Rom J Morphol Embryol 2021, 62(3):793–800 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.62.3.17

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

Endodontic fillings evaluated using *en face* OCT, microCT and SEM

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Abstract

Objectives: The goal of endodontic therapy is mechanically cleaning and shaping the root canal system, the removal of organic and inorganic debris followed by sealing with permanent filling materials. *Materials and Methods*: Therefore, the aim of this *in vitro* study was to engage three imagistic methods: (*i*) *en face* (*ef*) time domain (TD) optical coherence tomography (OCT), (*ii*) micro-computed tomography (µCT), and (*iii*) scanning electron microscopy (SEM), in terms of their efficiency in assessing the quality of endodontic fillings. So far, is settled that *ef*OCT images can identify defects/voids in several of the investigated root canal fillings and identify gaps of 50 µm. *Results*: The results delivered by µCT technology also showed several imperfections of the endodontic filling but also at the interfaces formed by the sealer and the root canal walls. SEM investigations highlights the complex form of the interface formed the endodontic filling material and the walls of the root canal, as well as shortcomings of the materials from several samples. Gaps of 50 µm are identified with *ef*OCT. *Conclusions*: The net advantage of OCT technology, in respect to the other two technologies consists in its non-invasiveness. The OCT axial resolution is also sufficient to see the material gaps. Another advantage of *ef*OCT investigations is that they allow real-time imaging of complex arrangement at the interfaces of the filling material with dentinal root.

Keywords: endodontic filling, *en face* optical coherence tomography, micro-computed tomography, scanning electron microscopy.

Introduction

Dental treatments use various technologies and materials with heterogeneous structure, and therefore problems related to physical, chemical, and mechanical compatibility between these materials still exists. Complex restorative dentistry involves a range of treatments that include crown restorations with adhesive restorative materials, endodontic therapy, crowns, bridges, dental implants, and bone augmentation. For successful restorative dentistry, all the techniques and materials must consider the biocompatible features of materials, which must simultaneously support the natural healing, and to consider at the same time the physiology of all oral tissues [1, 2]. It would therefore be ideal to accurate analyze the possible deficiencies of any technique, dental material, and treatment, to prevent future complications or treatment's failure [3–5].

The success of any endodontic therapy depends by the diagnostic established at the beginning of the treatment plan, on the detailed knowledge of tooth morphology, endodontic cavity design and endodontic protocols for instrumentation and filling the root canal system. The endodontic infection is controlled by the rigorous removal of the organic and inorganic debris, by shaping the root canal, and by choosing materials with high chemical and physical properties [6, 7]. The success of the endodontic therapy depends by the sealing potential of the endodontic filling and the adhesive dental materials dedicated to crown restoration [8].

The main goal of endodontic fillings is to three-dimensional (3D) adequately seal [9] the endodontic main and secondary canals, to inhibit percolation and micro-leakage of periapical exudates and to inhibit the reinfection of the endodontic space. The best possible sealing must create a favorable biological environment that will support the healing process of the distressed periapical tissue [10, 11].

The complexity of the tooth anatomy and root canal, the tissular changes caused by dental preparations [12], as well as the treatment objectives, is the initial determinants in deciding the best method for achieving the 3D filling of the root canal. Cold lateral condensation and warm vertical compaction of gutta-percha are validated filling techniques, which passed the test of time [13, 14]. Expansion of medical research domain, lead to new approaches in endodontics like endodontic irrigating solutions based on gold (Au) and silver (Ag) nanoparticles [15] associated with thermo-plasticized endodontic materials. The new endodontic protocols promise to offer a higher quality sealing and improved prognostic on long term [16].

The 3D filling of root canal system is the main goal in endodontics. A high-quality 3D sealing must be upheld by qualitative dental materials and accurate instrumentation techniques. The competency of endodontic filling can be assessed *in vitro* by destructive and non-destructive investigation methods like dye penetration tests, bacterial penetration tests, penetration of radioisotopes, proteins and endotoxin penetration tests, gas chromatography, electrochemical leakage tests, fluid-transport model, fluorometric tests. Beyond X-rays and cone-beam computed tomography (CBCT), which are saved to *in vivo* and clinical evaluation, the research domain is now focusing for *in vitro* imagistic investigation like scanning electron microscopy (SEM), spectrophotometry, micro-computed tomography (μCT) [17–22].

