Applications of micro-computed tomography in endodontic research

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One of the greatest advances in endodontic research is the micro-computed tomography (micro-CT). Micro-CT is based on multi-slice X-ray images that are digitally grouped into a three-dimensional (3D) image. In comparison to SEM, confocal microscopy and stereomicroscopy, the micro-CT has the advantage of providing tridimensional reconstructions without the requirement of sectioning the samples. Furthermore, the small voxel size of micro-CT result in higher resolution than cone-beam computed tomographic. Micro-CT can be used to evaluate volume and/or area using scanning pre and post endodontic treatment. In this work, some applications of micro-CT in endodontic research are presented.

Keywords Micro-computed tomography; Root canal treatment

1. Introduction

The results obtained from controlled studies support advances and enhancements in different fields of knowledge. The development of novel technologies is required to amplify the excellence and consistency of scientific researches. Endodontic treatment involves several steps, which can determine the success or failure of therapy. Cleaning and shaping procedures are performed to eliminate microbial infection from root canal system [1]. This phase of treatment is executed with manual or rotary instruments [2] associated with irrigating solutions [3]. After the reduction of microorganisms from root canal system, an obturation material is used to completely fill the cleaned space and prevent fluid infiltration [4, 5]. Several studies are performed to analyse the ability of materials and techniques accomplished during endodontic therapy. However, there are limitations related to some methodologies used. The introduction of X-ray micro-computed tomography has substantially improved perspectives of endodontic researches. This technology has been widely applied to evaluate anatomy, techniques and materials related to the endodontic treatment [6-8].

The X-ray micro-computed tomography (micro-CT) was developed in the early of 1980s [9]. The micro-CT is a non-invasive and non-destructive method to obtain two- and three-dimensional images [10]. Its operation is based on multiple X-ray converging on the sample and captured by a sensor. The projected X-ray is converted into digital images. The volumetric pixel (voxel) provided by micro-CT range in 5-50 μm [9]. Smaller voxel size generates image with higher resolution. The distance of axial scanning step can be previously determined using software. This adjustment determines the resolution of image obtained, but also affect the time of exposure. Decrease of the distance between scanning steps demand longer time of X-ray exposure. Furthermore, the multiple images created require high memory in a computer to store the dates [11]. Depending on the material to be scanned, it is necessary longer time of scanning to make it visible.

Micro-CT present several advantages in comparison with other methods, but otherwise has some limitations. Scanning electron microscopy, stereomicroscopy and confocal laser microscopy can be used for superficial analysis but do not provide 3D images without the requisite of sectioning the samples. Contrary of these microscopic methods, micro-CT allow the use of the same sample for different tests without destruction of the sample [12]. This characteristic is very important particularly when is required to evaluate volume pre and post instrumentation, quality of root canal obturation or removal of the material from root canal (retreatment). Others advantages of micro-CT are the possibility of repeated scanning [13] and the manipulation of image using specific software. On the other hand, a limitation of micro-CT is the impossibility of using for *in vivo* studies due to the radiation level of exposure. Moreover, micro-CT permits the examination of specimens of limited size, which restrict some analysis. Instead, cone beam computed tomography (CBCT) could be used in patients despite its lower resolution [14].

Some applications of micro-CT in endodontic research include the analysis of internal anatomy of teeth [6, 15, 16], instrumentation of root canal [17], root canal fillings [18], retreatment [19], physical and biological properties of materials. The success of endodontic therapy is directly related to the identification of all root canals for its adequate cleaning [15].The analysis of internal anatomy is important for knowledge of the complexity of root canal system and planning the treatment[15]. Instrumentation techniques and instruments are evaluated to improve the removal of contamination from root canal. Root canal filling techniques and sealers should be appropriately adapted into root canal walls and the quality of obturations is commonly studied. Microorganisms are the main cause of persistent apical periodontitis and the retreatment is indicated in some cases to decontaminate the root canal system. The aim of this work is to describe the applications of X-ray microtomography in endodontic research.

