

Comparison of the penetration of three endodontic sealers into dentinal tubules with scanning electron microscopy

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ABSTRACT

Objective: To compare *in vitro*, using a scanning electron microscope, the penetration of three endodontic sealers: made of epoxy resin (AH Plus®), of polydimethylsiloxane (Roekoseal®), and of calcium hydroxide (Apexit Plus®) into the dentinal tubules at 3 mm and 7 mm from the root apex, with the lateral compaction technique in uniradicular lower premolars. **Materials and methods:** *In vitro* study. Thirty-six teeth were prepared and divided into three groups of 12 teeth each. All the teeth were prepared and each group was obturated with three different endodontic sealers. Subsequently, the teeth were cut transversely at 3 mm and 7 mm from the root apex; then they were prepared to be taken to the scanning electron microscope to observe the penetration of the sealers in the dentinal tubules. **Results:** ANOVA test was used to compare the 3 groups and Student's t-test was used to evaluate the penetration of each of the sealers at 3 mm and 7 mm. Tukey's *post hoc* test was also performed to evaluate between sealer groups. When comparing the 3 groups of endodontic sealers, greater penetration was found with the Roekoseal® sealer at 3 mm with a statistically significant difference, ANOVA test ($p = 0.04$). When comparing each of the sealers at 3 mm and 7 mm, significant differences were only found ($p = 0.04$) in AH Plus®, showing better penetration at 7 mm with respect to 3 mm; and when the sealer groups were compared, both at 3 mm and 7 mm, no statistically significant differences were found. **Conclusions:** All three sealers evaluated *in vitro* penetrated in the dentinal tubules. At 3 mm, the Roekoseal® sealer outperformed the other two sealers; and at 7 mm, there was no significant difference between them.

Keywords: root canal filling; root canal filling materials; dental marginal adaptation.

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INTRODUCTION

Root canal obturation is based on the use of semi-solid gutta-percha cones accompanied by an endodontic sealer (ES), since these cones alone do not completely seal the root canal system (1). ES are inert or therapeutic substances that complement the obturation of the root canal, fixing and adhering the gutta-percha cones to each other and to the dentin walls of the root canal, thus eliminating empty spaces. ES, together with the cones, provide the essential hermetic sealing especially for the apical zone. Sealants must be stable and must be able to enter the side, secondary and accessory canals of the canal system where gutta-percha cannot enter (2, 3).

ES should also penetrate the dentinal tubules, which is considered a desirable result, as it increases the interface between the canal walls and the gutta-percha, thus improving the hermetic seal (3). A significant advantage of ES penetration is the isolation of the nutrient source to viable bacteria within the dentinal tubules, which favors their antibacterial effects (4, 5).

Numerous variables, including the existence of the dentin mud layer, dentin permeability, dentin tubule diameter, root canal branches, and the physical and chemical characteristics of the sealant, can influence the depth of ES penetration into the dentin tubules (6).

Several ideal characteristics or requirements that ES should possess have been established. Grossman (7) mentions the following: provide good adhesion between gutta-percha and root canal walls; have a tight seal, easy to insert into the root canal; adequate working time; be sterile or easy to sterilize prior to placement; be radiopaque; possess dimensional stability upon setting; do not pigment the dental piece; do not suffer corrosion; be bacteriostatic, or do not facilitate bacterial growth; be well tolerated by periapical tissues (biocompatibility); be insoluble in oral fluids but soluble in a solvent so that it can be removed if required. Lioni (8) defines that ES must be homogeneous when mixed, to promote better adhesiveness, be resorbable in case of overfilling, stimulate the formation of repair tissue, and without being cytotoxic, mutagenic or carcinogenic.

This study will contribute with knowledge to identify which ES has the best penetration capacity in the root canal irregularities and dentinal tubules, in order to use the appropriate one, according to the diagnosis and the treatment plan proposed, thus helping the achievement of the patient and the professional. In this

sense, the purpose of this study was to compare the *in vitro* penetration in micrometers of three ES, one based on epoxy resin, another on polydimethylsiloxane and the last one on calcium hydroxide, in the dentinal tubules, for which a scanning electron microscope was used.