The essential disadvantage of invasive investigation methods is the destruction of the samples. The sectioning process comes along with a loss of information about the sample and if the cut of the sample is not made in the interested area, the investigation regarding the microleakage is not conclusive.

Admitting that cone-beam μCT is an encouraging technology for endodontic therapy, still the radiation doses are higher than currently used for conventional intraoral panoramic imaging technologies [23]. This is a limitation of the CBCT technology.

The competence of optical coherence tomography (OCT) to image and map with high-resolution hard and soft oral tissues was proven by research studies. OCT is a non-invasive and non-radioactive optical diagnostic method based on low-coherence interferometry. The dominant advantage of OCT is that can provide micro-structural details which are reserved to this imaging technique [24]. OCT distinct feature is that is highly efficient for investigations of materials defects present not only on surface layer, mainly in the profound layers without damaging the samples. OCT finds applicability in the investigation of dental hard tissue, dental restorative materials, interface defects that support the microleakage phenomenon present at the interfaces formed by dental materials and dental hard tissues [25, 26]. By virtue of non-invasively investigation mode and ability to analyze the profound layers of the material, OCT is a future potential tool for *in vivo* imaging in dental medicine and endodontics [27, 28]. The *ef* version of OCT proves that real time evaluation of root canal filling adapting to the morphology of the root canal can be made non-invasively in real time [4, 5, 7, 29, 30].

Aim

This current *in vitro* study is assessing the quality of endodontic fillings by using next imagistic approaches: *ef*OCT, μCT and SEM.

Materials and Methods

The study fulfills the Declaration of Helsinki of 1975, revised in 2013. The protocol of the present *in vitro* study was approved by the Scientific Research Ethics Committee of Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania (Approval No. 25/28.09.2018).

For this study, 14 recently extracted caries free human teeth were selected. Before sample preparation, the teeth were cleaned with ultrasounds by debris and soft tissue. The samples were stored in distilled water with Thymol crystals prior preparation and imagistic investigation. To confirm the presence of a single canal for each root, the teeth were X-ray investigated from buccal and proximal view.

For the access cavities, preparations are made with dental rotary instruments, high-speed diamond burs and water spray. The next step was the instrumentation of the root canal with nickel–titanium (Ni–Ti) endodontic rotary instruments (System GT, Dentsply Tulsa Dental, Tulsa, Oklahoma, USA) following the standard protocol [31] (Figure 1, a and b).

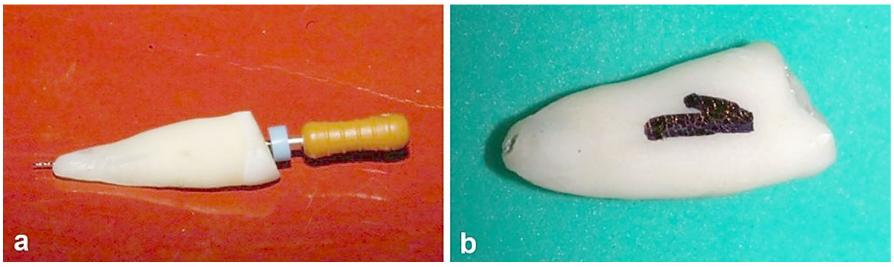

Figure 1 – *(a) Preparation of the root canals; (b) Prepared sample No. 1.*

(*i*) The access cavity is drill, cleaned and the orifices of endodontic canals are exposed. The patency and length of the root canal is made with a K-file No. 10 and the mechanical preparation is continued with Nos. 15 and 20. If any resistance is encountered, K-file No. 10 is replaced with No. 06 or No. 08. All canals are shaped with 6% taper GT instruments, according to the size of the apical foramen.

(*ii*) Each canal is firstly irrigated with 5% Sodium Hypochlorite, followed by alternative irrigation with 5% Sodium Hypochlorite and 17% Ethylenediaminetetraacetic acid (EDTA) for a total time of 30 minutes. All canals are instrumented with 6% taper GT instruments, according to the size of the apical foramen using the crown down technique. The root canals are progressively widened in the coronal and middle thirds. The instrumentation ends with apical preparation considering the size of the apical foramen. Every root canal was dried using 6% paper points fitted to the size of apical foramen until complete drying.

