in the active contour evolution partial differential equation and running the evolution interactively (24).

3D Reconstruction and Quantitative Visualization of Hard Tissue Formation

After segmentation, the 3D triangle-based surface of the hard tissue and the root canal was reconstructed without being smoothed to preserve its raw volume measurement. The volumes of the root hard tissue and root canal were calculated starting from the cementoenamel junction up to the apex pre- and postoperatively. Geometry quantification was performed in MeVisLab using the framework described by Gao et al (25). The root canal diameter (thickness) was measured and color

coded, and then the root hard tissue thickness measurement (starting from the cementoenamel junction up to the apex) was expressed as a color-coded map showing the calculated minimal distances from the canal surface to the external root surface (25).

Volumetric Comparison and Statistical Analysis

All data were analyzed with statistical software package STATISTICA 8.0 (StatSoft Inc, Tulsa, OK). In the first step, the accuracy of the volume measurements using CBCT imaging was evaluated by comparing the segmented volumes for hard tissue and root canal of the same samples to the volume segmented from μ CT scans using the Wilcoxon matched pairs test. Pearson correlation analysis was performed to examine the potential linear relationship between the segmented volumes (hard tissue and root canal separately) from CBCT and μ CT imaging. The degree of agreement between the segmented volumes from CBCT and μ CT imaging was compared using the method of Bland and Altman (26). In this method, the difference between the measurements is plotted against their mean because the mean is considered to be the best estimate of the true values. The volumetric changes after RET were tested by comparing the root hard tissue volume pre- and postoperatively starting from the cementoenamel junction up to the apex using the Wilcoxon matched pairs test.

Results

In Vitro Validation

Hard tissue and root canal segmented volumes for the samples were used to test the correlation between the μ CT and CBCT volumes;

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a strong correlation was found (hard tissue at 80 μm and 160 μm : $r = 0.99$, $P < .0001$; root canal at 80 μm and 160 μm : $r = 0.99$, $P < .001$). The comparison of the measurements of segmented volumes (hard tissue and root canal) from μCT and CBCT imaging

did not show any significant differences (Wilcoxon test, $P > .05$) (Fig. 2A and B).

The degree of agreement between μCT and CBCT segmented volumes was analyzed using the Bland-Altman method. The plots for the

Figure 2. *In vitro* validation. (A) A box plot comparison between segmented volumes in mm^3 (hard tissue) from μCT imaging at 30 μm and CBCT imaging at 80 μm and 160 μm . (B) A box plot comparison between segmented volumes mm^3 (root canal) from μCT imaging at 30 μm and CBCT imaging at 80 μm and 160 μm . (C and D) A Bland-Altman plot between the hard tissue segmented volumes from μCT imaging at 30 μm and CBCT imaging at 80 μm and 160 μm (the difference between the measurements is plotted against their mean). (E and F) A Bland-Altman plot between the root canal segmented volumes from μCT imaging at 30 μm and CBCT imaging at 80 μm and 160 μm (the difference between the measurements is plotted against their mean).

TABLE 2. Summary of RET Outcome

Case number	Follow-up period (months)	Healing of periapical lesion	Apical obliteration	Apical foramen diameter before RET* (mm)	Apical foramen diameter after RET* (mm)
1	24	Yes	Yes	1.63	0.50
2	16	Yes	Yes	2.02	0.0
3	12	Yes	Yes	4.48	0.20
4	25	Yes	Yes	3.84	0.3
5	20	Yes	Yes	3.38	1.13

RET, regenerative endodontic treatment.

*Measured in the axial view at the most apical part.

hard tissue and root canal volumes showed an agreement between the μ CT and CBCT segmented volumes. The segmented hard tissue volumes from CBCT imaging at 80 μ m and 160 μ m were slightly bigger (4.20 mm³ and 5.12 mm³) than the segmented volume from μ CT im-

aging at 30 μ m (Fig. 2C and D). Furthermore, the segmented root canal volumes from CBCT imaging at 80 μ m and 160 μ m were slightly smaller (−0.35 mm³ and −0.95 mm³) than the segmented volume from μ CT imaging at 30 μ m (Fig. 2E and F).

