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Scanning electron microscopy assessment of tubular penetration depth of root canal sealers combined with different obturation techniques

Procena dubine prodora u dentinske tubule materijala za punjenje kanala korena zuba u kombinaciji sa različitim tehnikama opturacije korišćenjem skenirajuće elektronske mikroskopije

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Abstract

Background/Aim. The ability to effectively and consistently penetrate dentinal tubules is considered a favorable factor for the evaluation of root canal sealers (RCSs). The aim of the study was to assess the penetration depth into dentinal tubules of three RCSs combined with four obturation techniques. **Methods.** The mesial canals of 66 extracted human mandibular molars were endodontically prepared and randomly allocated into 12 experimental groups depending on the RCS type used (AH Plus™, EndoREZ™, Sealapex™) as well as the obturation technique applied [cold lateral compaction, cone-fit, carrier-based (with heated gutta-percha), warm vertical compaction]. Using scanning electron microscopy, transversal root cross-sections were analyzed, and the maximum depth of RCS penetration was measured (396 sections, in total, corresponded to the apical, middle, and coronary third). **Results.** Group AH Plus™/warm vertical compaction yielded the highest penetration depth – 1,165 µm, followed by EndoREZ™/cone-fit – 1,154 µm; the lowest depth was measured for EndoREZ™/warm vertical compaction – 502 µm. The mean value of the maximum penetration depth of RCS yielded 1,204 µm in the coronary thirds, 1,005 µm in the middle thirds, and 770 µm in the apical thirds. The AH Plus™ RCS penetrated deeper into dentinal tubules when the obturation techniques with heated gutta-percha were applied, while the opposite findings were obtained for the EndoREZ™ RCS. **Conclusion.** According to our research, the RCS penetration depth appears to be influenced by the RCS type used, as well as the obturation technique applied.

Key words:
microscopy, electron, scanning; root canal filling materials; root canal obturation.

Apstrakt

Uvod/Cilj. Sposobnost efikasnog i konzistentnog prodora u dentinske tubule smatra se favorizujućim faktorom za procenu materijala za punjenje kanala korena zuba (MPKKZ). Cilj rada bio je da se proceni dubina prodora tri MPKKZ u dentinske tubule, u kombinaciji sa četiri različite tehnike opturacije. **Metode.** Mezijalni kanali 66 ekstrahovanih mandibularnih molara su endodontski pripremljeni i nasumično podeljeni u 12 eksperimentalnih grupa, u zavisnosti od vrste upotrebljenog MPKKZ (AH Plus™, EndoREZ™, Sealapex™), kao i od tehnike opturacije (hladna lateralna kompakcija, *cone-fit* tehnika, sa čvrstim nosačem gutaperke, topla vertikalna kompakcija). Upotrebom skenirajuće elektronske mikroskopije analizirani su poprečni preseci korenova zuba i zabeležena je maksimalna dubina prodora MPKKZ za svaku trećinu korena (ukupno 396 preseka koji su odgovarali apikalnoj, srednjoj i kruničnoj trećini korena zuba). **Rezultati.** U grupi AH Plus™/topla vertikalna kompakcija, postignuta je najviša dubina prodora – 1 165 µm, a zatim u grupi EndoREZ™/*cone-fit* – 1 154 µm; najniža dubina prodora izmerena je u grupi EndoREZ™/topla vertikalna kompakcija – 502 µm. Srednja vrednost maksimalne dubine prodora svih MPKKZ iznosila je 1 204 µm u kruničnoj trećini, 1 005 µm u srednjoj trećini i 770 µm u apikalnoj trećini. MPKKZ AH Plus™ prodirao je dublje u dentinske tubule u kombinaciji sa tehnikama opturacije sa zagrejanom gutaperkom, dok su suprotni rezultati zabeleženi kod MPKKZ EndoREZ™. **Zaključak.** Prema rezultatima sprovedenog istraživanja, dubina prodora MPKKZ u dentinske tubule zavisi od vrste MPKKZ, ali i od primenjene tehnike opturacije.

Ključne reči:
mikroskopija, elektronska, skenirajuća; zub, materijali za punjenje korenskog kanala; zub, punjenje korenskog kanala.