2. Materials and Methods

2.1 Internal anatomy

To analyse the internal anatomy it is not necessary a specific prepare of tooth before scanning. Extracted tooth is stored in 10% formalin solution. Tooth is positioned into a patten and scanned with a desktop x-ray micro focus CT scanner (SkyScan 1174v2, SkyScan, Kontich, Belgium). The equipment scan by using 50 kV x-ray tube voltages, 800 µA anode current, and a detector based on a Charge Coupled Device camera (CCD) of 1.3 Mp (1304 x 1024 pixel). This device enables the scanning of the sample up to 15 mm height with isotropic spatial resolution, which may vary between 6-30 micrometres. Commonly, one sample is scanned at a time. The equipment has a 1 mm of aluminium filter positioned in front of the X-ray source for changing the sensitivity of polychromatic radiation. This whole system is connected to a computer used in the control, data acquisition, reconstruction and analysis of the attributes of the images. The image capture parameters to use are variable according to the sample, in relation of a voxel size and degree rotation step. For example to scanning a maxillary first molar with approximately 20 mm length, may use 22µm with 0.7-degree rotation step using a 180-degree rotation, this parameter will provide suitable images for a study of the anatomy of the root canal system. The result of this scanning consisted of 327 .tiff images.

All digital data produced must be elaborated by reconstruction software (NReconv1.6.4.8, SkyScan) to obtain cross sectional, to resulting in complete representation of the internal microstructure of each sample. The CTan software (CTan v1.11.10.0, SkyScan) is used to processing and analysis of the images, utilized for the linear or 3D measurements. Initially by a binarization process, which uses the mathematical operations to change values of the pixels of the sample to be analysed. In the binarization is separated from the segments that correspond to the dentin and the root canal, in the case of evaluating the root canal anatomy, or the filling material and dentine, in the case of evaluating the root canal filling. The binary values are adjusted according to the raw images. In this process it is possible to divide the image into regions, recognizing them as objects independent of each other and background. To get a binary image where black pixels represent the background and the regions of white pixels, the objects of analysis. Different plug-ins can be used according to the desired analysis. In the CTan software, when were scanned two or more samples, they are present in the same image but could be separated to individually analyse. This was limited to the area of interest (ROI) for each sample and the new ROI data was saved in separate folders. For a qualitative evaluation, the double team cubes algorithm may create samples of three-dimensional models, and P3G in the format from the program CTVol v.2.2.1.0 (SkyScan, Kontich, Belgium), can be made realistic visualization of three-dimensional models.

2.2 Instrumentation

Conventional access to the root canal system is performed using high-speed diamond burs 1014 (Sorensen, SP, Brazil). Initial micro-CT scanning (SkyScan 1174v2, SkyScan, Kontich, Belgium) is performed at this stage to compare the volume of canal pre and post instrumentation. To analyse root canal shaping is suitable to use a high resolution with a rotation step ranging in 0.5-1.0 µm. After scanning, the working length is established measuring the position of a size 10 K-file (DentsplyMaillefer, Ballaigues, Switzerland) when it reached the apical foramen and then subtracting 0.5 mm. Then, the canals are negotiated a size 20 K-file. The root canals are shaped using a rotary system and 2 mL of 2.5% sodium hypochlorite (NaOCl) to irrigate the canal. After instrumentation, the canals are irrigated with 2 mL of 2.5% NaOCl for 1 min using passive ultrasonic irrigation with an intermittent flush technique. Then, the post instrumentation scanning is performed and the scanning pre and post instrumentation overlapping. The same teeth and the initial micro-CT scanning can be used to sequentially analyse the root canal filling.

