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Imaging methods to evaluate the quality of root canal system filling by endodontic sealers used in *in vitro* studies

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ABSTRACT

Three-dimensional obturation of the root canal system is the primary goal of endodontic treatment. Endodontic sealers should fill any irregularities in the root canal system and penetrate as deeply as possible into the dentinal tubules. They should also ensure that the gutta-percha points adhere well to the dentinal walls of the root canal without leaving voids at their margins.

The purpose of this study was to evaluate contemporary imaging techniques used for *in vitro* testing of selected specimens consisting of tooth scrapings after endodontic treatment with gutta-percha and selected endodontic sealers: AH Plus-Dentsply Sirona, Well Root ST-Vericon, Korea, and AH Bioceramics Sealer-Dentsply Sirona. The following imaging techniques were used in the evaluation: scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), micro-computed tomography (micro-CT), surgical microscopy, and light microscopy.

A comparison of the imaging methods (SEM, CLSM, micro-CT, surgical microscopy, light microscopy) showed significant differences in their suitability for assessing the penetration depth of endodontic sealers into dentinal tubules *in vitro.*

Based on the analysis of the imaging methods, it was concluded that confocal microscopy and SEM were the most objective and accurate methods for visually assessing the quality of root canal filling *in vitro*. However, the high cost of specimen preparation and the limited availability of specialized equipment are factors that limit their use in clinical settings.

Keywords: root canal sealers; intratubular penetration; SEM; CLSM; micro-CT; adhesive interface.

INTRODUCTION

Three-dimensional obturation of the root canal system is the primary goal of endodontic treatment [1]. Endodontic sealers should fill any irregularities in the root canal system and penetrate as deeply as possible into the dentinal tubules. They should also ensure that the gutta-percha points adhere well to the dentin walls of the root canal, and without gaps or voids at their margins and in the lumen of the filled root canal [2]. The ideal sealer should ensure good adhesion to the root canal wall, provide an airtight seal, have no shrinkage after bonding, be insoluble in tissue fluids, and be biocompatible with the surrounding tissues [3]. Currently available and used endodontic sealers include zinc oxide-eugenol sealers, calcium hydroxide sealers, glass ionomer sealers, methacrylate resin sealers, and the relatively recently introduced calcium silicate sealers [4].

The dentin-sealer interfacial bond can be assessed using various imaging techniques [5]. In scientific studies, scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), micro-computed tomography (micro-CT), and light microscopy are most commonly used to evaluate the dentin-sealer interfacial bond and the depth of penetration into the dentinal tubules *in vitro*. In the clinical setting, one may also use the surgical microscope to evaluate the homogeneity and uniformity of the root canal filling.

Scanning electron microscopy is used to evaluate the sealer-dentin interfacial bond and the depth of penetration of the sealer material into the dentinal tubules [6, 7, 8]. Specimens for analysis in the SEM should be properly prepared by dehydration, demineralization, polishing, and observation in a high vacuum, which nonetheless can produce artifacts that result in the creation of artificial gaps that prevent proper, objective analysis of the bond [9, 10].

Confocal laser scanning microscop is used to evaluate the bond and allows assessment of the depth of sealer penetration into the dentinal tubules, even in a moist environment [11, 12]. A fluorescent dye – rhodamine B [13] or alcohol-substituted porphyrin [14] should be added to sealers analyzed by CLSM to allow the excitation of the material through specific wavelengths and into the visible spectrum [15].

Increasingly, micro-CT analysis is being used for *in vitro* testing of specimens. It is a non-destructive analytical method that provides objective data that can be examined both quantitatively and qualitatively. Volumes of gutta-percha and sealers can be calculated using special software. It is also possible to locate specific details using visual image analysis. With this technology, we can describe gutta-percha, endodontic sealers, homogeneity of canal filling, and voids. We can also visualize all anatomical structures of the tooth with high accuracy and spatial resolution [16].

The purpose of this study was to compare contemporary imaging methods used for *in vitro* examination of selected specimens, i.e. tooth scrapings after endodontic treatment with gutta-percha and selected endodontic sealers. The following imaging techniques were used in the evaluation: SEM, CLSM, micro-CT, surgical microscopy, and light microscopy.

MATERIALS AND METHODS

The study was conducted on human teeth extracted for periodontal or orthodontic reasons. Patients voluntarily agreed to participate in the study. Anatomically, the teeth were round or oval-round canals (1:1.2 diagonal ratio) in single-rooted teeth. These teeth had not been previously endodontically treated, had a fully formed anatomical apex, and the diameter of the apex opening was not less than ISO 10 and not more than ISO 20.