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Introduction

In endodontic therapy, root canal sealers (RCSs) have been used to fill the interface between the core filling material and the root dentinal walls but also the lateral canals, dentinal tubules, and intracanal irregularities. Various obturation techniques have been proposed in order to achieve a three-dimensional canal filling, but the obturation of a complete root canal system is still a challenge for clinicians. The ability to effectively and consistently penetrate dentinal tubules is considered a favorable factor for RCSs [1–4]. Namely, sealer tags into dentinal tubules increase the contact surface area between the filling material and the dentinal wall, improving the retention rate of filling, increasing the fracture resistance of endodontically treated teeth by reinforcing the root, and potentially reducing the risk of microleakage. Sealer into dentinal tubules may entomb the remaining bacteria, while its chemical constituents can exhibit an antibacterial effect [5–7]. Measuring the depth of penetration into dentinal tubules is one of the methods used for evaluating the performance of a sealer [8]. The extent of sealer tags seems to be influenced by the presence of a smear layer, the root canal dimensions, dentine permeability (number and diameter of dentinal tubules), obturation technique applied, temperature, and humidity. The physical and chemical properties of sealers, such as particle size, viscosity, flowability, solubility, surface tension, and contact angle between the sealer and the root dentin, also play an important role in the effectiveness of tubular penetration [1, 5, 9, 10].

Micrographs obtained by scanning electron microscopy (SEM) allow for a detailed observation of dentine tubules and the integrity of sealer tags inside them, as well as precise measurements at a wide range of magnifications. The main disadvantages of this method are the high risk of producing artifacts during specimen preparation and the observation limited only to the specimen's surface [2, 7, 11]. Sealer penetration depths are commonly examined using a confocal laser scanning microscope. This method allows visualization of sealer penetration even below the dentine surface as the sealer is labeled with fluorescent organic dye [3, 6, 12–14]. As Donnermeyer et al. [15] demonstrated, the staining of sealer with a fluorescent dye (Rhodamine B) is not an adequate method for the evaluation of sealer penetrability into dentinal tubules as dye diffuses passively into the tubules and may give incorrect results. Taking all the above into consideration, SEM analysis is preferred for assessing the sealer penetrability into dentinal tubules.

Due to its favorable physical, chemical, and biological properties, AH Plus™ (Dentsply DeTrey, Konstanz, Germany) has become a "gold standard" for evaluating research in endodontics. AH Plus™ is an epoxy resin-based RCS with a setting time of eight hours, allowing long infiltration of dentinal tubules [11, 16]. On the other hand, EndoREZ™ (Ultradent Products Inc., South Jordan, Utah, USA) is a self-priming, methacrylate-resin-based sealer with hydrophilic characteristics and a short setting time (20–30 min). According to the manufacturer, EndoREZ™ needs to be applied to a slightly moistened canal in an oxygen-free environment to prevent inhibition of the polymerization process. The dual nature of the EndoREZ™ setting results in a higher polymerization shrinkage, which may adversely affect material adaptation to the dentinal wall. Sealapex™ (Kerr, Salerno, Italy), as the third sealer examined in this study, is based on calcium hydroxide, has a high biological potential, does not possess adhesive properties, and sets slowly [17, 18].

The aim of the study was to ascertain the degree of sealer penetration into dentinal tubules based on the micrographs obtained by SEM.

Methods

Specimen preparation

The use of extracted human teeth for the study was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad, Serbia (approved on 11 March, 2013). The mesiobuccal and mesiolingual canals of permanent first human mandibular molars extracted for orthodontic, periodontal, or prosthetic reasons were used in the research. The selected mesial roots were characterized by a fully formed apex and exhibited type IV anatomic configuration according to the Vertucci [19] classification, along with 10°–30° canal curvature, as measured by the Schneider [20] technique. Endodontically treated teeth, roots with calcifications, cracks, perforations, fractures, resorptions, and immature apices were discarded. Preoperative digital radiographs were taken using a standardized parallel technique (buccolingual and mesiodistal directions) in order to verify the morphology of the canals, discarding the samples with inappropriate features and severe curvatures. Freshly extracted teeth were cleaned using hand curettes and were stored in distilled water at 4 °C until required for the experiment, no longer than a month. As 12 groups were required for the experiments, a sample of 66 mandibular molars with two mesial canals was selected (132 in total), as determined by the total number of ways to choose two different groups out of 12 groups, i.e., 12*11/2.