Figure 3. Case 1: a 9.9-year-old girl presented with pain related to dens evaginatus on the maxillary right central incisor. (A–C) A CBCT scan showed an immature apex, periapical lesion, and excessive buccal bone loss (yellow arrow). (D) A clinical picture before treatment. (E–G) A coregistered CBCT scan 24 months after RET showing periapical and buccal bone healing and formation of hard tissue apically. (H) A clinical picture 24 months after RET (note the gray discoloration in the cervical region caused by MTA) (blue arrow). (I) Three-dimensional reconstruction showing the extent of buccal bone loss (red arrow). (J) Three-dimensional segmentation for the maxillary right central incisor (yellow) and the contralateral tooth (blue). (K) Three-dimensional reconstruction 24 months after RET showing buccal bone healing (green arrow). (L) Three-dimensional segmentation showing apical maturation after RET (note the progressive nature of apical hard tissue formation compared with the contralateral control). (M and N) Three-dimensional volumetric analysis of hard tissue changes after RET showing newly formed hard tissue apically (black arrow) and increased root thickness in the buccolingual dimension (purple color on the root surface and purple arrows).

Figure 4. Case 2: an 8.9-year-old girl presented with pain and swelling related to the maxillary right central incisor (7 months after dental trauma in a car accident). (A–C) A CBCT scan showed an immature apex, periapical lesion, and bone loss related to the maxillary right central incisor. (D–F) A coregistered CBCT scan 16 months after RET showing healing of the periapical lesion and thickening of the root walls with root canal obliteration (yellow arrows). (G) Three-dimensional reconstruction before RET showing the size of root canal and rootdentin thickness. (H) Three-dimensional reconstruction showing the nonuniform nature of hard tissue deposition on the canal walls and progressive root canal obliteration (white arrow). (I and J) Three-dimensional volumetric analysis of the root (sectioned from the cementoenamel junction showing nonuniform hard tissue gain after RET [purple and dark green color on root surface] and apical blunting [black arrow]).

Clinical Application: RET Outcome

Table 2 summarizes the results of RET. The mean follow-up period was 19.4 (± 5.4) months. All subjects showed clinical evidence of resolution of signs and symptoms at follow-up visits related to the treated teeth. All teeth were free of any signs of soft tissue pathology; all teeth reacted normally to palpation and percussion tests and showed normal mobility. CBCT evaluation showed complete healing of periapical lesions with bone formation (Figs. 3A–N and 4A–F). Table 3 summarizes the results of the 3D analysis; all teeth showed thickening of the root canal walls and continuing root maturation (apex formation). All teeth showed hard tissue formation on the internal walls of the root canal with 2 teeth showing progressive apical obliteration (Fig. 4B and D). 3D volumetric comparison of the root hard tissue showed significant hard tissue formation ($P < .05$); the mean volume of newly formed hard tissue was 27.9 (± 10.5) mm³ (Table 3).

Discussion

This study reports a novel method for the evaluation and analysis of the RET outcome. The presented data confirm the accuracy of volumetric measurements from CBCT data compared with μ CT data. Liu et al (17) reported a slight deviation of segmented teeth volumes from the physical volumes within -4% to 7% using manual segmentation strategies and without any smoothing operations. Furthermore, Michetti et al (19) reported slightly smaller segmented root canal volumes from CBCT data (less than 3%) compared with the volumes measured from digitized images from histologic sections.

The current study applied volumetric measurements from CBCT data obtained for pre- and post-RET to analyze treatment outcome in a standardized quantitative method. All teeth included in the current study showed hard tissue volume gain demonstrating the success of

TABLE 3. A Summary of 3-dimensional Analysis

Case number	3-dimensional thickening of the root walls	Volumetric change* (mm ³)	Increase in root length* (mm)	Pattern of hard tissue deposition on root canal walls
1	Yes	+19.9	+1.4	On apical 1/3, on buccal and palatal walls, apex formation
2	Yes	+21.8	+0.8	On apical and middle 1/3, random deposition, apical blunting
3	Yes	+36.4	+5.5	Root canal obliteration from apex till middle 1/3, apex formation
4	Yes	+19.6	+0.8	On apical 1/3, on buccal and palatal walls, apical blunting
5	Yes	+41.9	+1.7	On apical 1/3, on buccal and palatal walls, apex formation

*Measured from the cementoenamel junction.