(*iii*) Each canal is filled using a proper Resilon master cone and compacted with Buchanan pluggers (down-pack phase).

(*iv*) The elected sealer for root canal filling is Resilon (Resilon–Epiphany, Pentron Clinical Technologies, USA), a thermo-plasticizer polymer of polyester. Resilon has a melting temperature of +60ºC.

Technique used for the endodontic filling was the continuous wave of compaction. This technique can be divided into two sequences: down-pack and back-pack phase.

Down-pack phase

It is chosen the adequate plugger (Buchanan pluggers, Analytic Technology) that reaches up to 3–5 mm to apical foramen. A Resilon cone of 0.06 taper that fitted the apical size of the preparation is used as master cone for down-packing.

Resilon is a thermoplastic synthetic polymer-based material destined to root canal filling. Its basic chemical composition is a polymer, polycaprolactone, which is biodegradable aliphatic polyester. Filler particles are a bioactive glass, Bismuth Oxychloride and Barium Sulfate. Resilon polymeric matrix consists of 25–40% Polycaprolactone (PCL) and 3–10% Dimethacrylates. The sealer is a semi-crystalline polymer with a melting point of +60ºC. The sealer release Na^{+} and Ca^{2+} ions. The material has greater radiopacity then dentin. Tensile strength, elastic modulus, and melting point of Resilon are approximately equal with gutta-percha, a favorable characteristic for preventing different contraction values after setting. The thermoplasticity of Resilon is higher than other systems that use thermoplastic gutta-percha like Endoflow and Obturaflow [32, 33].

Resilon bonding material (Epiphany Primer) was injected in the root canal (Figure 2a) and the excess of sealer was eliminated with paper points (Dentsply Maillefer, Ballaigues, Switzerland).

The Resilon Epihany master cone was coated with Resilon sealer and positioned into the instrumented root canal (Figure 2b). The cone was shortened to the orifice cavum with the tip of a plugger connected to the System-B heat source (Analytic Technology) set on 160±10ºC and on full power mode. With a single passage, the heated plugger is pressed through Resilon master cone up to 3 mm short of the roots' apical foramen. The plugger is inactivated but the apical pressure is preserved. The plugger advances toward apical foramen to inter-counter any shrinkage of the sealer during cooling. After cooling, the plugger is heated for one second to be possible the separation from Resilon mass and to be removed without displacing endodontic material. The excess of Resilon is removed with paper points and the root canal is backfilled by injecting the thermo-plasticized Resilon.

Back-pack phase

Root canals walls are coated with paper point immersed in Resilon sealer. The Resilon Epihany pellets are heated in the Obtura II device (Obtura Spartan, Fenton, MO) and injected into the root canal. The already plasticized Resilon is condensed into the middle part of the root canal with pluggers that adapt to the size of endodontic canal. Another Resilon application and compaction is necessary until the root canal is completely filled up to the endodontic orifice (Figure 2c).

(*v*) The 2 mm coronal part of the sealer is light cured for 40 seconds, and the coronal seal is achieved. The self-curing time in the root canal is between 30–60 minutes. The coronal seal of the samples was made with a composite resin.

The imagistic investigation took place afterwards, along the following steps:

(*1*) The teeth were evaluated radiologically with the endodontic filling adapted to the morphology of the root canal (Figure 2d).

(*2*) Time domain (TD) OCT is working in *ef* mode and was engaged to image the instrumented and filled samples. *Ef*OCT can provide real time images in two orthogonal planes (B- and C-scans). The method is suited to convey tomographic information and identify the individual morphology of the root canal [34, 35].

OCT system is operating at 1300 nm at a working distance of 2–3 cm. The depth resolution is 10 μm (in air). To guide the OCT investigation the technology is supplied with a confocal channel operating at 970 nm.

The samples are positioned in a holder device which allows 3D micrometric movements (Figure 3).