Prior to instrumentation, the teeth were cut at the cemento-enamel junction using a low-speed diamond disc (Edenta AG, AU/SG, Switzerland) to access the orifices of mesiobuccal and mesiolingual canals; distal roots were discarded. Each root was assigned a unique number; the mesiobuccal canal was marked by a longitudinal groove made along the root buccal surface to distinguish the mesial canals under SEM. Working length (WL) was determined by introducing a #10 K-File (Dentsply Maillefer, Ballaigues, Switzerland) into each canal until the tip of the file was seen at the apical foramen and subtracting 0.5 mm from the measured length. The canals were prepared by a single operator in a crown-down manner, using rotary nickel-titanium endodontic instruments driven by an endodontic motor (X-Smart Plus, Dentsply Maillefer, Ballaigues, Switzerland). Glide paths were created using a #16/02 ProGlider (PG, Dentsply Maillefer, Ballaigues, Switzerland), and canals were shaped with ProTaper Next X1 (17/0.04) and X2 (25/0.06) up to the WL using the recommended settings (300 rpm; 2 Ncm torque). Each file was lubricated by Glyde (Dentsply Maillefer, Ballaigues, Switzerland); between files, the canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) solution delivered through a plastic syringe and 30-G side-vented irrigation needles (KerrHawe Irrigation Probe, KerrHawe SA, Bioggio, Switzerland). Apical patency was maintained by introducing the #10 K-File up to the WL between each file. For smear layer removal, the canals were irrigated with 10 mL of 17% ethylenediaminetetraacetic acid (EDTA, I-dental, Siauliai, Lithuania) and 10 mL of NaOCl; both solutions were activated using EndoActivator (Tips small 15/.02; Dentsply Maillefer, Ballaigues, Switzerland). The canals were finally rinsed with 10 mL of saline and gently dried using absorbent paper points (F2 Paper Points, ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland). Throughout the whole process, the roots were kept moist by wrapping them into saline-soaked gauze. The prepared roots were randomly allocated into three experimental groups, depending on the sealer type used, and four further subgroups were created within each, according to the obturation technique applied, resulting in 12 groups, each with 11 canals (Figure 1). Two samples were taken as controls to verify the presence/absence of a smear layer and dentinal permeability obtained after the final rinse.

Obturation techniques were performed in an incubator at 37 °C environment as described previously [21]. Three RCSs were used in the study (Table 1). Components of AH Plus™ and EndoREZ™ were packed in double syringes and automatically mixed; Sealapex™ was mixed according to the manufacturer's instructions using a precise weighing scale that allowed the extrusion of equal amounts of both components. AH Plus™ and Sealapex™ were introduced into the canals using a #20 K-File coated with the chosen sealer by a counter-clockwise rotation, inserting a length 1 mm shorter than the WL. According to the manufacturer, EndoREZ™ was dispensed into canals through a narrow syringe (Skini™ syringe) connected to a fine-tipped cannula (NaviTip™), placing 2–3 mm less than the WL while withdrawing the syringe until the sealer level reaches the canal orifice. The excess material was removed with dry paper points (F1, ProTaper, Dentsply Maillefer, Ballaigues, Switzerland).