For the study, 15 premolar teeth were used, of which 13 were single-rooted, single-canal teeth, and 2 were double-rooted, double-canal teeth.

The root canals were prepared mechanically with Poldent Azzure instruments using a crown-down technique to a tool size of 35.04 ISO for the full working length and filled with 3 different endodontic sealers: AH Plus-Dentsply Sirona, Well Root ST-Vericon, Korea, and AH Bioceramics Sealer-Dentsply Sirona, and 2 obturation techniques: single cone (SC) and continuous wave technique (CWT). A total of 19 canals were prepared and filled in the premolar teeth.

The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin, KB-006/48/2022, dated November 16, 2022.

Scanning electron microscopy and specimen preparation of endodontically filled teeth before scanning electron microscopy analysis

The roots of the teeth, after filling the root canal system with the selected sealer and obturation technique, were stored in a greenhouse at 37°C and 100% humidity for 7 days to allow the sealer to set. Following the protocol of Teixeira et al. for the fixation and dehydration processes, the specimens were demineralized with 6 mol/L hydrochloric acid for 30 s, and deproteinized with 2% sodium hypochlorite solution for 10 min. They were then washed with distilled water, dried at 37°C for 24 h, and placed in a vacuum chamber. The roots were then cut with a diamond disk perpendicular to the long axis at a distance of 5 mm from the tip of the anatomical root. A disc-shaped section of approx. 1–2 mm thickness was obtained. The specimen was then cleaned in an ultrasonic bath, dried, and subjected to preliminary analysis under a video-track light microscope to confirm that the sealer and gutta-percha were not damaged during specimen preparation at the cutting stage [8, 9].

The specimens were embedded in chemically hardened resin and ground on special discs designed for ceramics. A conductive layer of approx. 300 A° was then deposited on [10]. The resin-encapsulated and ground preparations were evaporated with a gold-palladium alloy by physical vapor deposition (PVD) using a JEOL JEE 4x vacuum sputtering machine. The vacuum during the formation of the coatings was $1 \cdot 10^{-4}$ Pa. The penetration of the sealer into the dentinal tubules was evaluated using a Hitachi SU-70 field emission SEM. Scanning electron microscope imaging of the specimens was performed using a backscattered electron detector BSE (composition contrast), where each shade of gray in the image indicates a different chemical composition.

Confocal laser scanning microscopy – specimen preparation

For CLSM observation, all sealers used in the experiment were mixed with a solution of 0.1% saturated alcoholic porphyrin [14]. The root canals were rinsed twice with alternating citric acid and sodium hypochlorite prior to filling, and then rinsing was terminated using sodium hypochlorite, isopropyl alcohol, or chlorhexidine [17, 18, 19]. After filling the root canal system with the selected sealer and obturation technique, the roots were stored in a greenhouse at 37°C under 100% humidity for 7 days to allow the sealer to set. All roots were then sectioned perpendicular to the long axis with a diamond disk under constant water cooling. Sections (1 mm thick) were cut at 1 mm, 5 mm, and 8 mm from the root apex, and the surfaces of the cut discs were polished with sandpaper of the selected grit size. The specimens were mounted on slides and examined under CLSM to evaluate the depth of sealer penetration into the dentinal tubules. Sealer labeling and imaging on a Nikon Ti-E inverted microscope with a Nikon A1 confocal system (Nikon, Tokyo, Japan) in TRICT mode were used to examine the depth of sealer penetration into the dentinal tubules [14]. After rinsing, the canals were dried and filled with labeled epoxy sealer. The fluorescein- or porphyrin-labeled sealer is characterized by excitation in the appropriate wavelength range and emission of photons at wavelengths different from the excitation. Excitation was achieved with a laser set at 488 nm (for fluorescein) and 561 nm (for porphyrin).

Scanning electron microscopy and confocal laser scanning microscopy analysis of specimens, grading scales used

In the study of the specimens, the analysis was performed by taking images of 4 standardized areas of each root segment at ×100 magnification. The images were digitally captured and allowed the evaluation and comparison of the adhesive bond of the sealers to the root dentin and gutta-percha walls. The intracanal penetration of the sealers into the dentinal tubules was also measured [20]. Specimens in SEM and CLSM were analyzed for adhesion of sealers filling the root canal system to root dentin walls. Sealer penetration into the dentinal tubules was also evaluated to assess the depth of sealer protrusions [20].

