Contrast-enhanced micro-CT to assess dental pulp tissue debridement: a series of cascading experiments towards method validation

G. De-Deus[1], F. G. Belladonna[1], D. M. Cavalcante[1], M. Simões-Carvalho[1], E. J. N. L. Silva[1,2], J. C. A. Carvalha[1], P. M. H. Dummer[3], M. A. Versiani[1], M. Zehnder[4]

[1] Department of Endodontics, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil
[2] Department of Endodontics, Grande Rio University, Duque de Caxias, Rio de Janeiro, Brazil
[3] School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK
[4] Division of Endodontology, Clinic of Conservative and Preventive Dentistry, Plattenstrasse 11, CH-8032 Zürich, Switzerland.

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Corresponding author:
Prof. Dr. Gustavo De-Deus
Rua Sambaíba 176/504 - Leblon, Rio de Janeiro, RJ, Brazil
ZIP CODE: 22450-140
Phone: (55) 21 99700-8254
e-mail: endogus@gmail.com

Introduction

Conventional analysis of histological sections and use of X-ray micro-computed tomography (micro-CT) are considered the gold standard methods to evaluate cleaning and shaping procedures during root canal preparation. Whilst micro-CT allows the mineralized tissues of the root canal removed by mechanical preparation to be identified and quantified, the amount of remaining (unmineralized) pulp tissue is usually assessed on histological sections. Thus, despite the usefulness of micro-CT technology, this method has been limited to the evaluation of changes along the canal walls, such as transportation and the creation of aberrations, such as zips, perforations, etc. Because of the penetrating power of X-rays, micro-CT techniques provide a 3D density map of specimens and tissues that strongly absorb this radiation (Alfaro *et al.* 2015, Cunha *et al.* 2015). However, it is unsuitable for imaging soft tissues, such as the dental pulp, as these tissues absorb X-rays to a very limited degree (Gignac & Kley 2014).

Recently, a correlative approach using histology as a complementary method for micro-CT assessment was employed in two studies to evaluate various chemomechanical protocols in root canals (Lacerda *et al.* 2017, Siqueira Jr *et al.* 2018). Both studies demonstrated histologically the presence of pulp tissue remnants attached to untouched canal walls previously identified by micro-CT analysis. Although this correlative approach using different methods may allow the assignment of causation mechanisms, histologic analysis usually allows only a few sections per root to be assessed, which provides only limited data and is inconsistent with the amount of information in hundreds of cross-sectional images usually produced by the micro-CT scanning of a typical root canal. Moreover, histological sectioning is a time-consuming and expensive procedure that needs to destroy the specimen.

It is obviously desirable to develop a reliable non-destructive experimental method able to simultaneously assess the quality and quantity of soft and hard tissues in a heterogenous specimen such as the human tooth. In other research areas, this limitation has been overcome by using various contrast agents such as osmium, gold, barium sulphate, and iodine-based dyes (Metscher 2009a, 2009b, Faulwetter *et al.* 2013, Pauwels *et al.* 2013). Overall, it has been demonstrated that an aqueous solution of Lugol's iodine, also called iodine-potassium iodide (I_2KI), is amongst the most effective means for rapidly differentiating a diversity of soft tissue types. Lugol's solution is a simple, cost-effective, nontoxic, and rapid staining option for contrast enhancement of soft tissues. Yet, its use has been limited to anatomical studies of a wide variety of biological specimens using a broad range of different concentrations of iodine and staining durations, depending on the

type of the tissue (Heimel *et al.* 2019). Currently, despite the fact that Lugol impregnation is the most widely used contrast agent in the anatomical study of soft tissues, it is still not clear whether this solution would be suitable in the micro-CT assessment of pulp tissue after root canal debridement *ex vivo*. One of the fundamental issues when using Lugol concerns sodium hypochlorite (NaOCl), the main endodontic irrigant used to dissolve necrotic pulp tissue (Zehnder 2006), which reacts with iodine (Vogel 1978) and could thus interfere with its impregnation and visibility on radiographic images.

The present communication reports on a series of cascading experiments seeking to introduce and explore the potential to visualize dental pulp tissue on micro-CT images using Lugol as a contrast-enhancing solution. The cascade experiments aimed to validate the impregnation protocol and the contrast-enhanced micro-CT (CE-CT) method whilst identifying the advantages and potential limitations of this novel methodology. The cascade experiments were design to:

- Evaluate the potential of NaOCl to reduce the degree of radiographic contrast associated with the Lugol-stained pulp tissue (radiopacity test);
- Verify the possibility that Lugol's solution affects the oxidizing ability of NaOCl (dissolution test);
- Assess the ability of Lugol's solution to impregnate pulp tissue properly by correlative imaging of Lugol-enhanced micro-CT and conventional histology (histological validation);
- Measure the remaining volume of Lugol-impregnated pulp tissue (volumetric micro-CT assessment).

Materials and methods

Impact of NaOCl on the radiopacity of Lugol's solution

The Lugol's solution (I_2KI) used for all the experiments had a concentration of 5% I_2 and 10% KI. To assess the impact of NaOCl on its radiopacity, a 1:1 dilution series was performed in distilled water and 5.25% NaOCl (1.5 mL total volume). The pure solution and its dilutions were pipetted into round transparent polystyrene dishes (Semadeni, Ostermundigen, Switzerland) with an inner diameter of 23.4 mm to a depth of 3.5 mm. Radiopacity was determined using a standard set-up as described previously (Hertig *et al*. 2017). In brief, electronic datasets were generated using a fixed unit (Trophy, Paris, France) at 65 kV, 8 mA, and 0.22 s with a focus-film distance of 25 cm and electronic sensors (Digora, Soredex, Tuusula, Finland). Images were analysed using ImageJ (Bethesda, MD, USA). Gray values were normalized in each image against an aluminium step wedge, with an individual standard curve for each image. Experiments were done in triplicates.

The relative radiopacity of the Lugol's solution and its dilutions is expressed as the aluminium equivalent (in mm) per mm solution.

Soft tissue dissolution test

Thirty slices of fresh bovine meat were adjusted to a similar weight (2 mg) and dimensions (4 x 4 mm) using a no. 15 surgical blade. Ten slices did not undergo any tissue processing, whilst the other 20 were fixed in formaldehyde for 24 h. After that, 10 of the fixed slices were further immersed in Lugol's solution for another 24 h. Then, all specimens were placed individually into flasks containing 40 mL of 5.25% NaOCl and the total time required for complete pulp tissue dissolution (in min) was recorded. All testing procedures were performed at room temperature. This investigation was not classified as an animal study because it had no influence on the premortal fate or the slaughtering process of the animals. Preliminary analysis of the raw data indicated the adherence to a Gaussian distribution (Shapiro-Wilk test, $P < 0.05$). Data was compared between groups using one-way ANOVA followed by Tukey's HSD test. Alpha-error was considered at 5%.