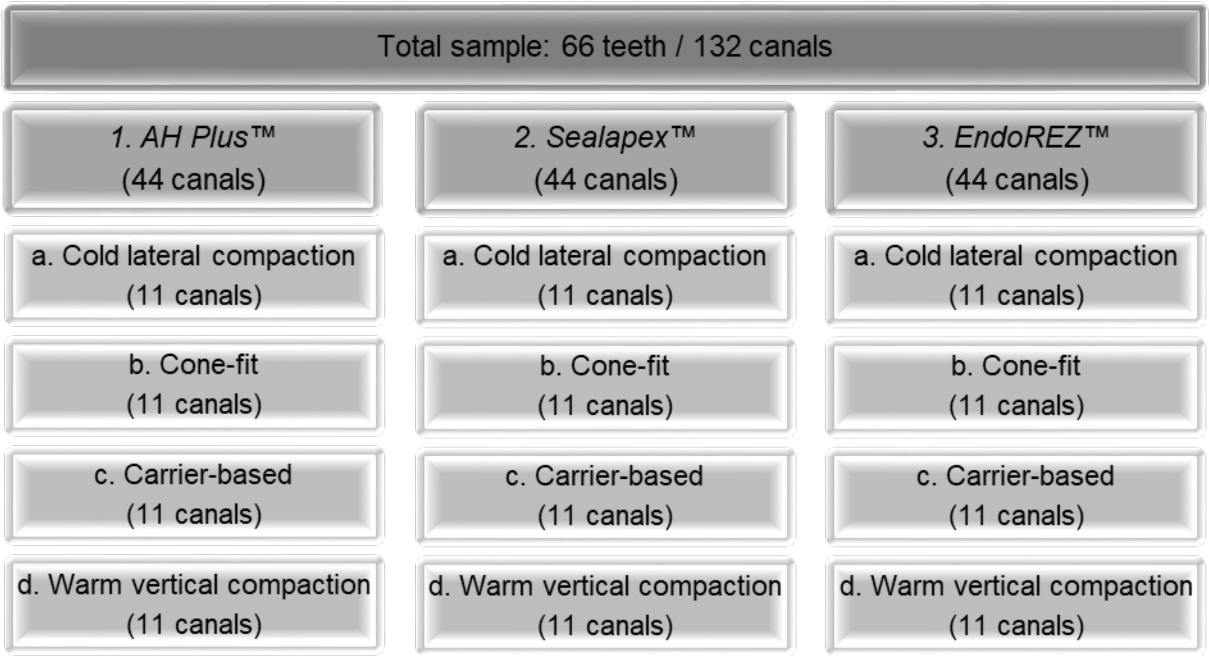

Fig. 1 – Schematic diagram of the experimental groups.

Table 1

The chemical composition of root canal sealers

Material	Type	Chemical composition	Manufacturer
AH Plus™	Epoxy resin	Paste A: epoxy resin, zirconium oxide, calcium tungstate, iron oxide pigments, aerosil Paste B: amines, zirconium oxide, calcium tungstate, silicone oil, aerosil	Dentsply De Trey, Konstanz, Germany
Sealapex™	Calcium hydroxide polymeric resin	Base: N-ethyl toluene sulfonamide, zinc oxide, silica, calcium oxide Catalyst: Isobutyl salicylate, methyl salicylate, polymethyl methacrylate, silica, titanium dioxide, bismuth trioxide	Kerr, Salerno, Italy
EndoREZ™	Methacrylate resin	Base: Diurethane dimethacrylate, benzoyl peroxide Catalyst: 2,2'- diethanol, triethylene glycol dimethacrylate	Ultradent, South Jordan, Utah, USA

In this study, four obturation techniques were used. The first technique used was cold lateral compaction. Master gutta-percha (GP) cone (ISO 25, Gutta-percha Points; Dentsply Maillefer, Ballaigues, Switzerland), fitted to the WL with apical tug-back, was inserted into a root canal previously coated with the chosen sealer. The remaining intracanal space was filled by lateral compaction of accessory, non-standardized GP cones using a #20 finger spreader (Dentsply Maillefer, Ballaigues, Switzerland). The excess GP was removed by a heated instrument. The second technique used was the cone-fit technique. Master GP cone (F2 ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland), fitted to the WL with apical tug-back, was inserted into a root canal previously coated with the chosen sealer. The excess GP was removed by a heated instrument. The third technique used was the carrier-based obturation technique (Thermafil). Upon heating in ThermaPrep 2 Oven (Dentsply Maillefer, Ballaigues, Switzerland) for 20 sec, GP obturator (F2 ProTaper Universal Thermafil Obturator, Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal with a constant apical pressure up to the WL. The coronal part of the obturator was manually stabilized and removed with a Thermacut bur (#12; Dentsply Maillefer, Ballaigues, Switzerland). Finally, the fourth technique used was the warm vertical compaction. Master GP cone (F2 ProTaper Universal Gutta-Percha Points, Dentsply Maillefer, Ballaigues, Switzerland) was inserted into a root canal previously coated with the chosen sealer, 0.5 mm shorter than the WL. The down-packing phase was performed with DiaPen (Pen type size – fine; DiaDent, Korea) heated at 200 °C (medium temperature setting) for 1–2 sec, inserting length 4 mm shorter than the WL. Back-filling was performed with DiaGun (DiaDent, Korea), filling the remaining intracanal space with melted GP (200 °C) using intermittent compaction with Heat Carrier plugger (No 1/2; Dentsply Maillefer, Ballaigues, Switzerland).

After obturation, the flowable composite (Filtek ™ Supreme Ultra Flow, 3M/ESPE, St. Paul, MN, USA) was applied over canal orifices and light-polymerized for 20 sec (Radii Plus, SDI, Bayswater, Victoria, Australia). The roots were stored at 37 °C and 100% humidity for 14 days to allow the material to set.

Scanning electron microscope analysis

All roots were sectioned perpendicular to the long axis at 3, 5, and 8 mm from the anatomic apex using a low-speed diamond disc under water-cooling (Edenta AG, AU/SG, Switzerland). Three cross-sections per root were created, with a thickness of 1 mm corresponding to the apical, middle, and coronary third, producing 396 sections in total. The coronal surfaces of cross-sections were chosen for analysis and were immersed in 17% EDTA solution (I-dental, Siauliai, Lithuania) for 10 min, followed by 4% NaOCl for 10 min in order to remove any residues of organic components around the sealer tags. The sections were finally rinsed with distilled water and gently dried. The prepared sections were dehydrated, mounted onto aluminum stubs, and sputter-coated with a gold coating (SCD050 Sputter Coater, BAL-TEC, PA, USA) under low-vacuum conditions.