A 5-point scale (0–4) by Tedesco et al. was used to evaluate the bond of sealers to the root dentin walls, where: 0 – absence of a continuous and homogeneous interface, with gaps in all areas; 1 – continuous and homogeneous interface in 1 area; 2 – continuous and homogeneous interface in 2 areas; 3 – continuous and homogeneous interface in 3 areas; and 4 – continuous and homogeneous interface in 4 areas [20].

Another scale by Tedesco et al. was used to evaluate the penetration of sealer tags into the dentinal tubules, where: 0 – no tags, 1 – tags <20 μm, 2 – tags 20–100 μm in length, and 3 – tags >100 μm [20].

This study also used a 3-point scale for the sealer-dentin interfacial bond according to Ray and Seltzer 1991 [21, 22], where 1 – very good adhesion, with a line of contact at the sealer-dentin interface with no gaps; 2 – good adhesion, with a curved contact line at the sealer-dentin interface and small gaps between sealer and dentin wall; and 3 – acceptable adhesion where gaps are frequently observed in the middle of the sealer-dentin wall and the sealer-dentin interface had an indistinct and highly curved contact line.

The Bolles et al. scale can be used for determining [23, 24] the depth of penetration of sealers in the CLSM analysis. The deepest penetration was measured from the canal wall to the point of maximum sealer penetration using a measuring device and ImageJ software (National Institutes of Health, USA). Measurements were taken twice to ensure consistency and repeatability. Sealer penetration depth was measured at 4 points on the clock face at 3, 6, 9, and 12 o'clock corresponding to the buccal, mesial, lingual, and distal directions, respectively [13, 25, 26].

Various studies use different units to express the depth of the penetration of endodontics sealers into dentinal tubules in the CLSM analysis; in our study measurements were made in microns (μm) [18].

Micro-computed tomography – specimen preparation

The canals of the premolar were filled with AH plus sealer using the lateral gutta-percha condensation method. After obturation, the tooth was stored in a greenhouse at 37°C and 100% humidity for 7 days to allow the sealer to set. Using a Skyscan 1174 microtomograph (Bruker, Belgium), the tooth was scanned with a 1.3 MP VDS camera with a resolution of 1024x1024, an X-ray source of 50 kV, an exposure time of 3400 s, a flip angle of 0.7°. A1 filter 0.5 and scan time 1 h 6 min. Reconstruction software NRecon ver. 1.6.10.4, for 2D analysis Data Viewer ver. 1.5.2.4 and for 3D CT Vox ver. 3.1.2.

Micro-CT analysis can be used to determine: the presence of voids, the percentage volume of root canal filling (the sum of gutta-percha and sealer volumes), the volume of internal voids distributed within the root canal, root canal filling material, external voids along the canal walls, and combined voids at the interface of a sealer and canal walls [27, 28, 29].

Surgical microscope and specimen preparation

A maxillary premolar, after filling the root canal system with the selected sealer and obturation technique, was stored in a greenhouse at 37°C with 100% humidity for 7 days to allow the sealer to set. The roots were then sectioned perpendicular to the long axis with a diamond disk under constant water cooling. A 1 mm thick section was taken approx. 5 mm from the root tip. The specimens were embedded in alginate paste. Observation of the specimen under a treatment microscope does not require any special preparation of the specimen [30, 31]. A Carl Zeiss surgical microscope was used to observe the surface of the specimen at a magnification of 2.5 times.

Light microscope and specimen preparation

The primary purpose of light microscopy observations is the qualitative and quantitative analysis of the microstructure of the specimen. It makes it possible to observe the microstructure of materials and to detect microdefects that cannot be seen with the naked eye or during macroscopic observations. First of all, it leads to the identification of the components that form it, determining the type, size, quantity, distribution, and morphology of the components that make up the microstructure. Among other things, studies using the technique of light microscopy make it possible to determine the shape and measure the size of the components in the structure of the observed materials; reveal defects in the microstructure in the form of microporosity, microcracks, inhomogeneity of the structure; and reveal and measure the thickness of the elements of the preparation with an altered structure. Using a light microscope requires appropriate preparation of the observed specimens, the surface of which should be ground and polished beforehand. For light microscopy studies, specimens prepared for scanning microscopy observation were used, but without the process of sputtering the gold-palladium conductive layer. No chemical etching was performed. Taking into account the different possibilities of contrast formation from the details of the microstructure in the microscopic image, modern light microscopes have the following possibilities for observing the microstructure: in a bright field of view; in a dark field of view; in polarized light and with phase contrast [32]. The specimens were examined on a Keyence VHX7000 reflected light microscope (observation of opaque specimens) using the dark field technique.