Micro-CT assessment of pulp tissue remnants

After the approval by the local ethics committee (protocol no. 12127319.3.0000.5243), eight non-carious single-rooted mandibular premolars and one mandibular molar with vital pulps, extracted for orthodontic reasons, were immersed in 10% buffered formalin and stored for up to 30 days at 15 ºC. Afterwards, the teeth were scanned at a pixel size of 14.37 μm using a micro-CT device (SkyScan 1173; Bruker microCT, Kontich, Belgium) set at 70 kV, 114 mA, 360° rotation around the vertical axis with a rotation step of 0.5°, frame average of 5, using a 1.0-mm-thick aluminium filter. NRecon v.1.7.16 software (Bruker micro-CT) was used to reconstruct the micro-CT projections into axial cross-sections using a ring artefact correction of 4, contrast limits ranging from 0.006 to 0.05, and beam hardening correction of 40%, resulting in 800-900 grayscale images per tooth from the cementum-enamel junction to the apex. To verify the canal morphology, the cross-sectional images were segmented using an automatic routine (De-Deus *et al.* 2020) in the FIJI/ImageJ software (Fiji v.1.51n; Madison, WI, USA), and the aspect ratio of the root canal, defined as the ratio of the major to the minor diameters, was measured in each slice from the orifice to the apical foramen. Then, canal volume (in mm^3) was calculated as the volume of binarized root canal within the volume of interest.

After conventional access cavity preparation, each tooth was immersed in Lugol' solution for 7 days and submitted to a new scan and reconstruction procedures using the previously mentioned parameters in order

to assess the impregnation of the entire pulp tissue by the contrast solution. This set of images was co-registered with the unstained dataset using the affine algorithm implemented on the 3D Slicer 4.6.0 software (http://www.slicer.org) (Fedorov *et al.* 2012) and the root dentine of the tooth following immersion was removed through a Boolean Operation to reduce the noise generated by the pulp tissue segmentation. Thus, the pulp tissue impregnated with Lugol's solution was observed and quantified (in mm^3) by the segmentation process with a specific threshold value, using the Object Counter tool available in the FIJI/ImageJ software. After that, the root canals of 5 out of the 8 selected premolars were chemomechanically prepared, whilst the other 4 sound teeth (3 premolars and 1 molar) were prepared for histological sectioning to confirm the presence of the pulp tissue remnants (control group).

Root canal preparation

The root canals were prepared up to the working length with Reciproc R25 instrument (VDW GmbH, Munich, Germany) driven by a VDW Silver motor (VDW GmbH) in the "RECIPROC ALL" preset programme using light apical pressure with a slow in-and-out pecking motion of 3 mm amplitude. After completing three pecking movements, the instrument was removed from the canal and its flutes cleaned by insertion into a sponge moistened with alcohol. The working length was achieved after 3 waves of instrumentation. Apical patency was performed with a size 15 K-file (Dentsply Sirona Endodontics, Ballaigues, Switzerland) throughout the preparation procedures. Irrigation was performed with a total of 12 mL of 5.25% NaOCl dispensed into the root canal with a 31-G NaviTip double side port needle (Ultradent Inc., South Jordan, UT, USA) taken up to 1 mm short of the working length throughout the preparation procedures. After root canal preparation, the specimens were re-scanned, reconstructed and co-registered using the aforementioned parameters. Then, the volume of pulp tissue remnants impregnated with Lugol's solution was calculated (in mm^3) and quantified as a percentage value based on the initial volume of the pulp tissue.

Histological assessment

After the experimental procedures described above, the specimens were demineralized in 22.5% (vol/vol) formic acid plus 10% (wt/vol) sodium citrate solution for a period of 2 to 3 weeks. The end-point was monitored radiographically. The specimens were then rinsed for 24 h in tap water, dehydrated and processed for routine histological examination. Teeth were embedded in paraffin blocks and serial 0.6 µm thick cross-sections were obtained every 1 mm from the cemento-enamel junction to the apex, resulting in 8

slices per tooth. The acquired sections were mounted on glass slabs and stained with haematoxylin-eosin. Histological images were visualized using an Axioplan 2 Imaging fully-motorized light microscope (Carl Zeiss Vision, Hallbergmoos, Germany).

Matching the Lugol-impregnated micro-CT images and histological slices

The acquired micro-CT slices containing the Lugol-impregnated pulp tissue were inspected along the Z-axis using a reference coordinate system based on a landmark-based registration algorithm (Analyze software; Biomedical Imaging Resource, Mayo Clinic, Rochester, MN, USA) to align them with the microradiograph images of the histologic sections. After selecting the corresponding images, a dimensional standardization adjustment was performed including automatic magnification, resizing and cropping, by means of a computer-assisted procedure. This procedural step allowed the examiners to reliably inspect the roots at the same levels and thus, qualitatively verify if the pulp tissue in the histological section matched its counterpart in the Lugol-impregnated micro-CT slice, confirming the efficacy of the impregnation protocol and scanning parameters. Two pre-calibrated examiners used a proforma with predefined criteria to analyse the degree of matching between Lugol-impregnated and histological images. The image analysis procedure was performed in a 34' high-quality computer monitor with the possibility of escalating images (up to 10x) and reversing the colour mode. To validate the analytical process, analyses were repeated twice at 10-days intervals to appraise the reproducibility.

Results

Impact of NaOCl on the radiopacity of Lugol's solution

Pure Lugol's solution had a radiopacity of 0.70 ± 0.09 mm Al/mm. Dilution in 5.25% NaOCl had a similar effect on radiopacity as the control procedure in water, with the higher dilutions in NaOCl showing slightly higher radiopacity (**Fig. 1**), which is based on the difference in radiopacity between the pure 5.25% NaOCl solution and water of 0.05 mm Al/mm. There was no discernible effect on radiopacity caused by the chemical interaction between the NaOCl and Lugol's solution, which was visible by clearing of the brown colour in the presence of NaOCl.

Dissolution test

Violin-plots illustrate the mean, minimum, and maximum values, as well as, the data distribution of tissue dissolution amongst the specimens (**Fig. 2)**. Tissue processing in formaldehyde and Lugol's solution did not affect the time required for the dissolution of fresh bovine meat ($P > 0.05$).

Histological validation

The overall quality of the staining protocol used is illustrated in **Fig. 3**. Correlative analysis between micro-CT and histological images confirmed the identity of the Lugol-impregnated pulp tissue in the micro-CT images. The results of the matched micro-CT and histological images are shown in **Fig. 4** and **Fig. 5**, confirming the quality of the staining protocol.

Volumetric micro-CT assessment

Additionally, segmented pulp remnants after root canal preparation were quantified and expressed as a percentage value. Instrumented root canal volume and non-instrumented canal areas acquired by the micro-CT method were also quantified (**Table 1**) and illustrated in **Fig. 6** and **Fig. 7**. The volume of the root canal and aspect ratio directly influenced the removal of pulp tissue during instrumentation. Teeth with aspect ratio values less than 3.5, expressed by the graphic curve and high volumes, were associated with smaller volumes of pulp tissue remnants.