Using micrographs obtained by an SEM (JEOL-JSM-6460LV, Tokyo, Japan), the depth of sealer penetration into dentinal tubules was measured. First, using an overall view obtained at low magnification (×50, ×100), the area with maximum sealer tag density was selected. Next, in this area at higher magnification (≥ ×400), the maximum sealer penetration depth was identified and marked digitally. Upon reducing magnification until the root canal wall was visualized, the image was captured, and, using a calibrated measuring tool (NIH Image Analyser), the distance from the marked point and root canal wall was measured (Figure 2). The depth of sealer tags was calculated in μm, but also in relative measure as a percentage of the total distance: inner canal dentinal wall – the outer root surface. The measurements were conducted by two operators blinded to the group being tested.

Data analysis comprised descriptive and comparative statistical methods. Although the values of the examined features are continuous, some samples did not meet the requirements for applying parametric comparative tests (Student's *t*-test, ANOVA), and, in those cases, non-parametric comparative tests were used (Mann-Whitney *U* test, Kruskal-Wallis ANOVA). Tukey HSD test for groups of different sizes was performed as a *post-hoc* test in ANOVA, whereas Multiple Comparisons of mean ranks were adopted as a non-

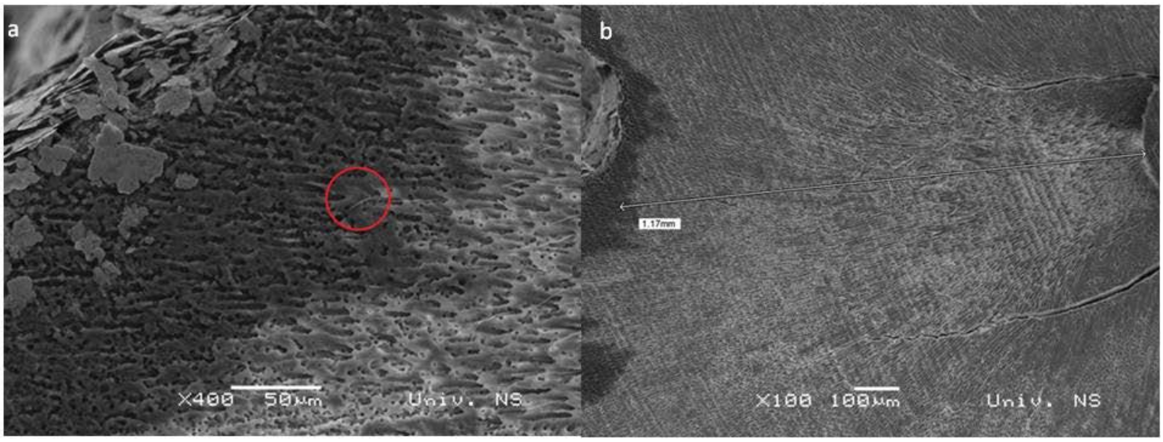

Fig. 2 – Scanning electron microscopy micrograph of sealer penetration into dentinal tubules: a) The deepest detected sealer tag; b) The deepest sealer tag measured from the dentinal wall.

parametric *post-hoc* test in Kruskal-Wallis ANOVA. The results were considered statistically significant if the corresponding *p*-value was below 0.05.

Results

The basic indicators of the descriptive statistics for the depth of sealer tags in all twelve groups are shown in Table 2. Presented data refer to the arithmetic mean of the maximum depth of sealer penetration into dentinal tubules for each group (coronary, middle, and apical third together) shown in percentage. Among all groups, statistically significant differences in the mean values of the variable were noted. Namely, group AH Plus™/warm vertical compaction yielded the highest penetration depth – 85.4% (1,165 μm), followed by groups EndoREZ™/cone-fit technique – 80.6% (1,154 μm), and AH Plus™/Thermafil – 77.6% (1,229 μm). The lowest penetration depth was measured for group EndoREZ™/warm vertical compaction – 46.2% (502 μm) (Table 2).