RESULTS

A comparison of current imaging techniques (SEM, CLSM, micro-CT, surgical microscopy, light microscopy) used to assess the penetration depth of sealers into the dentinal tubule lumen revealed significant differences in the usefulness of the aforementioned imaging techniques.

Scanning electron microscopy

A photograph of a gutta-percha-filled and sealer-filled Well Root ST bicuspid premolar taken in cross-section 5 mm from the root apex under electron microscopy shows the dimension of the penetration depth of the sealer into the dentinal tubules. The analysis showed that the above method of evaluating the depth of penetration of the sealer into the dentinal tubules is very useful and accurately shows the root canal space occupied by the gutta-percha point, the sealer, the adhesion of gutta-percha and sealer to the canal lumen. The depth of sealer penetration into the dentinal tubules was measured to compare the depth of sealer tags. In the test performed with the single point method: gutta-percha with Well Root ST sealer (Fig. 1a), a maximum penetration depth of 350 µm was obtained and with the method: vertical condensation of gutta-percha by CWT heat with AH Bioceramics sealer (Fig. 1b), the maximum penetration was 15 µm. The SEM imaging technique used allows the observation of porosity and possible voids at the periphery of the gutta-percha point and root dentin walls (Fig. 1). In the specimens shown in Figure 1, according to the Tedesco et al. scale [20], which evaluates the sealer-dentin interfacial bond, the score was 4, i.e. homogeneous bond with no gaps over the entire surface of the specimen. When examining the penetration of the sealer into the dentinal tubules, a score of 3 was obtained in the specimen in Figure 1a according to the scale of Tedesco et al. [20], and a score of 1 was obtained in the specimen in Figure 1b.

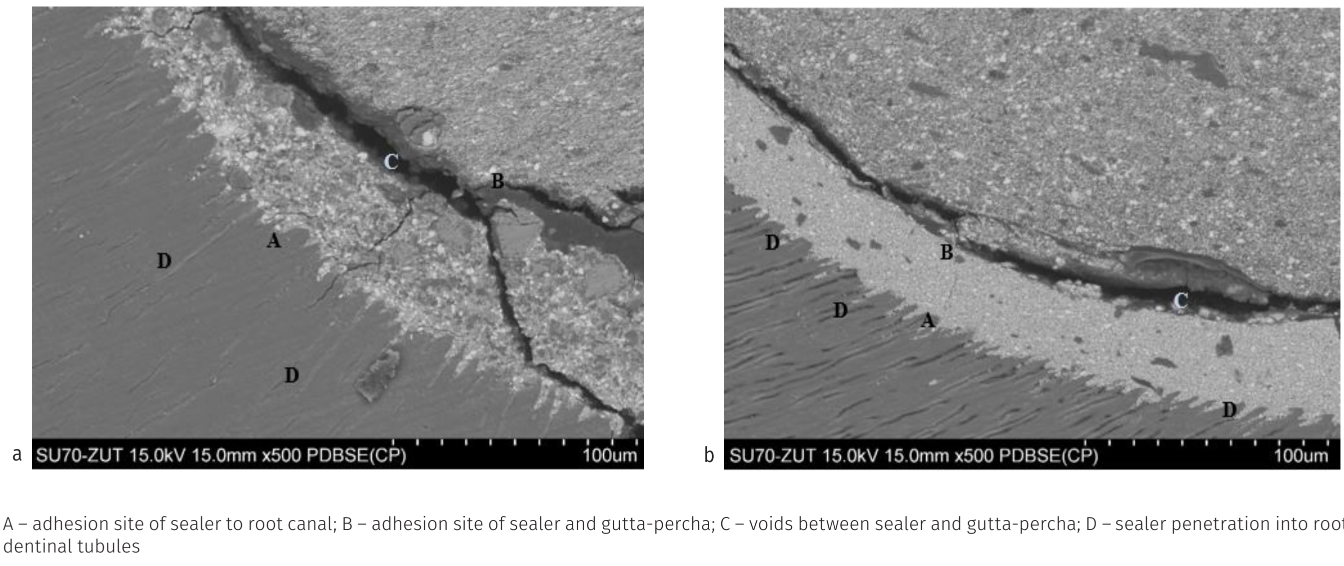
In order to evaluate the connection of the sealer with the root dentin walls, we also used the Ray and Seltzer scale, whose score in the specimens in Figure 1 was 1. This means very good adhesion of the sealer to the root dentin walls over the entire surface of the tested image of the specimens, without gaps, and maintaining the continuity of the connection.