(*3*) μCT was first employed to validate the *ef*OCT imagistic evaluation of the endodontic instrumented and endodontically filled teeth.

The custom build μCT system [36] consists of a high resolution micro-angiographic detector [37], a micro-focus Ultrabright Oxford X-ray tube and a XYZ+rotary stage for sample's stand. The detector has a 1024×1024 matrix with 45 μm effective pixel size [38]. The detector resolution is better than 10 lp/mm and field of view (FOV) is ~4.5 cm. The samples were scanned with a tube voltage of 40 kVp and tube current of 1 mA, at a magnification between 2× and 1.1×. The parameters are adjusted to the sample size. After the acquisition, the samples were reconstructed using a standard Feldkamp–Davis–Kress algorithm [39]. Each data volume was $(512)^3$ voxels with an isotropic voxel of 45 μm^3. The scanning was made from occlusally toward apical foramen.

(*4*) The samples were investigated by SEM connected with an energy-dispersive X-ray (EDX) analyzer. The device is Inspect S (Figure 4) (FEI, North America NanoPort, Hillsboro, Oregon, USA) and holds a high magnification microscope. The images of the samples are generated by a focused scanned electron beam. The primary electrons are generating a low energy, but the secondary electrons are the ones that generate the topography of the specimen. Ionized atoms are discharged by electron shell to shell transmission which is further driven to the X-ray emission or by Auger electron ejection. The discharged X-rays bear the characteristics of the elements of the sample and are measured by the EDX detector. EDX analyzer provides information about quantitative composition of the analyzed sample.

The system is supplied with a wolfram (W) filament in the electronic cone. The working parameter is 200 V at 30 kV and the beam current is >2 μA.

On a standard specimen with Au particles separated by carbon (C) particles, the resolution is 3.0 nm in both high- and low-vacuum operational modes. The focusing range is between 3–99 mm (in this case 9.6 mm) and magnification is from 6× to >1 000 000× on a standard liquid-crystal display (LCD).

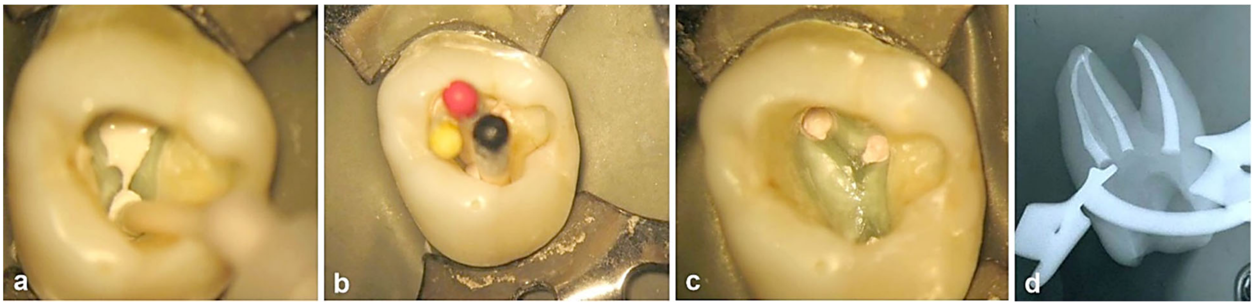

Figure 2 – ***(a–c) Clinical aspects of the endodontic filling technique; (d) Radiographic evaluation of one of the prepared samples.***

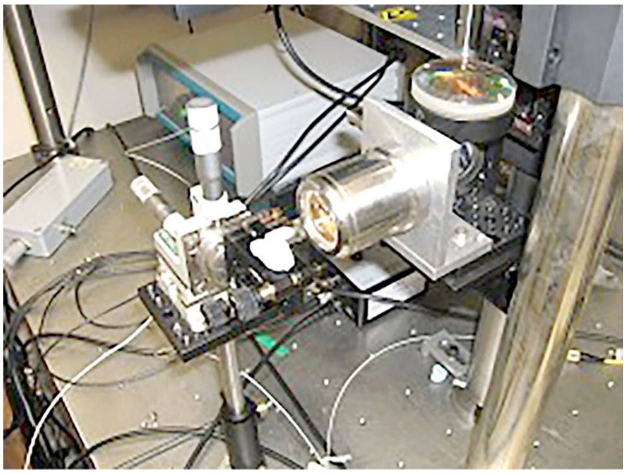

Figure 3 – ***Holder device of OCT system positioned in front of the interface optics lens. OCT: Optical coherence tomography.***