2.3 Obturation and retreatment

Initially the root canals of the teeth are instrumented using endodontic instruments and 2.5% sodium hypochlorite (NaOCl). At the end of the cleaning and shaping procedure, the canals are irrigated with an ultrasonic unit with 2 mL of 2.5% NaOCl for 1 minute, this procedure is repeated three times. Then, the root canals receive a final flush of 2 mL of 17% EDTA (pH 7.7) for 3 min. This procedure is performed to improve the adaptation of the filling materials to the root canal walls and to allow the penetration of the sealer into dentinal tubules. Finally, the canals are washed with a final rinse of 2 mL distilled water and dried with paper points. The root canals are filled using an endodontic sealer and gutta-percha cones. Teeth are stored at 37^{o}C with 100% humidity to allow the sealers to set completely. Root canal fillings require a high-resolution image, resulting a long time of scanning. High-resolution is obtained with a rotation step ranging in 0.3-0.6µm. After scanning the root canal filling, it is possible to use the same sample to analyse retreatment. For this evaluation, the volume of the obturated canal is measured and, after removal of the filling material the volume of residual material is measured. The volume of the root canal filling and the residual filling material are compared.

3. Results and Discussion

Novel technologies are continuously introduced to enhance endodontic researches. The X-ray micro-computed tomography has been used to evaluate several stages related to the endodontic therapy [6, 18-20]. Micro-CT provides high-resolution images that can be grouped creating a three-dimensional image, analysed using software [6]. The opportunity of sequential analysis without the destruction of samples provided a new perspective to endodontic researches. Thus, it is possible to subsequently evaluate the internal anatomy [6], to shape the root canals and verify the cleaning ability [20], to obturate the canals and calculate the filled area [8] and then desobturate using a retreatment system and analyse its effectiveness [19].

Anatomic complexities difficult cleaning procedure and sometimes are the main causes of failure of treatment [21, 22]. During a long time the analysis of internal anatomy was performed using diaphanization method. This technique consisted in access of the pulp chamber, dissolution of pulp tissue with sodium hypochlorite, decalcification of mineralized structure with hydrochloric acid and injection of gelatin coloured with dye into the root canal [23]. The analysis using diaphanization can provide an idea of three-dimensional structure of root canals [23]. However, this method is considered out-dated and has the disadvantage of do not allow further use of the sample. Others methodologies have been used to evaluate the internal anatomy. Stereomicroscopy was used to examine the mesio-buccal roots of maxillary first and second molars using serial sections of root [24]. Cone beam computed tomography (CBCT) was used for clinical analysis of internal anatomy [15]. Recently, micro-CT has been used to study anatomical complexities of the root canal system (Fig 1). Some specific complexities as C-shaped canals [16] and isthmuses [25] can be verified using three-dimensional reconstructions (Fig. 2). Additionally, the volume of the canal can be measured using the initial scan and used to compare after shaping procedure.