Discussion

This communication introduces a novel staining method for dental pulp tissue in the context of the micro-CT assessment of root canal debridement with obvious beneficial uses for future research in this field. The proposed non-destructive method is able to provide high-resolution images and 3D information on soft pulp tissue and dentine simultaneously thus allowing the longitudinal and quantitative volumetric assessment of root canal cleaning and shaping procedures. Since Gysi & Röse (1894) published the first high-quality photomicrographs depicting details of the vascular, lymphatic and nervous elements of the pulp-dentine complex of a mandibular molar, and Kölliker (1896) provided the first description of the dental pulp, named by him as *Pulpa dentis*, in his classic book on the minute structures of the tissues and organs of the body, many studies have investigated teeth using histological methods. Using this technique, Hatton *et al.* (1928) were the first to demonstrate that the canal was only superficially cleaned and much of the pulp tissue was not removed after preparation with stainless steel instruments. However, it was only after Walton (1976) published a

seminal study assessing the amount of the remaining pulp tissue after the cleaning and shaping procedures that paraffin-based histological sectioning became the standard method to determine the efficacy of debridement procedures within the root canal space.

In intact teeth with vital pulps, normally used as a control in histological sectioning studies, the pulp tissue is attached to the entire perimeter of the root canal (De-Deus *et al.* 2010, 2011) whilst the tissue remnants in the experimental groups confirm which areas along the canal walls were not mechanically debrided or where irrigation protocols were ineffective. Since pulp remnants may serve as a substrate for bacteria and could negatively affect the quality of canal filling procedures (Ricucci *et al.* 2009, 2011), it theoretically endorses this experimental variable, remaining pulp tissue, as an accepted surrogate endpoint for the quality of debridement procedures within the root canal space. However, the processes and resultant workload for specimen preparation that embraces sectioning, staining, imaging, and the final comprehensive histological assessment remains a cumbersome and labour-intensive technique. Specifically, in the context of endodontic laboratory research, the decalcification of mineralised tooth tissues is a time-consuming and complicated step and it is challenging to achieve high quality specimens without damaging the pulp tissue. This becomes even more important as, in general, histological sectioning of decalcified hard tissues is prone to induce considerable tissue distortions, processing glitches and structural artefacts. For example, tissue shrinking by up to 3% occurs with bone tissues (Lane & Ráliš 1983, Henson *et al.* 1994), whilst dehydration of soft tissue can create shrinkage up to 11% (Rown *et al.* 2002). Thus, even with the useful insights available in the literature on this topic, it is of note that quantitative microscopy data from histological sectioning were derived from tissues that presumably shrunk during specimen preparation. Moreover, histological sectioning techniques invariably lead to the loss of specimens, rendering longitudinal experiments over time impossible. Hence, histology may be considered as an archaic method compared to the volumetric and quantitative approach achieved by non-destructive imaging methods (**Table 2**), even though to date it remains the only available experimental model that allows the simultaneous assessment of both mineralized hard and soft tissues of teeth at their ultrastructural level and, accordingly, is able to shed light on this important research area (De-Deus *et al.* 2008, 2010, 2011).

Several studies using non-destructive micro-CT technology have demonstrated that the preparation of root canal walls by endodontic instruments activated in either rotary or reciprocating motion is not ideal (Paqué *et al.* 2010, Paqué & Peters 2011, Versiani *et al.* 2013, De-Deus *et al.* 2015, Zuolo *et al.* 2018). Mechanical preparation with these instruments is limited as they tend to prepare only the central aspects of root canals to create a round shape, leaving most of the buccal and lingual extensions of these complex spaces untouched,

even when attempting lateral movements such as when using a brushing motion (Paqué *et al.* 2010, Paqué & Peters 2011, Versiani *et al.* 2013, De-Deus *et al.* 2015, Zuolo *et al.* 2018). Although micro-CT can provide valuable and accurate measurements regarding the position and amount of dentine removed during canal preparation, it does not provide information on pulp tissue or microbial biofilms that may remain attached to the root canal walls, particularly in areas not reached by the mechanical action of the instruments, such as isthmus, fins, anastomoses, and accessory canals (Versiani *et al.* 2013). This means that micro-CT has been essentially limited to the evaluation of the changes to the dentinal walls since, in its essence, micro-CT is unsuitable to image soft tissues as they are virtually "transparent" for X-rays. This limitation is related to the inability of this bone research-derived radiographic method designed to depict denser elements such as calcium, to detect non-radiolucent soft tissues (Rüegsegger *et al.* 1996). However, there has been significant progress in micro-CT based research in other biomedical areas, including different types of bench-top scanners, capture of phase-contrast information, more rapid and more effective scan acquisition protocols and reconstruction algorithms. Taken together, such developments can be exploited to image soft (non-calcified) tissues overcoming its inherent limitation. For that, specific soft tissue visualization enhancement can be achieved using radio-opaque contrast agents to attain X-ray attenuation, the so-called contrast-enhanced micro-CT technique (CE-CT). In short, CE-CT is suitable to assess heterogeneous tissues such as teeth.

Contrast agents are composed of specific chemical agents with a high molecular weight able to naturally bind to soft tissues to create "contrast" in an effective way. The contrast agent used in the present study was the inorganic Lugol's Iodine (I_2KI), which was first introduced by Metscher (2009a), who tested several sample fixation protocols and the staining potential of several commercially accessible compounds for a number of types of soft tissues. To date, it has been demonstrated that Lugol's Iodine has a high affinity for glycogen (Fennerty 1999) and targets epithelial cells and mouse soft tissue (Degenhardt *et al.* 2010, Baverstock *et al.* 2013). Thus, the potential of Lugol's Iodine to achieve pulp tissue imaging with micro-CT was tested in the current study. Through a series of trials with various impregnation protocols, teeth with conventional access cavities immersed in Lugol's solution for 7 days allowed the effective impregnation of pulp tissue (**Fig. 3** and **Fig. 7**). However, two aspects regarding the use of the CE-CT method to analyse pulp remnants longitudinally after root canal irrigation with NaOCl solution may be of concern. The first aspect relates to the possibility of NaOCl reacting chemically with the iodine of the Lugol's solution. This chemical interaction was verified and Lugol's solution did not significantly reduce the oxidizing ability of NaOCl. The second concern was the potential of NaOCl to reduce the degree of contrast associated with the Lugol-impregnated pulp tissue. The

radiopacity test revealed that Lugol's solution was suitable for impregnating pulp tissue as the NaOCl did not interfere with its radiopacity. Taken together, these results confirmed that Lugol's solution can be used as a contrast agent for testing pulp tissue as substrate for the analysis of NaOCl-based irrigation protocols. A further analysis focused on the validation of the Lugol's solution in identifying pulp tissue properly. For that, paraffin-based histological sectioning was used to confirm whether the pulp tissue impregnated with Lugol's solution was visible on micro-CT scans. An experimental approach was then developed to compare the histological sections with their corresponding images acquired from the micro-CT stacks, overcoming typical alignment problems in this type of correlative analysis. The results confirmed the correct identification of pulp tissue in the Lugol-impregnated micro-CT images and thus proved the quality of the impregnation protocol (**Fig. 4** and **Fig. 5**).