Figure 4 – ***The Inspect S device used for SEM/EDX analysis. SEM/EDX: Scanning electron microscopy/ Energy-dispersive X-ray.***

High vacuum mode (10–5 mbar) is engaged for imagistic and microanalysis of conventionally prepared specimens. Low vacuum mode (<270 Pa) is engaged and used for imagistic and microanalysis of unprepared samples. The FOV respects the same parameters in high and low vacuum (18 mm at the largest working distance).

Results

Our *in vitro* study evaluated the quality of endodontic fillings and its adaptation to the morphology of the endodontic complex space with three different imagistic technologies: (*i*) *ef* TD OCT, (*ii*) µCT, and (*iii*) SEM.

X-ray imagistic investigation of endodontic filings made with Resilon appears to have favorable clinical prognostic. The high contrast of the filling material evident on the X-ray, show a qualitative sealing of the endodontic filling and lack of gaps and free interface and at the apical end of the filling. On the X-ray investigation, the possible micro-leakage phenomena appear to be absent because of perfectly sealing of the apical end root. The material filling perfectly seals the apical end.

Findings of current study are materialized in the images achieved by *ef*OCT and show defects at the interface formed by the root canal wall and endodontic filling material. For some investigated samples, the defects are inside the endodontic filling material, such as shown in Figures 5–7. These investigations can be used to identify gaps as small as 50 µm.

The contrast between the endodontic filling and dentin is evident. The endodontic filling has higher opacity. In the apical end of the filling, a defect is localized close to the interface formed by dentin and endodontic filling. The Resilon sealant slightly and non-uniformly covers the external apical end of the root. The size of the defect is estimated to be 0.225 mm inside the apical end (Figure 5).

Figure 6 shows two gaps in the apical end, which are laterally positioned toward the dentin walls of the root canal. The high contrast between the filling material and dentin, clearly contour and validate the presence of the defects.

Figure 7 shows an image from a stack of 44 C-scan images acquired at a differential depth of 10 µm measured *in air*; 3D reconstruction of the apical end images the root canal walls that surround the root canal sealer and gutta-percha cone. The gap-like apical end defines the limits of the area susceptible to microleakage.

The *ef*OCT imagistic evaluation of the human teeth with endodontic treatment was first validated by µCT investigation. Figure 8 shows the µCT investigation of the same sample presented in Figure 6: two-dimensional (2D) cross-sectional image of the apical end filling demonstrates the two defects of different diameters, which are marked by arrows. The defects are at the interface, localized between the wall of the dental root canal and endodontic sealing – because are being identified next to the dentin walls of the root canal. The endodontic filling is highly radio-opaque in comparison with the dentin and because of this characteristic the gaps are clearly defined.

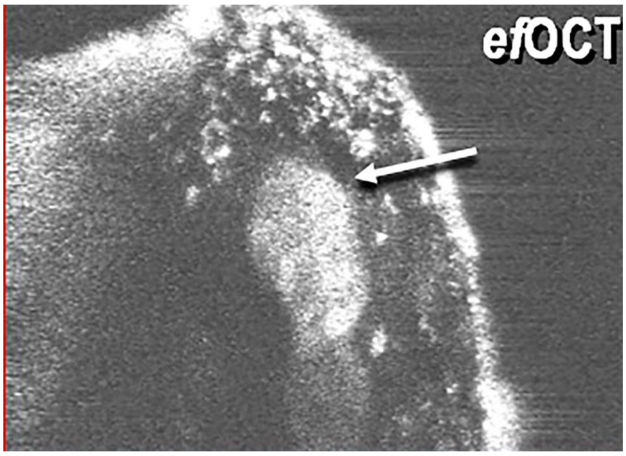

Figure 5 – ***C-scan image (0.95×0.95 mm), approximately 0.225 mm in depth measured in air, inside the apical root, 18° lateral scanning size.***