MATERIALS AND METHODS

The study was *in vitro*. A pilot study was conducted and the mean comparison formula was used to determine the sample size of 12 uniradicular premolar teeth for each ES group. All teeth had to meet the following inclusion criteria: uniradicular human teeth, teeth with a fully developed canal, teeth with a single canal, teeth with an adequate length, with a minimum of 15 mm of root length remaining after decoronation. And the exclusion criteria were as follows: teeth with coronary and/or root fractures, teeth with previous endodontic treatment, teeth with calcified and/or atresic canals, teeth with moderate or severe curvatures, teeth with root resorptions, and teeth with immature apices.

The teeth were placed in a jar containing a 1% sodium hypochlorite solution for four hours (9). Hand scrapers were used to carefully remove calculus or soft tissue remnants from the root surface. Subsequently, the teeth were preserved in jars containing saline solution at room temperature until the time of use (10). To confirm the presence of a single canal, periapical radiographs were taken of the selected teeth in the vestibulo-lingual and proximal direction.

Procedure sequence

The teeth were cut at the cemento-enamel junction with a carborundum disc (dentorium) using a Lynx™ low speed motor (USA). The length of the tooth was standardized to 15 mm. A number 10 K file (Maillefer-Dentsply) was introduced into the root canal until the tip is visible in the apical foramen. This measurement was reduced by 1 mm to obtain the working length. With the radiovisiograph (RVG), the corresponding conductometry was performed with a number 15 K file, and in some teeth a number 20 file was used.

Biomechanical preparation was performed with Mtwo® system rotary files (VDW, Munich-Germany) according to the manufacturer's recommendations. It was instrumented up to a 40.04 Mtwo file, and finished with an ISO 45.02 manual file, at a working length of 14 mm. Irrigation was performed using NaviTip needles with 2 mL of 2.5% sodium hypochlorite (NaOCl) for each instrument used. Once the biomechanical

preparation was completed, a K ISO 15.02 file was extruded through the apical foramen to make sure that the root canal was not obstructed. Final irrigation of the root canals was performed with 3 mL of 17% EDTA (Biodinamica Quimica, Brazil) for 3 min, followed by 3 mL of 2.5% NaOCl to remove the smear layer (11, 12).

Three groups of 12 teeth each were formed to be filled with three different ES: group 1 with ES AH Plus® (Maillefer-Dentsply), group 2 with ES Roekoseal® (Coltene-Whaledent) and group 3 with ES Apexit plus® (Vivadent-Ivoclar). In terms of the Roekoseal® ES group, 3 mL of 95% ethyl alcohol was added to the final irrigation (10, 13). Two teeth were prepared and used as negative controls, which did not undergo root canal obturation. The root canals were then dried with standardized sterile paper cones; conometry was performed with a gutta-percha cone number 45.02, tested and adjusted to the working length.

The obturation of canals was performed with the lateral compaction technique using standardized gutta percha and the respective ES. The application of the ES in the root canal was performed with the same chosen gutta-percha master cone number 45.02. For the lateral compaction, a digital spacer size B (Maillefer-Dentsply) was used entering 1-2 mm short of the working length, and accessory gutta-percha cones number 20.02 were used. Excess gutta-percha was removed using a hot instrument and the corresponding vertical compaction was performed; the coronal part of the canal was sealed with Coltosol® F cement to an average depth of 4 mm.

Each specimen was placed in a respective test tube, previously coded by each ES group containing saline solution. They were then placed in an incubator at the microbiology laboratory of the Universidad Peruana Cayetano Heredia (UPCH) for two weeks at 37 °C and 100% humidity for complete setting of the ES (10, 12, 14, 15).

Subsequently, specimens were cut transversely at 3 mm and 7 mm of the root apex with a carborundum (dentorium) disk, with constant water cooling to 5 °C, to avoid frictional heat (10). To remove all inorganic debris produced during cutting, all samples were cleaned with a 17% EDTA bath for two minutes and then with 3% NaOCl, for two minutes as well (11, 15). The specimens were left at room temperature to allow drying for 12 hours.