Confocal laser scanning microscopy

Images taken by confocal microscopy technique show cross sections of the root of a premolar tooth at 5 mm and 8 mm from the top of the root starting from 10 µm from the surface of the specimen to 35 µm inside the specimen. The maximum penetration depth of the sealant was 25 µm (Fig. 2).

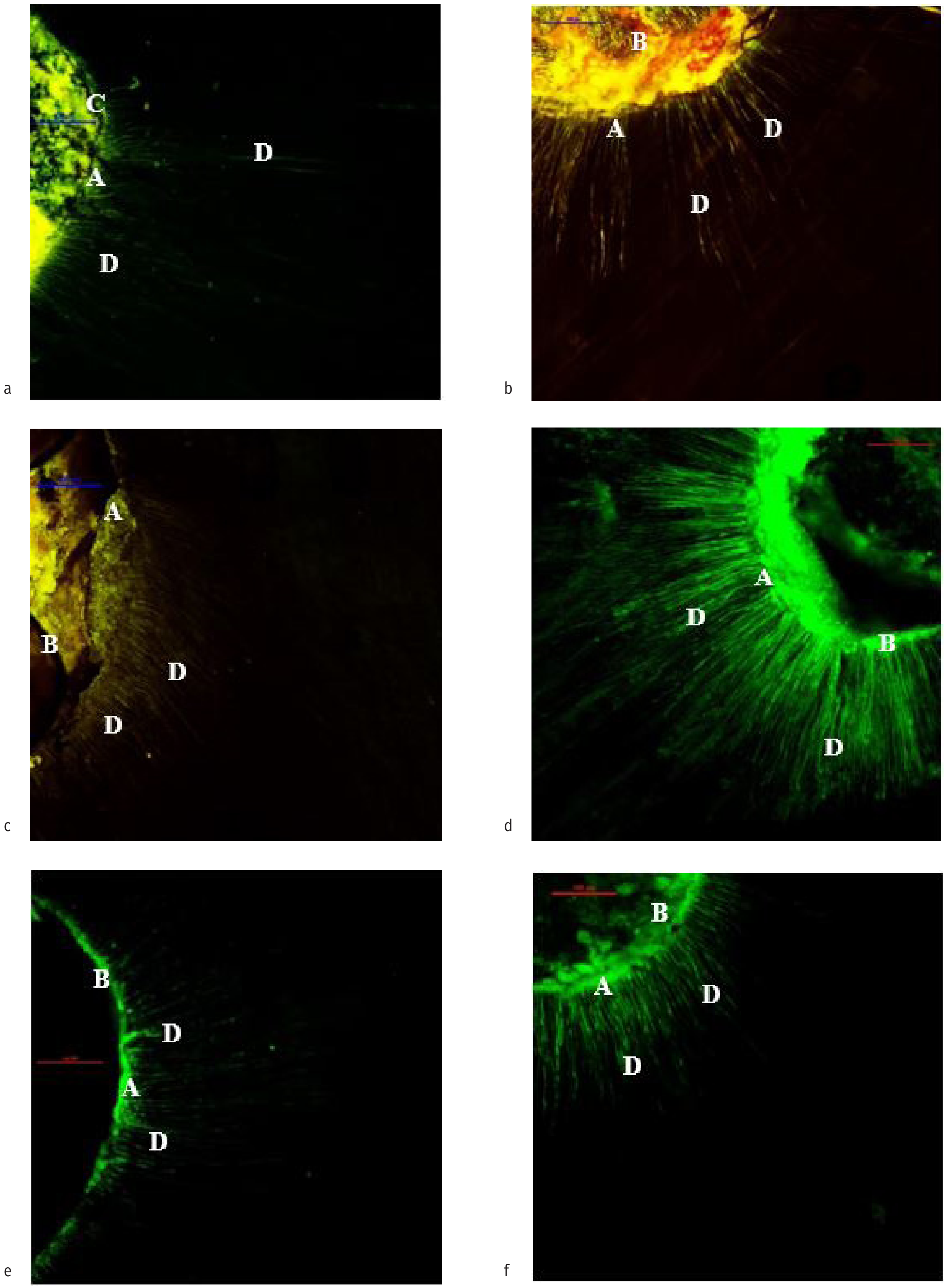
Each sample was imaged in 4 quadrants in a clockwise direction with a slice thickness of 25 µm. Digital images were obtained by averaging an overlay of 50 stacks taken in 0.5 µm increments starting at 10 µm from the sample surface to a depth of 35 µm from the sample surface. Resin tag lengths were measured in ImageJ 1.45 s software (National Institutes of Health, USA) in all 4 quadrants for each section. For each image, a series of 32 measurements were taken in fixed fields arranged radially around the channel axis every 2.8 degrees, and the measurement result for each field was the average value of the resin tag length in that field.