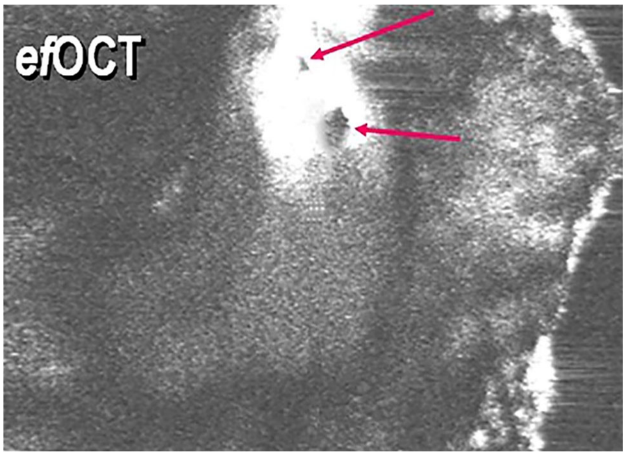

Figure 6 – ***C scan image (0.95×0.95 mm), approximately 0.975 mm inside the apical root measured in depth in air, 18° lateral scanning size.***

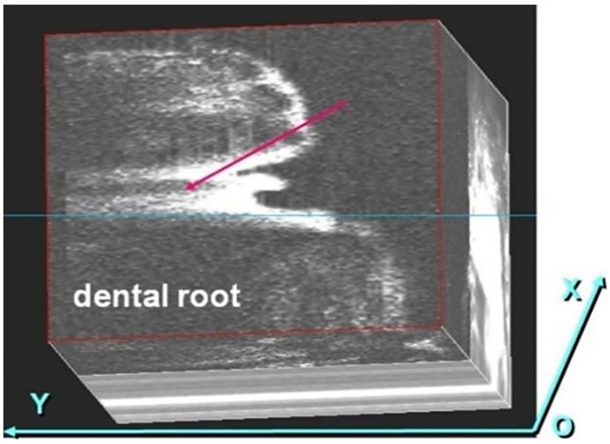

Figure 7 – ***OCT investigation of a root canal filling using the dual efOCT/confocal system, showing defects inside the endodontic material (red arrows). B-scan, approximately 0.7 mm inner the apical third of the root in OY and 0.453 mm in OX, 18° lateral scanning. efOCT: En face optical coherence tomography.***

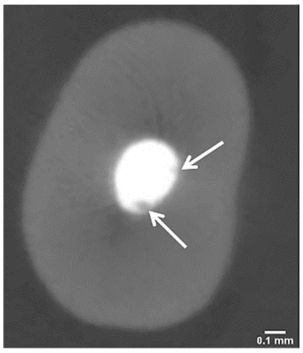

Figure 8 – ***µCT investigation of the same sample presented in Figure 6. µCT: Micro-computed tomography.***

On the SEM imagistic investigation of the apical endodontic end can be seen a tight connection of material filling on gutta-percha matrix coated with the sealer at a magnification of 180×. It is present a tight connection of the dentin with Resilon polymeric filling material. A thin layer of sealant is covering the apical end. The arrows on the image presented in Figure 9 point out defects as gaps between the endodontic filling layers and localized close to the dentin-filling material interface.

The EDX microanalysis of the SEM investigation from Figure 9 is presented in Figure 10. From the mentioned Figure, the defects are validated. The EDX proved that at the interface between the material and the possible gap (observed in black in the Figure 9) there are different elements with the proportions presented in Figure 10. This means that there are no artifacts in the interested area.

Discussions

A comprehensive overview of the causative factors of non-resolving periapical lesions that are seen as asymptomatic radio-translucencies after endodontic treatment, emphasizes the presence of residual micro-organisms in the apical area of the root canal system. This is the major cause of persistent apical periodontitis for poorly and properly treated cases [18]. The apical third of the endodontic filling in the root canal is the most susceptible area to micro-leakage. Due to complex and not completely accessible morphology apical infiltration is still common in endodontic treatments and thus raises concerns about the quality of the filling provided by the filling materials currently available. The appearance of the spaces and the interface like gaps in the root canal is primarily attributed to the quality of the interface configured by the adhesive endodontic material and dentin, as well as the difficult and unfavorable configuration of the endodontic morphology [7, 29, 30].