The results yielded by the analysis of each sealer separately are given in the following text. Using AH Plus™, a statistically significant difference was noted between the mean values of maximum penetration depth achieved *via* different obturation techniques ($p = 0.0000$). AH Plus™, in combination with warm vertical compaction, showed a significantly higher penetration depth compared to groups 1a ($p = 0.000008$), 1b ($p = 0.000008$), and 1c ($p = 0.00043$), while no significant differences were noted among other pairs. Sealapex™, in combination with warm vertical compaction, resulted in a significantly lower penetration depth into dentinal tubules compared to the combinations involving cold lateral compaction ($p = 0.005631$) and carrier-based techniques ($p = 0.003658$), while no significant differences were noted among other pairs. EndoREZ™, in combination with warm vertical compaction, had a significantly lower mean maximum depth of penetration compared to the combinations with cold lateral compaction ($p = 0.000138$), cone-fit technique ($p = 0.00014$), and carrier-based technique ($p = 0.000148$). In combination with the cone-fit technique, EndoREZ™ had a significantly higher depth of penetration compared to the combination with the carrier-based technique ($p = 0.004883$). There were no significant differences between the other pairs.

The results obtained when obturation techniques were analyzed separately were discussed in the following text. There were no significant differences between the mean maximum depth of tubular penetration of AH Plus™, Sealapex™, and EndoREZ™ using cold lateral compaction and carrier-based obturation technique. Using the cone-fit technique, a significantly higher penetration depth into dentinal tubules was noted for EndoREZ™ compared to AH Plus™ ($p = 0.006759$) and Sealapex™ ($p = 0.000167$). The penetration depth of all tested sealers differed significantly using warm vertical compaction. The highest penetration depth was measured for AH Plus™, followed by Sealapex™, and finally EndoREZ™ (for 1d and 2d, $p = 0.00011$; 1d and 3d, $p = 0.000002$; 2d and 3d, $p = 000011$).

The mean value of the maximum penetration depth of AH Plus™, Sealapex™, and EndoREZ™ yielded 79.3% (1,204 μm) in the coronary thirds, 73.2% (1,005 μm) in the middle thirds, and 64% (770 μm) in the apical thirds. By analyzing each group, statistically significant differences in the maximum penetration depth between coronary, middle, and apical thirds of the root canals can be observed. In almost all groups, higher penetration depth in the coronary thirds compared to the middle thirds was noted, while the lowest value was measured in the apical thirds. The results are presented in detail in Table 3.

The SEM images of the control group showed complete smear layer removal and open dentinal tubules produced by the final irrigation protocol (Figure 3). The representative SEM micrographs (Figures 4–8) depict the dentinal tubular infiltration of AH Plus™, Sealapex™, and EndoREZ™ achieved in different groups.

Table 2

Basic indicators of descriptive statistics for the penetration depth of AH Plus™, Sealapex™, and EndoREZ™ into dentinal tubules for all experimental groups

Group	Arithmetic mean		95% CI limit		SD	Min–Max	Median
	%	μm	lower	upper			
RCS AH Plus™							
a	75.02	1,098.00	71.86	78.19	8.78	59.30–89.20	75.65
b	73.76	1,183.00	70.17	77.34	9.61	55.30–93.70	75.95
c	77.59	1,229.00	74.79	80.40	7.77	60.70–90.80	78.15
d	85.43	1,165.00	83.79	87.07	4.55	76.50–92.00	85.70
RCS Sealapex™							
a	74.70	1,113.00	71.68	77.71	8.22	59.30–93.10	75.10
b	70.81	997.00	67.79	73.83	8.10	53.40–85.40	70.80
c	75.03	985.00	71.07	79.00	10.42	50.70–90.10	79.10
d	66.35	778.00	62.57	70.13	9.55	52.20–81.60	65.70
RCS EndoREZ™							
a	75.07	1,010.00	71.58	78.56	9.85	58.30–93.20	78.80
b	80.63	1,154.00	77.87	83.39	7.78	65.00–92.30	81.10
c	73.89	883.00	70.79	77.00	8.16	55.80–84.50	75.30
d	46.21	502.00	40.99	51.42	10.15	21.30–62.70	48.10
Total	74.10	1,008.00	72.90	75.29	11.48	21.30–93.70	76.90

RCS – root canal sealer; a – cold lateral compaction technique; b – cone-fit technique; c – carrier-based technique; d – warm vertical compaction technique; CI – confidence interval; SD – standard deviation; Min – minimum; Max – maximum.