The figure shows the sections of a root cross-section at a distance of 8 mm (Fig. 2a, b, c) and 5 mm from the root apex (Fig. 2d, e), taken using the porphyrin emission imaging technique in 2 channels (green and red laser). Depending on the substance used for root canal irrigation, i.e chlorhexidine (Fig. 2a, d), isopropyl alcohol (Fig, 2b, e), and sodium hypochlorite (Fig. 2c, e), we observed different penetration depths of the resin sealer into the dentinal tubule lumen.



A – adhesion site of sealer to root canal; B – adhesion site of sealer and gutta-percha; C – voids between sealer and gutta-percha; D – sealer penetration into root dentinal tubules

FIGURE 1. Scanning electron microscopy images: (a) CP method: gutta-percha with Well Root ST sealer; (b) continuous wave technique method and AH Bioceramics sealer



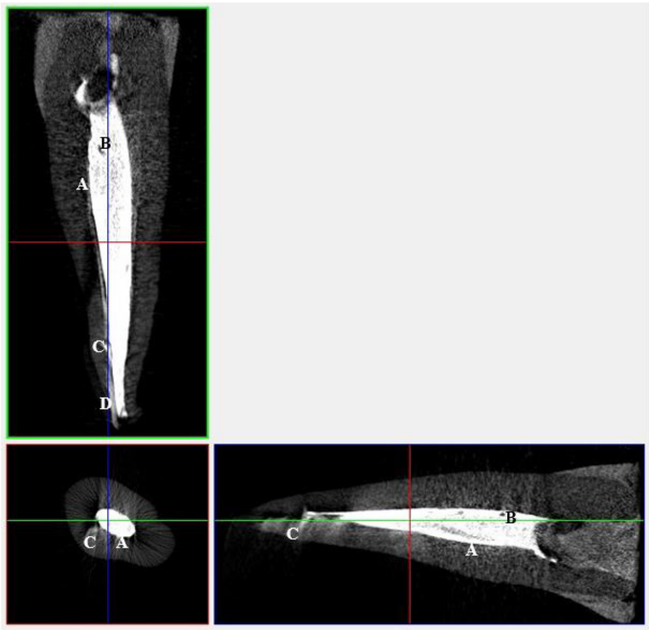
A – site of sealer adhesion to the root canal; B – site of sealer adhesion to gutta-percha; C – voids between sealer and gutta-percha; D – site of sealer penetration into dentinal tubules

FIGURE 2. Photographs taken by confocal laser scanning microscopy. A section of an endodontically filled premolar with gutta-percha and fluorescein- or porphyrin-labeled epoxy sealer (a–f)

Micro-computed tomography

The micro-computed tomography images show a premolar tooth after endodontic treatment and filled with a lateral condensation of gutta-percha and AH Plus sealer (Fig. 3).

The analysis showed good adhesion of the gutta-percha and sealer to the root canal lumen, visible penetration of the sealer into the accessory canals, and voids between the sealer and the canal wall.