(*a*) Regarding the results obtained with these investigations, several aspects are notable: OCT is employed as a non-invasive method in assessing with micro-depth resolution the micro-leakages of apical space after root canal fillings. In contrast, common methods of investigation, except X-rays and CBCT, which are still the exclusive methods used for *in vivo* assessment, the quality of endodontic treatment is evaluated with invasive technologies and leads to the destruction of the probes and loss of information [17].

(*b*) OCT does not present the problem of radiation exposure that limits the applicability of radiology and µCT.

(*c*) The dual confocal microscope (CM)–TD OCT system allows precise location of sectioning planes inside the sample. For conventional methods, such radiology, the investigation is less conclusive in respect to micro-leakage detection.

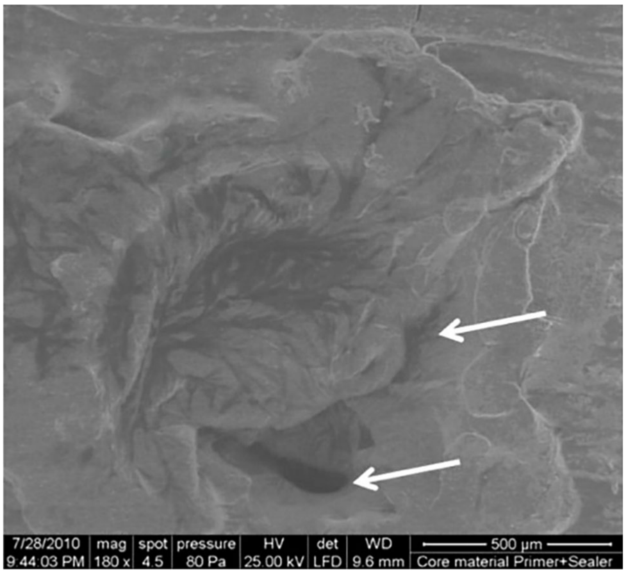

Figure 9 – ***SEM image: complex appearance aspect of the interface formed by the endodontic filling material and walls of the root canal (the same sample as that presented in Figures 6 and 8). SEM: Scanning electron microscopy.***

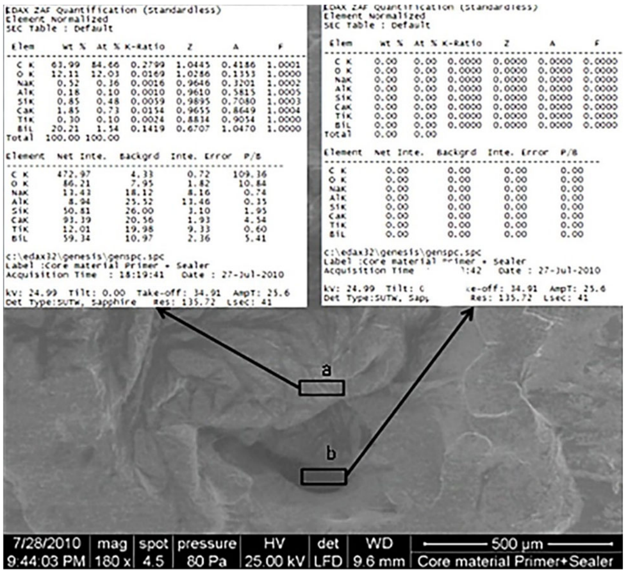

Figure 10 – ***The EDX microanalysis of the sample investigated by SEM in Figure 9. The composition of the structure in the selected areas is presented above the figure, showing the existence of different materials (a) or voids (b). EDX: Energy-dispersive X-ray; SEM: Scanning electron microscopy.***

(*d*) The continuous adjustment capability of the sectioning plane of the CM–TD OCT system has been illustrated, as an example, by showing *ef* slices from different depths in Figures 5 and 6. The *ef* view can be used to display a complete sweep in depth through the sample, thus allowing precise identification of defects inside the investigated material. 3D reconstructions, as shown in Figure 7 allow an entire view and clear display of gaps in the apical root canal filling [5, 7, 15, 29].