Table 3

Results of the Kruskal-Wallis ANOVA test for the penetration depth of AH Plus™, Sealapex™, and EndoREZ™ into dentinal tubules in the coronary (1), middle (2), and apical third (3) of the canal

Group	H-statistics	Degrees of freedom	*p*-value	*Post-hoc* test
RCS AH Plus™				
a	17.0501	2	0.0002	level 1 is significantly different from levels 2 and 3
b	15.786	2	0.0004	level 1 is significantly different from levels 2 and 3
c	15.8505	2	0.0004	level 3 is significantly different from levels 1 and 2
d	14.4999	2	0.0007	level 1 is significantly different from level 3
RCS Sealapex™				
a	22.5219	2	0.0000	level 3 is significantly different from levels 1 and 2
b	17.3372	2	0.0002	level 1 is significantly different from levels 2 and 3
c	13.7759	2	0.001	level 1 is significantly different from level 3
d	19.5621	2	0.0001	level 1 is significantly different from levels 2 and 3
RCS EndoREZ™				
a	17.9072	2	0.0001	level 3 is significantly different from levels 1 and 2
b	23.8586	2	0.0000	level 1 is significantly different from levels 2 and 3
c	13.6763	2	0.001	level 1 is significantly different from levels 2 and 3
d	6.3137	2	0.04	level 3 is significantly different from level 1

ANOVA – analysis of variance. For abbreviations of other terms, see Table 2.

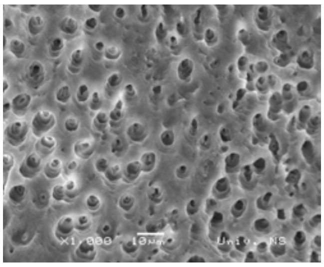

Fig. 3 – Control group: removed smear layer and open dentinal tubules.

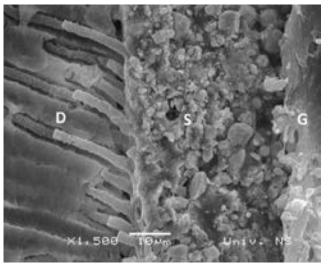

Fig. 4 – The penetration of AH Plus™ sealer with cone-fit technique at the middle third of the root; sealer tags are visible penetrating dentinal tubules. D – dentin; S – sealer; G – gutta-percha.

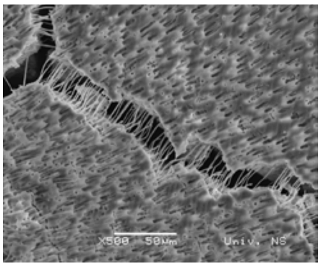

Fig. 5 – The penetration of AH Plus™ sealer with Thermafil at the middle third of the root; a micro crack can be seen as a result of the tooth specimen preparation for scanning electron microscopy; sealer tags are visible inside the crack.

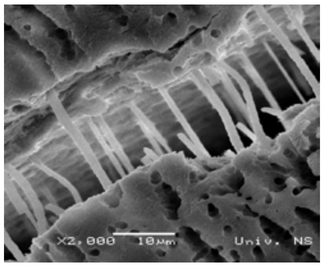

Fig. 6 – The penetration of EndoREZ™ sealer with cone-fit technique at the coronary third of the canal; a micro crack filled with sealer tags can be seen.

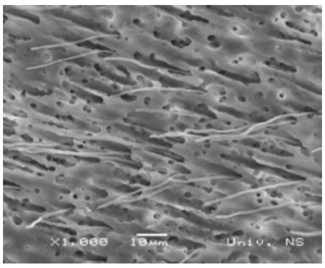

Fig. 7 – The penetration of Sealapex™ sealer using Thermafil at the apical third of the canal; extensive sealer penetration into dentinal tubules can be observed.

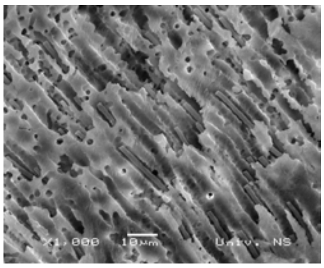

Fig. 8 – The penetration of AH Plus™ sealer with cone-fit technique at the middle third of the root; sealer tags penetrate deep into dentinal tubules.

Discussion

In the present study, the tubular penetration depth of RCSs was evaluated using SEM. Statistically significant differences were obtained between the groups. The sample standardization in the present study was achieved by focusing on mandibular molars with two mesial canals obturated using different combinations of sealers and techniques, whereby a sample of 66 mesial roots with two canals was segregated into 12 groups, allowing pairwise comparison between groups, as 12*11/2 = 66 represents the total number of ways to choose two different groups out of 12. In this way, the influence of factors such as internal root anatomy and dental permeability on the research results was minimized, i.e., all tested sealers had the same conditions to penetrate into dentinal tubules. The results were presented in μm, but also in percentages, i.e., the maximum sealer penetration depth with respect to the total distance: canal wall – root cement. This method eliminated any influence of factors such as the tooth size (i.e., the cross-section diameter) on the measurement results because, as can be seen in Table 2, the comparative values of the measurement results expressed in μm do not correspond exactly to the values expressed in percentages.