A – the site of the sealer's adhesion to the walls of the root canal; B – voids in the lumen of the gutta-percha-filled canal; C – filling of the accessory canal with sealer

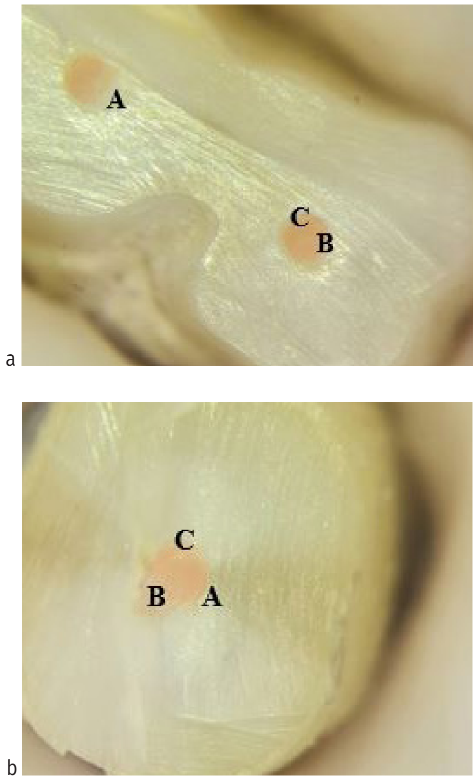
FIGURE 3. The micro-computed tomography images of a premolar. Cross-section through the frontal (top photo), transversal (middle), and sagittal (bottom) planes

Surgical microscopy

Surgical microscope images show a cross-sectional view of the maxillary first premolar at a distance of 5 mm from the root apex. The canals were filled with Well Root ST bioceramic sealer using the single gutta-percha point method (Fig. 4).

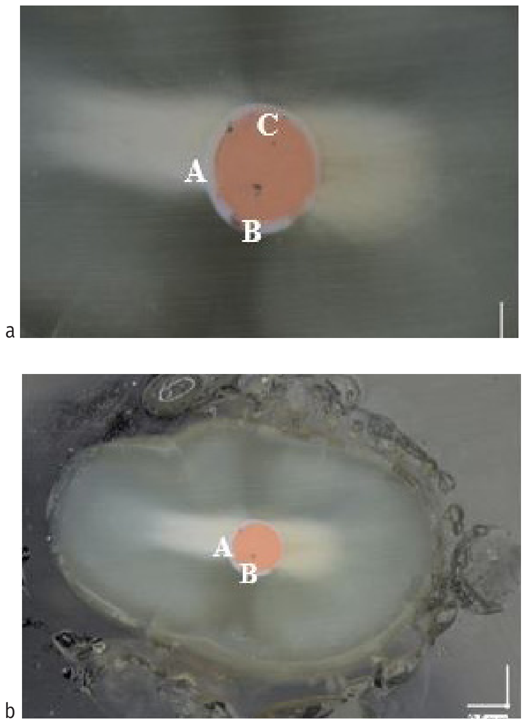
Light microscopy

Light microscopy images show a cross-section of a mandibular lateral incisor filled with AH Bioceramics Sealer by CWT at a distance of 5 mm from the root apex. The adhesion of the sealer to the root canal wall was evaluated, as well as the area where the sealer and gutta-percha meet, with visible voids between them. Homogeneity and uniformity of the filling in the lumen of the gutta-percha point can be observed (Fig. 5).



A – adhesion site of sealer to root canal; B – adhesion site of sealer and gutta-percha; C – voids between sealer and gutta-percha

FIGURE 4. Photographs taken with a surgical microscope: (a) right maxillary first bicuspid. Canals filled with Well Root ST bioceramic sealer using the single gutta-percha point method, magnification x2.5; (b) upper right maxillary second molar. Canal filled with AH Plus epoxy sealer using the single gutta-percha point method, magnification 1.6



A – site of adhesion of sealer to root canal; B – site of adhesion of sealer and gutta-percha; C – voids between sealer and gutta-percha

FIGURE 5. Photographs taken by light microscopy, mandibular right premolar tooth filled by continuous wave technique with AH Bioceramics Sealer: (a) magnification 50x, (b) magnification 100x

DISCUSSION

The objective of the conducted research was to evaluate the effectiveness of various microscopy techniques, including light microscopy, SEM, CLSM, micro-CT, and operative microscopy, in assessing the depth of penetration into dentinal tubules by selected endodontic sealers, depending on the filling method applied: SC and CWT. This pilot study is part of a larger research endeavor, encompassing the optical analysis of sealer penetration in 78 single-rooted teeth.

The research project was made possible through a collaborative effort among multiple national academic institutions, fostering cooperation between researchers from medical and technological universities. The primary motivation for initiating a multi-center study stemmed from the lack of access to specialized materials and equipment infrastructure at the home university, which are essential for conducting such experiments. Furthermore, the initiative aimed to foster collaborations with other scientific research institutions, thereby facilitating the exchange of knowledge and experiences.