It is of note that, particularly for Endodontic research using the remaining pulp tissue as an outcome parameter, the CE-CT approach has the clear advantage of not focusing on an ultrastructural detailed evaluation of the soft tissue. Instead, CE-CT easily enables quantitative assessment of the remaining pulp tissue as a whole in longitudinal (overtime) experiments (**Fig. 6** and **Fig. 7**). From a qualitative standpoint, hundreds of cross-sections produced per tooth by CE-CT may render a better understanding of the close relationship between the internal anatomy of root canals, and mechanical shaping and irrigation protocols. This is because CE-CT delivers 3D high-resolution models, which contain true-to-life information on the dimensions, structural quantification, and anatomic features of heterogeneous tissues, e.g. dentine and pulp tissue. At the same time, this method allows the evaluation of the preoperative distribution of the pulp tissue throughout the canal space before the experimental procedures even after 7 days without any fixation protocol. This is an important point as the amount and location of the pulp tissue may act as a confounding factor, affecting the outcome of the experiment. In this way, the use of the CE-CT method in teeth with vital pulps seems to be valid and reproducible, since pulp tissue was distributed along the entire root canal system in all sound teeth. Future studies using this innovative method should include the comparison of different irrigant solutions (inert vs active solutions) overtime and preparation protocols on the dissolution/removal efficiency of the pulp tissue from the root canal system. Further improvements on this method would also allow it to be applied in *in vivo* research using CBCT, for instance. At the moment, the present methodology requires the contrast agent to be in contact with pulp tissue for at least 7 days and, in an *in vivo* approach, it would also require pre- and post-operative tomographic imaging, which clearly needs to come comply with acceptable ethical research principles. Definitely, the present protocol should be validated in vivo using CBCT. On the

other hand, it can be safely applied *in vivo* by using non-carious and non-restored teeth with vital pulps scheduled for extraction without pre- or post-operative scans. For example, after confirming the vital condition of the pulp by conventional tests, the chemomechanical protocol can be applied *in situ* and the contrast solution injected into the pulp canal space and the coronal access cavity restored to ensure the Lugol's solution remains within the root canal space. Then, the tooth can be extracted, stored, and evaluated through micro-CT imaging after one week.

In summary, the current study thus concentrates on providing preliminary but original evidence to support non-destructive longitudinal CE-CT studies using remaining pulp tissue as an outcome parameter. It was demonstrated that CE-CT combines, in a single method, the main advantages of micro-CT imaging technology (mineralized tissue evaluation) and traditional histological methods (non-mineralized tissue evaluation) to study root canal debridement procedures embracing the possibility of assessing, identifying and measuring those canal areas not affected by either mechanical preparation or irrigation protocols. Worthy of note is the fact that CE-CT allows mechanical canal preparation and irrigation protocols to be studied independently or the combined synergetic effect of chemical-mechanical procedures.

Conclusions

Lugol's solution allowed the visualization of pulp tissue on micro-CT images. Lugol's solution was unaffected by NaOCl and did not interfere with the dissolution of the fixed and stained soft pulp tissue. In practical terms, the contrast-enhanced micro-CT imaging technique with Lugol's solution presented here allows the effect of chemical dissolution and the mechanical removal of pulp tissue by cleaning and shaping procedures to be evaluated independently or together making it a most useful technique in laboratory-based Endodontic research.

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Figure legends

Fig. 1. Radiopacity of Lugol's solution as a function of dilution in either distilled water or 5.25% NaOCl.

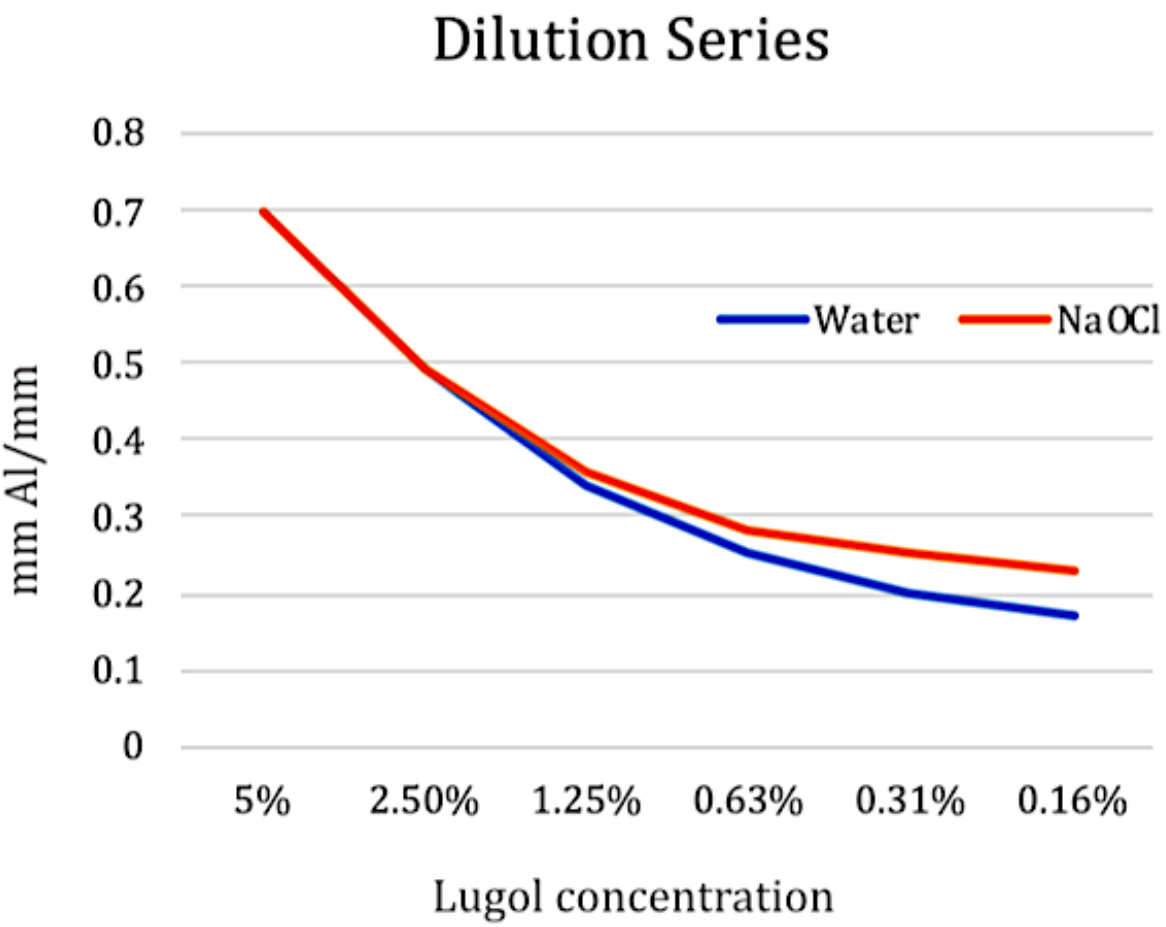


Fig. 2. Violin-plots illustrating the mean, minimum, and maximum values, as well as, the data distribution of the tested specimens; nonfixed tissue, fixed tissue and fixed and Lugol-stained tissue.