(*e*) The TD OCT working in *ef* mode provides complementary results in terms of the qualitative evaluation of the root canal fillings, compared with the established imaging methods. Comparison of images obtained with OCT (Figure 6), μCT (Figure 8), and SEM (Figure 9) demonstrates that either of these methods can be used to assess the leakage. The OCT and μCT identify the defects due to the air or adhesive materials trapped inside. Air or adhesive materials appear similar in the OCT images, as well as in μCT images. Therefore, the SEM/EDX method was essential to identify that the material defects are voids and their origin [30, 36, 40–42].

Moreover, artifacts can be corrected or reduced with the reconstruction algorithms. The results can show a significant difference between the measurement volumes among the Rx devices and μCT device. Thus, μCT could be used as the "gold standard" in evaluating the artifacts based on root canal sealer.

The limitation of this study was that the study was based only on backpack and down-pack, Thermafil technique root canal sealers. Further tests should be made with the other filling techniques and other sealers. Further studies should be conducted to enable full comparisons using both different filling techniques and scanning at different voxel sizes [41, 42].

(*f*) The SEM/EDX protocol acts as a valuable tool for the investigation of the interfaces formed by the endodontic material, to validate the results obtained by the other methods. The analysis made in Figure 10 shows different types of materials that exist in the area where the endodontic material is considered. The preparation of a sample for SEM analysis is one of the most critical aspects of this investigation method [30, 43].

There are two methods to prepare the samples. The first method uses the original samples in a dehydration and drying procedure that may create artifacts in the hard tissue. The dehydration and drying procedures may create artifacts in the samples' morphology and differences in observation analysis. The indirect approach is more conservative and follows a protocol consisting of taking an impression of the sample with a highly accurate impression material. A positive model is manufactures and examined. The second method preserves the original sample characteristics [40].

An imperfect seal at the interface between the root-end filling and the cavity margin is a common feature after endodontic sealing. It could be hypothesized that the presence of gaps would favor a continuous bacterial leakage from the infected root canal system to the periapical tissue and sustain the periapical tissue inflammation. In this case are available nutrients that might easily diffuse into the apical canal tubules and support the metabolism of surviving bacteria. The phenomenon is emphasized when the root-end filling is absent. After the endodontic therapy, an undetected root canal or a secondary canal which was not filled by the sealer maintain the infiltration and infection of complex endodontic system.

Conclusions

With OCT technology, we can achieve a better and safer axial resolution. The resolution is increasing when the source spectrum is wider. Even though the development of endodontic dental materials is in continuous progress, at present, it cannot meet all the requirements for 3D hermetic sealing of the root canal system. Therefore, post-endodontic treatment is essential. This study manages to show that the presented imaging technology is optimal to be use for assessing and the defects present in root canal filling. Using μCT technology, defects such as gaps within the root canal fillings and at the interfaces between root canal walls are confirmed. The SEM assessments reveal the observation of the interested areas by mapping the surface. *Ef*OCT investigations proved to be qualified to visualize complex layered structures restrained at the interface between the filling material and the dental hard tissue, as well as in the apical region.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

Adrian Gheorghe Podoleanu and Meda-Lavinia Negruţiu contributed equally to the conceptualization and supervision of this *in vitro* research.

Consent for publication

Consent for publication of the results of this study was obtained from all the participants. All the specimens used in this research are extracted teeth for orthodontic purpose and the patients gave their consent for using the teeth as specimens for research purposes.

Data Availability Statement

Data is available upon request from the corresponding author.

Acknowledgments

This work was performed in the frame of a PhD grant of the Victor Babeş University of Medicine and Pharmacy, Timişoara, Romania, No. 4133/01.10.2018 (Contract No. 16082/02.11.2018). Virgil-Florin Duma and Cosmin Sinescu acknowledge the support of the Romanian Ministry of Research, Innovation and Digitization, CNCS/CCCDI–UEFISCDI, project PN-III-P2-2.1-PED-2020-4423, within PNCDI III. This paper is based on results presented by the authors in conference proceedings papers, included in the References list.

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Received: November 3, 2021 *Accepted: January 20, 2022*