The influence of a smear layer on the sealer penetration depth was examined by many authors whose findings indicate that its effective removal results in deeper penetration [7, 9, 22]. On the other hand, sealer tubular penetration can serve as an indicator of the degree of the smear layer removal [23]. In the present study, the smear layer was removed by flushing with 17% EDTA, followed by 1% NaOCl and finally saline solution, which is consistent with the methods adopted in other studies and is in line with the recommendations of American Association of Endodontics for the work in clinical endodontics [1, 5, 24].

Dentinal tubules can be observed on longitudinal or transversal root sections. In teeth with curved canals, e.g., maxillary and mandibular molars, the longitudinal cross-section is difficult to perform, increasing the risk of producing artifacts, especially in the apical third [1, 2, 5, 25]. In the present study, transversal cross-sections of the roots were used, providing insight into the complete circumference of the corresponding third of the canal. Regional variations in the sealer penetration depth have been established by many authors, with a common finding that the lowest penetration depth is achieved in the apical third of the root canal [8, 11, 16]. The findings obtained in the present study are in line with these observations, as a significantly lower penetration depth of all tested sealers was measured in the apical third, while the variations between the coronary and middle thirds of the root were not significantly different. The apical third of the root is anatomically the most variable region, having fewer dentinal tubules of a smaller diameter, which are often closed or occluded with cement tissue, so the lowest measured depth of sealer tags was expected [5, 16].

Although manufacturers claim that RCSs are convenient for all obturation techniques, it is undeniable that the physicochemical properties of the sealer are affected by the heat application [2, 10, 26]. It was demonstrated in a previous study that the rheological properties of RCSs are highly temperature-dependent. Namely, the complex viscosity of AH Plus™ was significantly decreased with temperature increase, while the complex viscosity of Sealapex™ and EndoREZ™ behaved the opposite [10]. These findings might explain the higher penetration depth of AH Plus™ in combination with heated GP obturation techniques and the lower values obtained with Sealapex™ and EndoREZ™. Low-viscosity materials have the potential to penetrate deeper into the dentinal tubules. It was also confirmed in the recent study that AH Plus™ is more suitable for thermal endodontic obturation techniques compared to EndoREZ™ and other tested sealers [27]. Viapiana et al. [2] noted that heat affects the amino groups of AH Plus™ sealer, resulting in lower compressive strength, while in another study, it was found that film thickness and the setting time of resin-based RCSs were affected by the heat [28]. Baldi et al. [29] showed that the physicochemical properties of epoxy-based sealers, such as flowability and setting time, depend on the tube segment (initial, middle, and final) from which the materials were squeezed and mixed. All of these findings may be the reason for the different behavior of the materials after the application of heat.

The depth of sealer penetration into dentinal tubules has been measured by many authors, yielding inconsistent findings. While the results obtained in the majority of these studies are aligned with those presented in this work, in terms of measured length [1, 7, 30], a significantly lower measured depth was noted in others [5, 11, 25]. These conflicting research results can be attributed to a wide range of factors, most notably differences in sample selection, sample size calculation, the methods of smear layer removal, obturation technique applied, and sample preparation for microscope analysis. A lack of validation of the experimental method is certainly a contributing factor to the inconsistency of the results [31]. It should be kept in mind that the maximum penetration depth was measured and compared in the present study; if the average values were calculated and compared, which is extremely difficult to measure, the penetration depth results would certainly be lower. The results yielded by the present study pertaining to the tubular penetration of AH Plus™ and EndoREZ™ are in agreement with those reported by Mamootil and Messer [1], who used the same evaluation methods as those adopted in this work, but also confirmed the sealer penetration into dentinal tubules *in vivo* getting similar results: over 1,000 μm for epoxy resin-based RCS.

Conclusion

Within the limitations of this study, it can be concluded that the penetration depth of RCSs into dentinal tubules varies depending on the sealer type used, as well as its handling, i.e., combination with different obturation techniques. SEM is a suitable tool for the analysis of sealer penetrability as it provides precise measurement at a wide range of magnifications and allows detailed observation of ultrastructural morphology within the root dentin. Clinically, the results obtained might be applicable in the material selection for endodontic therapy and its proper handling.

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