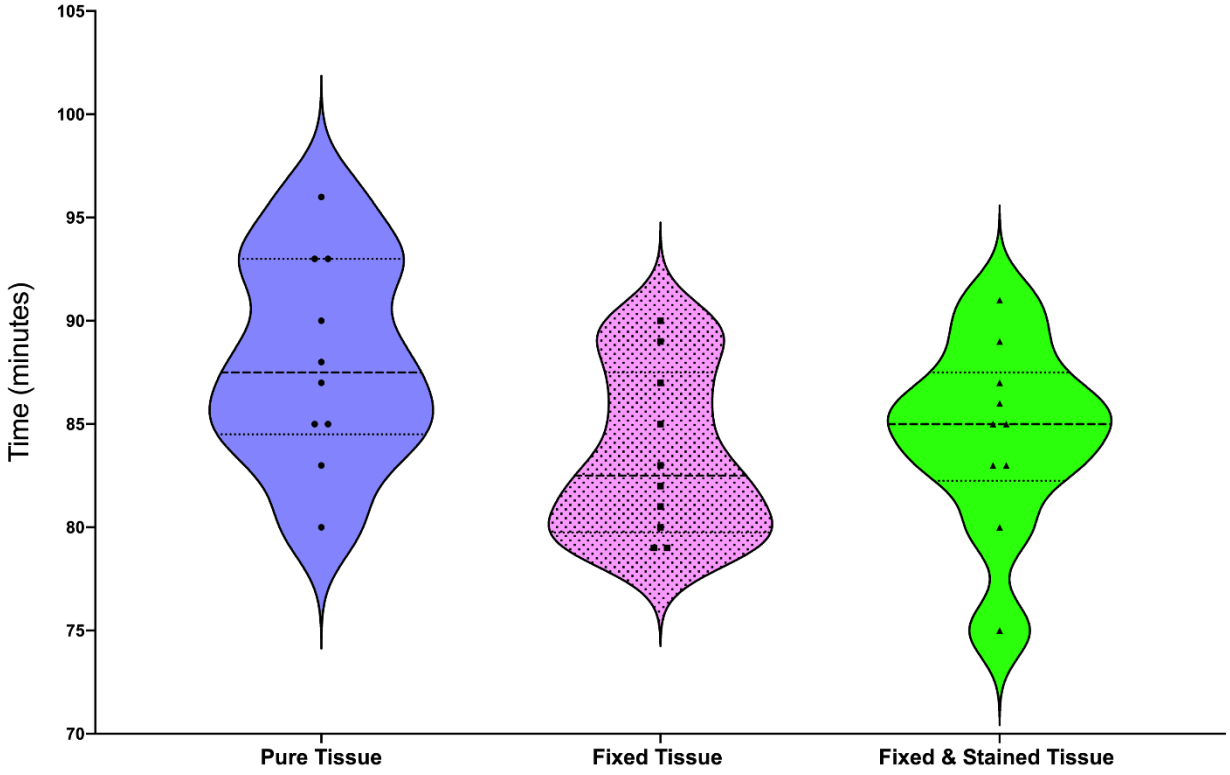


Fig. 3. 3D models of no-stained and Lugol-stained pulp tissue where it is possible to observe the efficacy of the staining protocol.