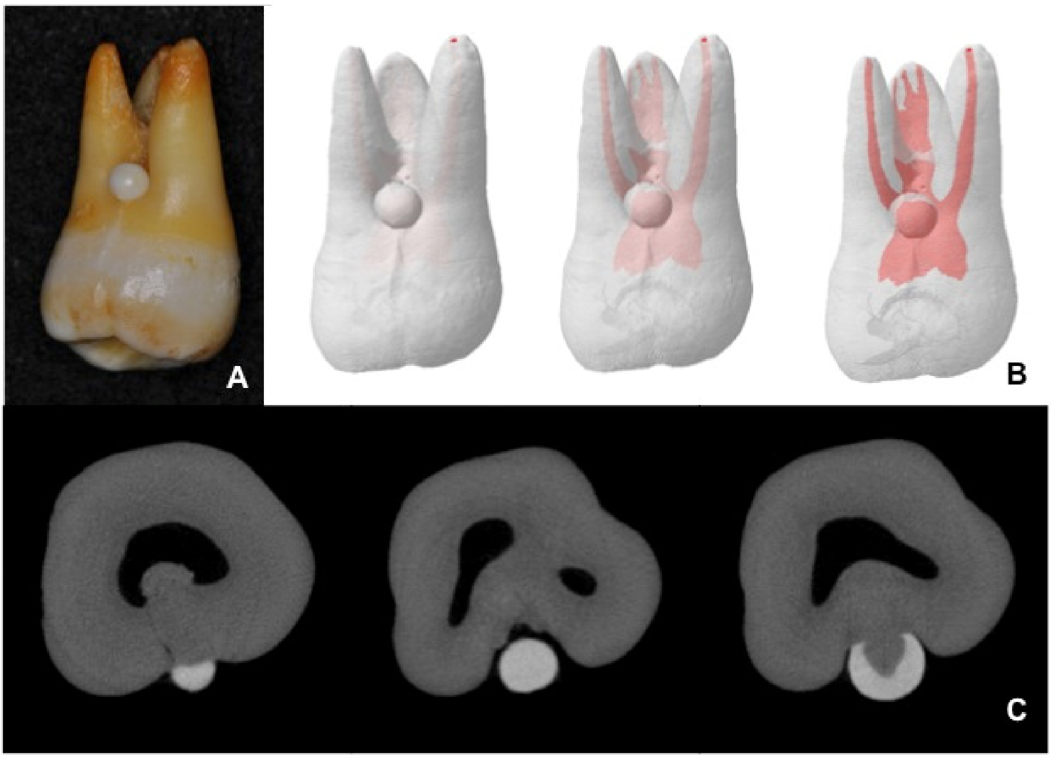

Fig. 1 The figures show a maxillary molar with a projection of enamel between the roots (enamel pearl). In (A) the external anatomy is represented by a digital photography. In (B) the internal anatomy of the root canal can be identified in a micro-CT reconstruction with different transparency gradations. In (C) the corresponding x-ray image provided by micro-CT allows the recognition of the root canal and the enamel projection at the middle third of the tooth. The software allows the analysis of the tooth in vertical or horizontal sections, without the necessity of the physical section of the sample.

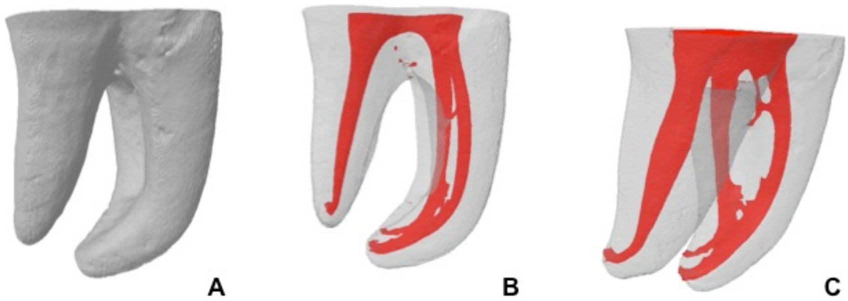

Fig. 2 Micro-CT reconstructions of a mandibular molar. In (A) the external anatomy is showed. In (B-C) the root canal can be recognized and the anatomical complexities are easily identified in due to the transparency. It is possible to verify an isthmus area in the mesial root.

Success of endodontic treatment is related to an adequate cleaning and shaping procedure to eliminate bacterial infection from root canal system [1]. Micro-CT can be used to evaluate the ability of endodontic instruments to clean the root canal system. The volume pre and post instrumentation are measured and the area cleaned by the instruments is determined [26]. The analysis of instrumentation procedure can be performed using different methods. Bramante *et al.*[27] have proposed a method to evaluate the instrumentation of the root canals. Teeth are moulded and sectioned before the root canal shaping. Each slice could be removed and photographed. The slices are originally positioned and the root canal instrumented. After shaping, the slices are again removed and photographed. The images of sections before and after instrumentation are compared to determine the instrumented area. Most recent methodologies are used in endodontic researches to evaluate the shaping procedure. Some of these techniques include stereomicroscope [28], scanning electron microscope [29] and morphometric evaluation [30]. In general using these methods, some sections are selected and the cleaned area measured. Contrary, micro-CT allows the analysis of the complete root canal without the requirement of sectioning. Scans pre and post instrumentation can provide date of the volume of canal cleaned. It is also possible to verify areas that were not reached by the instruments, and consequently pulp tissue has persisted [7] (Fig. 3).