The issue of leakage, due to its crucial importance in endodontics, has been the subject of numerous research works using optical methods such as SEM, CLSM, micro-CT, and light microscopy, as well as operating microscopy [33, 34]. The above methods allow the spatial evaluation of the quality of the filling of the root canal system and the bond between the sealer and gutta-percha and the root dentin walls. The evaluation in SEM and CLSM allows the observation of areas of interest by surface mapping, to obtain a large amount of information that positively influences a more complete evaluation of the structures under study, i.e. to determine the distribution and thickness of the sealer layer and the continuity of the connection and adhesion of different surfaces, including the presence of voids and the size of any gaps [35]. Compared to conventional SEM, CLSM has the advantage of providing detailed information on the presence and distribution of sealers or bonding systems within dentinal tubules or at the site of dentinal tubule protrusions in root canals at relatively low magnification and in non-dehydrated specimens by using rhodamine- or porphyrin-labeled sealers soaked in 0.1% alcohol. In addition, the method uses undrained specimens or hard tissues that do not require special sectioning techniques and can be visualized at different depths [12, 36, 37]. Confocal microscopy also has the advantage of measuring from an area of 10–35 microns below the surface of the specimen. It is therefore less random in terms of the grinding angle of the specimen relative to the axis of the dentinal tubules and therefore provides images that are more representative of sealant penetration into the dentinal tubules.

Scanning electron microscopy and CLSM analysis, on the other hand, require the destruction of specimens by cutting and an appropriate preparation protocol for observation. During the cutting process, information about the specimen is lost, and if the specimen is not cut properly, proper analysis is not possible. In addition, these methods are time-consuming for researchers. In contrast, micro-CT is a reproducible method and does not require a special protocol for specimen preparation. Micro-computed tomography has been used to evaluate defects such as fissures, porosity, and inhomogeneity within root canal fillings and at the interface of the sealer with the dentinal tubules of the root canal [38, 39, 40].

However, despite the continuous development of specialized endodontic root canal sealers, they still do not meet all the requirements for airtight spatial sealing of the root canal system and repeated endodontic treatment is still necessary to prolong the duration of tooth retention in the oral cavity. The disintegration of the sealer filling the lumen of the root canal, in addition to gutta-percha, is still a common cause of reinfection, exacerbation of inflammation in the periapical tissues, and pain. Endodontic retreatment has a higher risk of treatment failure than the original treatment and can be the cause of tooth loss. Therefore, a thorough visual assessment of the penetration depth of selected sealers will help refine the appropriate method of preparing the root canals for final filling. It will also allow the selection of the obturation method for the type of sealer based on its chemical composition and physical properties.

Our own SEM study performed after filling the canals with gutta-percha and sealer of a single canal premolar tooth showed that this method is very useful for evaluating the depth of penetration of the sealer into the dentinal tubules, accurately showing the root canal space occupied by the gutta-percha point, the sealer, and the adhesion of the gutta-percha and the sealer to the lumen of the dentinal tubules. Similarly, in SEM studies performed after filling canals with gutta-percha and RSA, Pawińska et al. found very good adhesion of silicone sealer to tooth tissue in all specimens and few, small gaps between sealer and gutta-percha [40].

The usefulness of microscopic studies to evaluate the sealer integrity of RSA silicone sealers was also demonstrated by Pawlicka et al. and Gencoglu et al. [41, 42]. In addition, SEM imaging of the contact surfaces of the different structures provides a very detailed indication of the areas of fissures and voids and shows surfaces where both fissures and areas of perfect adhesion of the materials to the root dentin coexist, allowing a detailed evaluation of sealer penetration [43, 44, 45]. Other authors confirm the usefulness of the confocal microscopy method for evaluating the tightness of endodontic filling materials [40].

The time of evaluation of the tested preparations also appears to be very important in terms of the usefulness of test methods for evaluating the quality of root canal filling by sealers used in *in vitro* studies [45]. Short-term and long-term evaluation of the ability to seal the canal should be considered as 2 separate characteristics, and the latter should rather be considered as a reference in the clinical setting.

CONCLUSIONS

The most useful and accurate methods for visually assessing the quality of root canal filling by sealers in *in vitro* studies are confocal microscopy and SEM. However, the high cost of specimen preparation and limited availability of equipment are factors limiting their use in clinical settings [45].

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