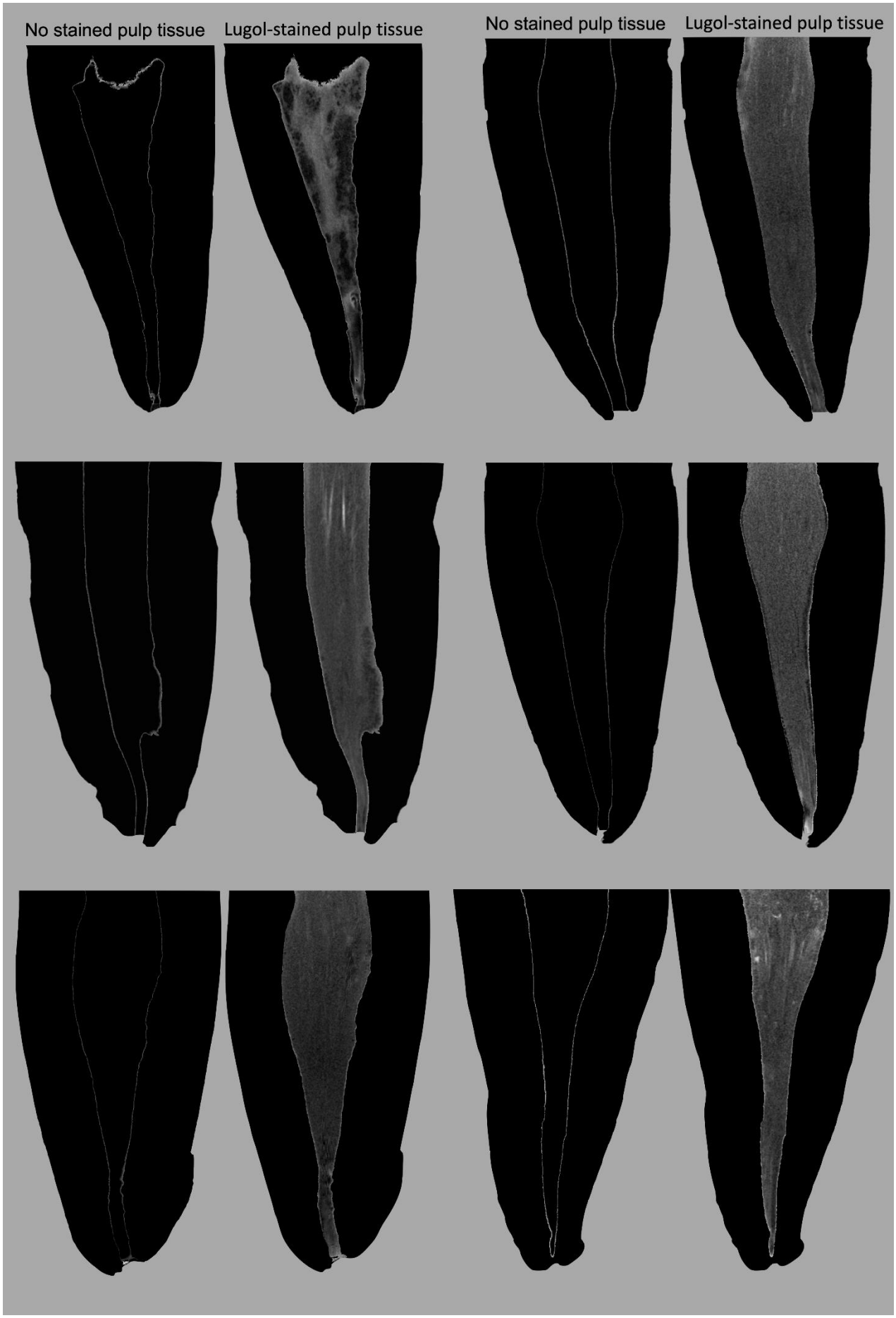


Fig. 4. Correlative analysis showing agreement between micro-CT and histological images, confirming the identity of the Lugol-stained of pulp tissue in a lower molar tooth. (a) Micro-CT image of non-stained pulp tissue; (b) Micro-CT image after the staining protocol with the Lugol's solution; (c) Distal canal in detail; (d) Corresponding histological slice attesting the full presence of the pulp tissue.

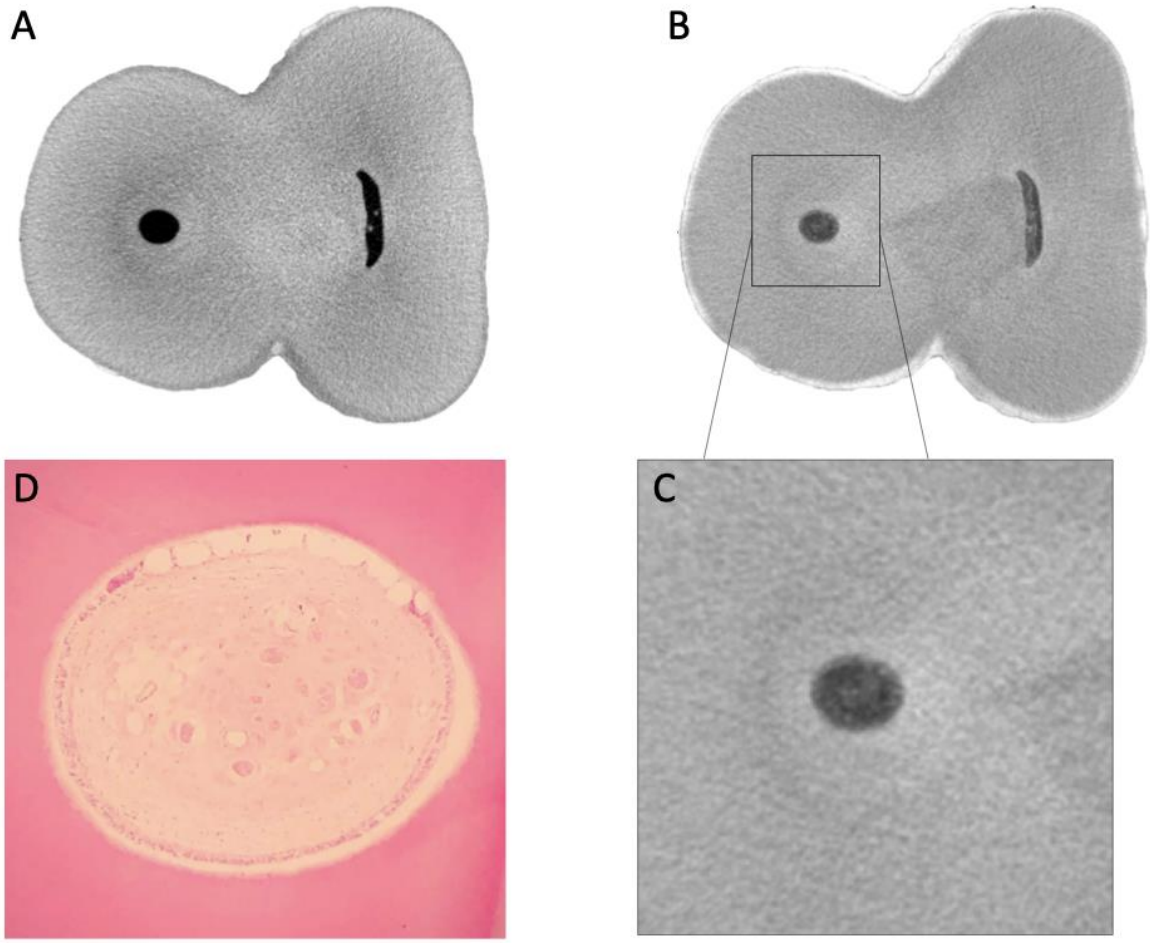


Fig. 5. Correlative analysis showing agreement between micro-CT and histological images, confirming the identity of the Lugol-stained of pulp tissue in a mandibular pre-molar tooth. (a) Micro-CT image of non-stained pulp tissue; (b) Micro-CT image after the staining protocol with the Lugol's solution; (c) Buccal extension of the canal in detail (d) Corresponding histological slice attesting the full presence of the pulp tissue.