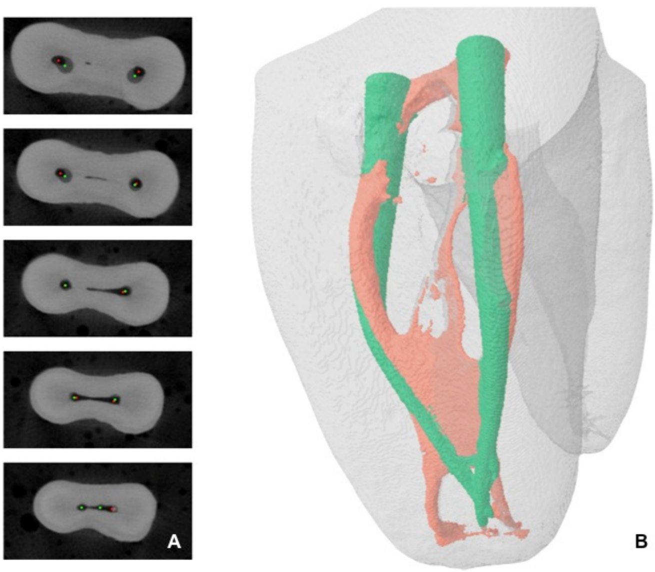

Fig. 3 The figures in (A-B) show a micro-CT scanning of mesial root of a mandibular molar. In (A) are represented horizontal sections at different levels of the mesial root. It is possible to identify some isthmus areas. In (B) the three-dimensional reconstruction of the instrumented canals are shown. Green represents the areas cleaned by endodontic instruments and red represents the areas untouched. It is possible to observe large areas of the root canals without instrumentation. The anatomical complexities had compromised the adequate cleaning.

The filling of the decontaminated root canal must adapt to the root canal space, including accessory anatomy [4]. In overall, obturation techniques are performed with gutta-percha associated with a sealer. An adequate root canal filling should present higher volume of gutta-percha and a thin layer of sealer at sealer/dentin interface. The presence of voids or gaps is not desirable for a satisfactory obturation due to the risk of fluid infiltration and consequent contamination of the root canal system. Several methods have been used to evaluate the adaptation and quality of root canal fillings.

Confocal laser scanning can be used to visualise sealer adaption by adding a fluorescent dye in the sealer [4]. This method is recommended when is necessary to analyse interfacial adaptation and verify the presence of gaps at sealer/dentin interface. Stereomicroscope can be also used to evaluate root canal fillings [31]. These two methods require sectioning the samples before analysis. Leakage tests are another method to analyse the adaptability of filling materials [32]. However, the models proposed are controversial and some authors indicated that they are not reliable [33]. Micro-CT has been proposed to evaluate the quality of obturation techniques. The percentage of volume of voids and gaps in the root canal can be calculated (Fig. 4). The analyses include demarcation of voids and gaps in the two-dimensional slices and then the reconstruction in a three-dimensional image. This process is considered prolonged and difficult, but very accurate. The main advantage of micro-CT to evaluate root canal fillings is the possibility of further analysis. After study of obturation, the material can be removed using a retreatment system and the ability of retreatment techniques studied [34, 35].