RET in stimulating hard tissue formation and root maturation. 3D root canal reconstructions showed progressive root canal calcification (obliteration) mostly in the apical part when compared with the contralateral teeth. This finding has been reported in previous studies on RET outcome (6, 7); root canal obliteration is a common finding after traumatic dental injuries and a desired outcome after autotransplantation of teeth acting as a sign of pulp revitalization (3–5, 27). Moreover, visualization of the pattern of hard tissue formation using color-coded maps for root dentin thickness before and after treatment clearly showed the nonuniform nature of hard tissue formation on root canal walls, which was unique for each tooth included in this case report. So far, the significance of the pattern of hard tissue formation is unknown. Future studies including a larger sample size or a multicenter study could implement the method described in the current study to evaluate the relation between baseline situation and the pattern of hard tissue deposition, thus exploring the determinants of success.

The segmentation strategy applied in the current report seems to offer the advantages of increasing accuracy and reducing working time. Using the livewire boundary technique, boundaries are extracted in one fifth of the time required for manual tracing but with 4.4 times greater accuracy and 4.8 times greater reproducibility (22). This proved to be of great value, especially for teeth in the posterior area of the mandible where segmentation is more difficult because of the cortical bone (17). Furthermore, using 3D semiautomatic active contour level set methods available in ITK-SNAP offers the advantages of a greatly reduced working time, high accuracy, and real-time interaction with the segmentation process in contrast to the commonly used low-level methods (region growing). Level set methods offer the possibility of adjusting the contour behavior, making it more suitable for segmenting areas with constrictions around the tooth apex.

This case series described the outcome of 4 patients who presented with 5 immature teeth with periapical infection and pulp necrosis. The RET outcome was in harmony with reported cases in the literature, showing resolution of periapical lesions, bone healing, hard tissue formation of the root canal walls, and root maturation (6–9, 12, 28). The treatment applied a biantibiotic mix instead of the commonly used triantibiotic mix to reduce the potential risk of discoloration because of the presence of minocycline in the triantibiotic mix (29). Furthermore, EDTA 17% was used in root canal conditioning after NaOCl irrigation and directly before the induction of bleeding into the canal to remove the smear layer, expose dentinal tubules and collagen fibrils, and release growth factors from the dentin matrix (21). EDTA is thought to enhance the outcome of RET through optimizing the conditions for cellular differentiation, tissue formation, and regeneration (21).

The aim of this study was to report the outcome of RET using 3D CBCT data and to analyze the outcome in a quantitative method. CBCT

images overcome the limitations of 2D images including the lack of the buccolingual dimension, masking of areas of interest by overlying anatomy (anatomic noise), and avoiding geometric distortion. A high spatial resolution and true volumetric information can be obtained because of the isotropic nature of CBCT images (16, 30). Moreover, it has been shown that dental trauma and developmental anomalies can be better assessed in 3 dimensions (16, 30–32). Nevertheless, CBCT imaging has its own limitation; the effective radiation dose delivered by CBCT devices available in the market varies enormously from around 10–1200 μ Sv (which is an equivalent of 2–240 panoramic radiographs) (33, 34). Therefore, a proper balance (optimization) between, on the one hand, costs and radiation dose and, on the other hand, information required needs to be maintained (16). Furthermore, the scanned area should not exceed the area of interest. This will limit the dose substantially, thus justifying its use (16, 31, 32). More research is still needed to validate the benefit of applying CBCT imaging in routine monitoring of RET cases.

Quantitative methods using 2D data were successfully reported and validated (12, 35). However, working with 2D data has its limitations such as image standardization and the lack of volumetric measurements. Moreover, 2D imaging does not account for the presence of intracanal calcifications, which is a common finding in cases treated with RET (35). Nevertheless, a standard quantitative method is highly needed to compare results from different studies and to be applied in multicenter prospective studies. Three-dimensional images offer the unique possibility of analyzing the pattern and position of hard tissue formation. The use of standardized 2D data from short-term monitoring combined with 3D data from long-term monitoring in a study with a large sample size might offer much needed information regarding the outcome of regenerative endodontic procedures. Finally, future research including a larger sample size and control group and comparison with the available 2D methods is needed to validate the clinical application of the method proposed in the current study.

Conclusion

Analysis of 3D data for teeth treated with regenerative endodontic procedures offers valuable insights into the treatment outcome and patterns of hard tissue formation.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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