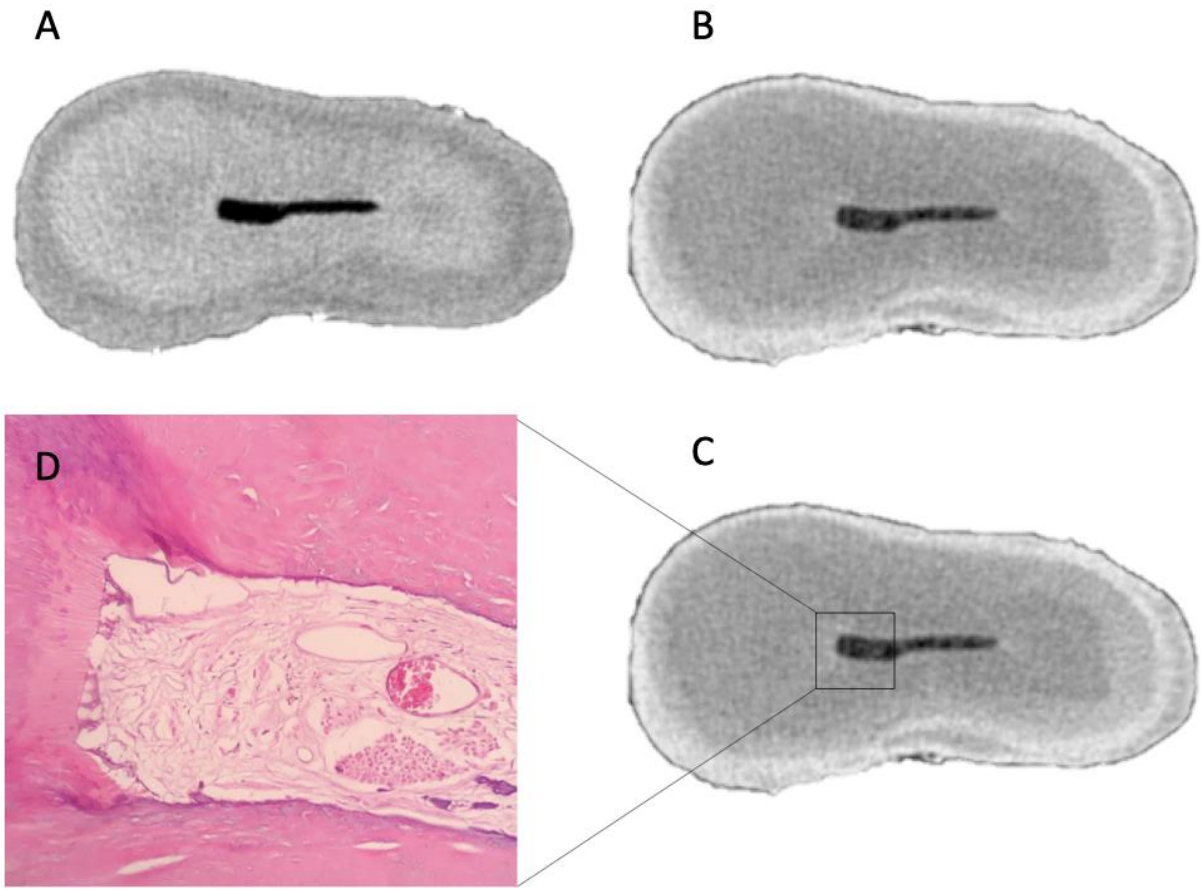
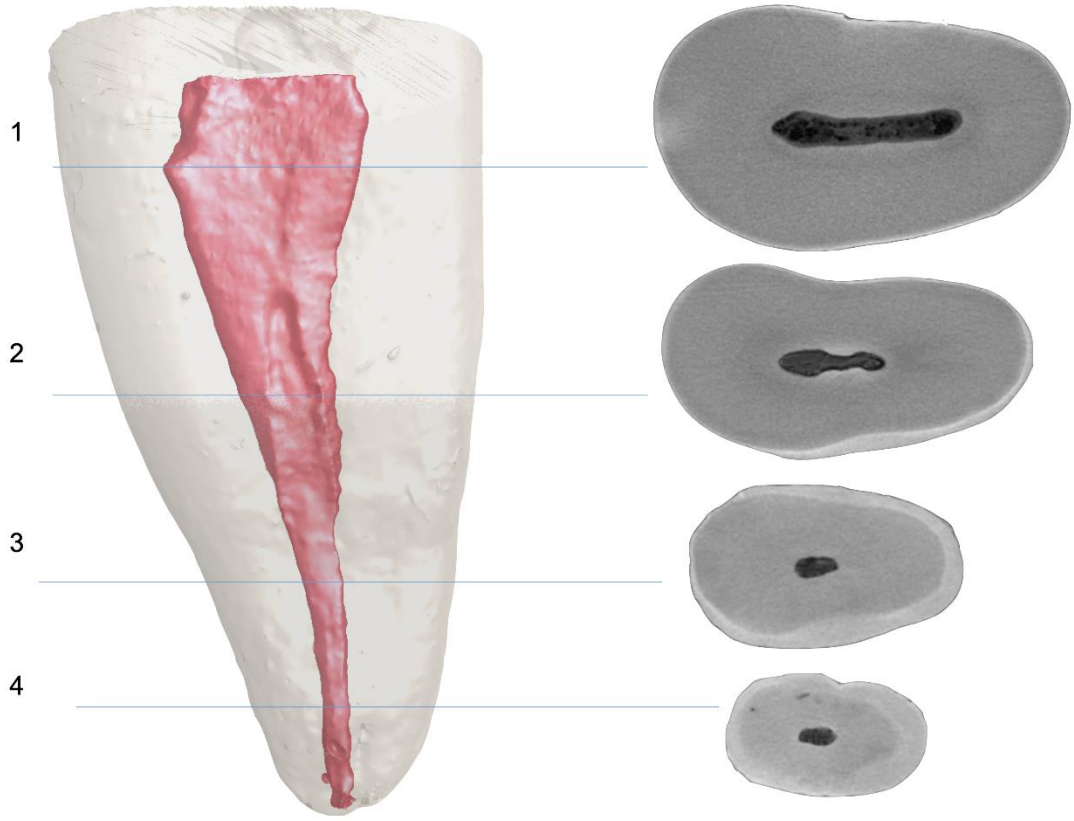


Fig. 6. 3D models of Lugol-stained lower pre-molar. (a) Lugol-stained pulp tissue (in red) before the canal preparation. Cross-section micro-CT images (1-4) showing the Lugol-stained pulp tissue besides the oval-shape of the canal; (b) 3D model after canal preparation where it is possible to observe the remaining pulp tissue in the buccal and lingual extensions of the root canal. Cross-section micro-CT images (5-8) showing the Lugol-stained pulp tissue in the irregularities of the canal space (yellow arrows).