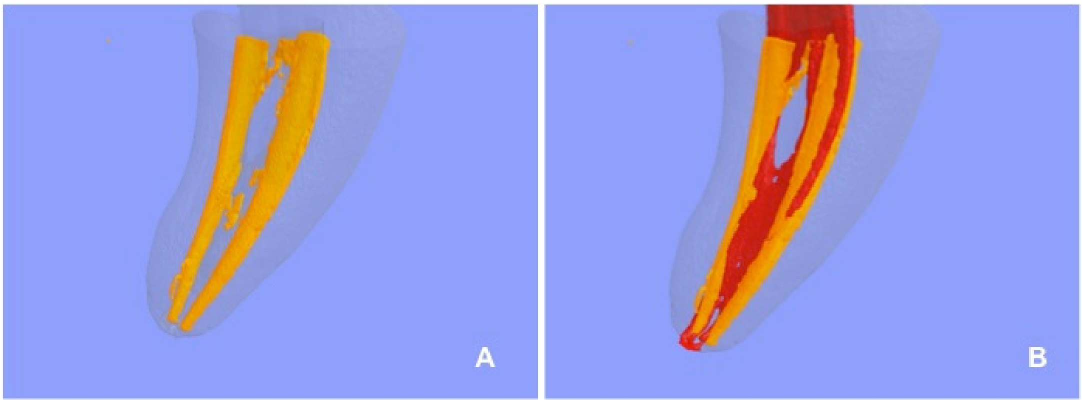

Fig. 4 Mesial root of a mandibular molar is represented in (A-B). The image (A) shows the root canal filling in yellow. The image (B) shows the root canal filling in yellow and the areas of the root canal that were not filled by the gutta-percha or sealer in red.

Recently, other applications for micro-CT in endodontic research have been developed. Previous investigations regarding the use of micro-CT to analyse solubility was performed. Volume pre and post immersion are used to determine the solubility rate of the material. It is an alternative to conventional solubility test that is commonly used in endodontic research [36] and can provide an accurate date of volume loss closer of clinical situation. Furthermore, micro-CT has a potential to analyse the root canal instruments (Fig. 5). It can be possible to verify deformation of instruments after several uses. Biological properties are not widely analysed with micro-CT, however it represent a promissory application. Volume of apical lesions can be assessed with scanning of fixed tissues (Fig. 5).

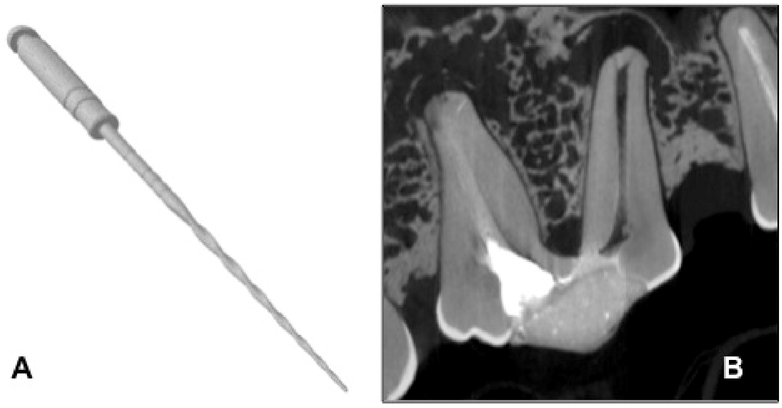

Fig. 5 In (A) an endodontic instrument (Mtwo, VDW, Munich, Germany) scanned by using micro-CT. The figure in (B) shows a dog' tooth with an evident periapical lesion round roots. The filling material into the root canal can be identified by its radiopacity.

Tree-dimensional images can be also made with cone beam computed tomography (CBCT). This method is considered non-destructive as the micro-CT. CBCT has been used in endodontic research to evaluate several variables [14]. This equipment has the advantage to be faster for scanning in comparison with micro-CT, and consequently the levels of radiation exposure are lower [37]. That characteristic of CBCT allows its use for clinical researches and *in vivo* analysis in animals [38, 39]. Nonetheless, micro-CT has higher resolution due to the lower voxel size [37]. Many times, for scientific researches it is more significant the resolution and quality of the image than the time required for the analysis. Despite CBCT is indicated to *in vivo* analysis, micro-CT is better recommended for laboratory researches. It can be concluded that micro-CT represent the ideal advice for laboratory researches. In comparison with microscopic methods of analysis, three-dimensional images provided by micro-CT are superior for laboratory analysis. The limitation of not allows the use in patients; do not limit its use in endodontic research.

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