A

Lugol-stained pulp tissue before canal preparation



B

Lugol-stained pulp tissue after canal preparation

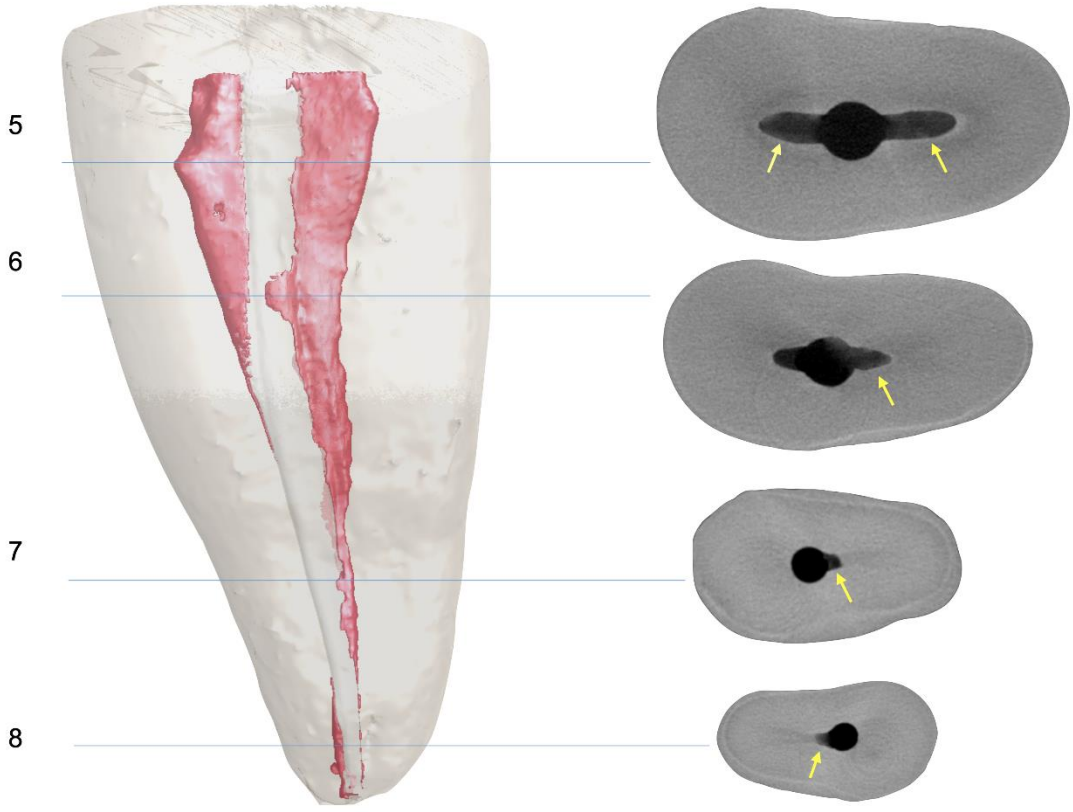


Fig. 7. Longitudinal and cross-sections images of the same (a) non-stained and non-prepared oval-shaped canal, (b) Lugol-stained non-prepared oval-shaped canal, and (c) Lugol-stained and prepared oval-shaped canal.

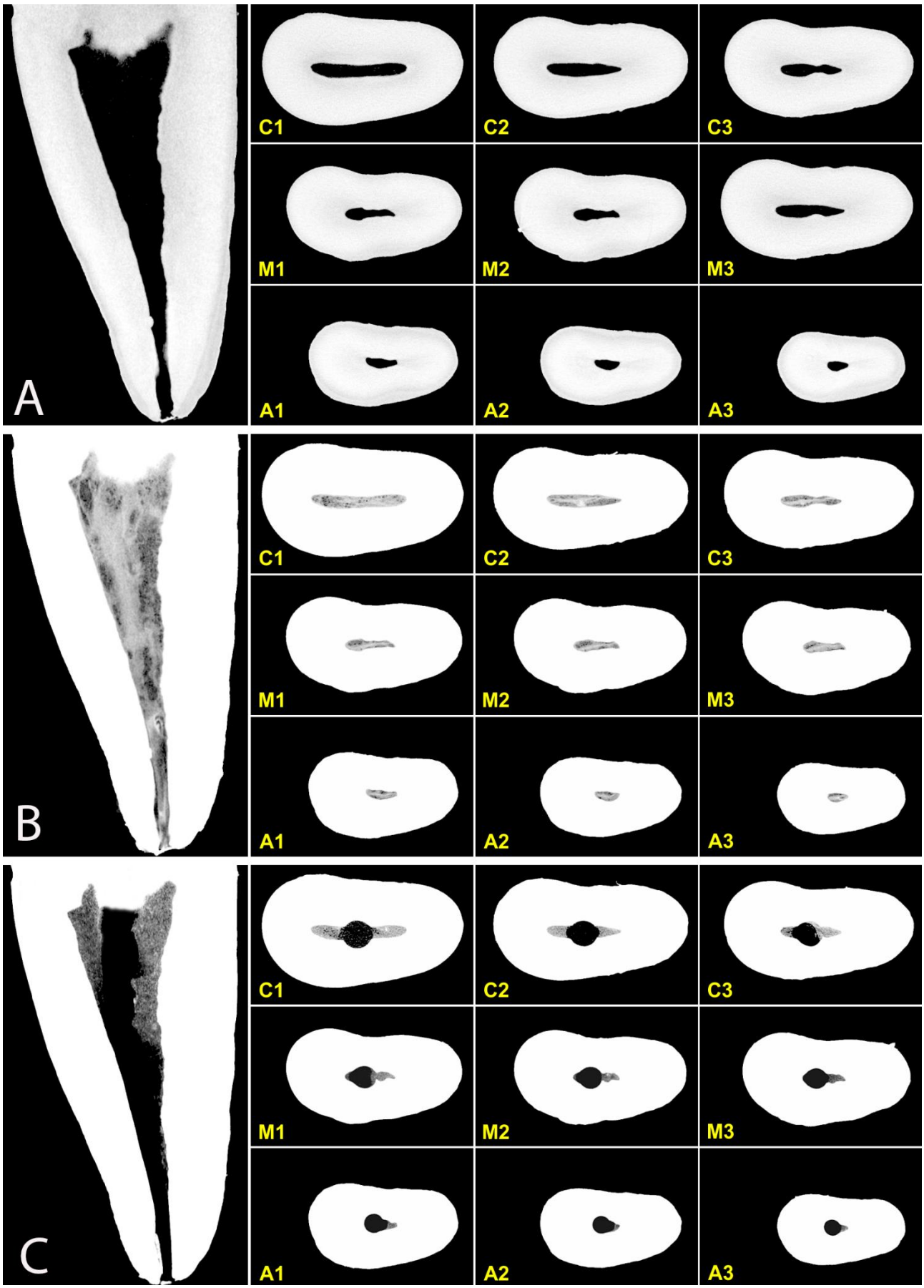


Table 1. Micro-CT quantification of the volume of root canal and pulp tissue of the fixed and stained (Lugol's solution) specimens before and after preparation procedures

Experimental steps	**Parameters**	**Specimens**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Before preparation	Canal volume (**mm^3**)	20.46	9.89	21.85	19.77	9.08	10.60	10.79	14.16
	Pulp tissue volume (**mm^3**)	19.36	8.72	20.84	18.03	8.76	10.35	10.67	13.99
After preparation	Canal volume (**mm^3**)	20.62	12.43	22.44	20.04	9.72	-	-	-
	Untouched canal walls (**%**)	90.40	58.10	95.76	96.73	72.18	-	-	-
	Pulp tissue volume (**mm^3**)	2.84	3.94	3.37	0.80	0.86	-	-	-
	Remaining pulp tissue (**%**)	14.67	45.18	16.17	4.44	9.82	-	-	-

Table 2. Comparative table between micro-CT, CE-CT and histological methodologies.

Method	Requires specimen preparation	Tissue Shrinking	Measurement Accuracy	Assess Hard Tissues	Assess Soft Tissues	Destructive	Labor-intensive	Number of Slices Produced	Ultrastructural Tissue Evaluation	3D images
Histological slicing	Yes	Yes	Poorly accurate	Yes after tissue decalcification	Yes	Yes	***	Usually few slices per root	Yes	No
Micro-CT	No	No	Highly accurate	Yes	No	No	**	Hundreds of slices per root	No	Yes
CE-CT	No	No	Highly accurate	Yes	Yes	No	**	Hundreds of slices per root	No	Yes

Supplementary material legend

Supplementary material 1. Video showing the same specimen after micro-CT scan without contrast, after the use of Lugol's staining protocol and after root canal preparation.