Sample processing

This was carried out in the specialized postgraduate equipment laboratory of the Faculty of Biological Sciences at Universidad Nacional Mayor de San Marcos (FCB-UNMSM). The specimens were taken to complete the drying process for 1 hour (Beltec Scientific PH - 050A). Afterwards, they were placed in discs duly coded for each group of ES, and then taken to the vacuum machine (SPI® - module Sputter Coater) for their respective gold coating for 4 minutes, ready for observation and measurement in the scanning electron microscope (SEM) (Inspect S50, FEI brand) (Figure 1). The measurement was performed by a professional expert in the use of the scanning electron microscope software of the same specialized postgraduate equipment laboratory of the FCB-UNMSM, thus allowing the obtaining of correct data.

The canal wall was located at a magnification of 1000x and the depth of the greatest entry of the ES into the dentinal tubules was measured at 1500x, having two reference points (the root canal wall and the most distant entry in the direction of the external root surface). This depth was measured linearly and expressed in micrometers, using the measurement tool of the XT Microscope Control software. The corresponding microphotography was taken at 1500x for its measurement (Figure 1).

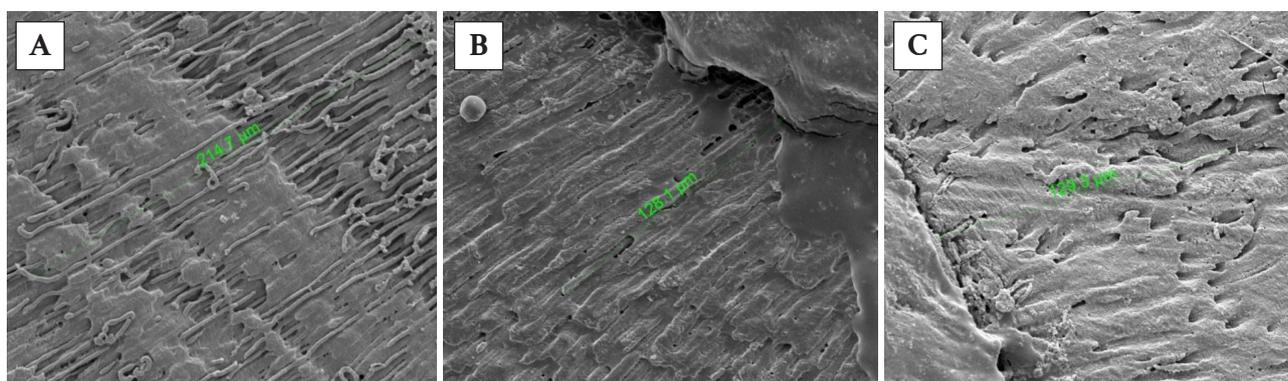


Figure 1. Penetration of the endodontic sealer into the dentinal tubules as seen under the scanning electron microscope. A) AH Plus® Sealer; B) Roekoseal® Sealer; C) Apexit Plus® Sealer.

Stata v. 11 software was used for a statistical analysis. Descriptive statistics were performed to determine the mean and standard deviation values of the penetration of each of the ES into the dentinal tubules. The assumptions of normality and homogeneity of variances of the dependent variable were analyzed. The p-value was set at 0.05. An ANOVA test was used to compare all sealer groups, and a Student's t-test was used to determine if there was a statistically significant difference when evaluating the penetration of each sealer at 3 mm and 7 mm. Tukey's post hoc test was also performed between sealant groups.

The project was implemented with the approval of the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia (CIE-UPCH) on June 9, 2017, and SIDISI code no. 100983.

RESULTS

When analyzing the three ES, at 3 mm it was found that AH Plus® sealer obtained a mean of 127.14 ± 54.55 ; Roekoseal®, a mean of 179.22 ± 43.71 ; and Apexit Plus®, a mean of 132.61 ± 59.24 . And at 7 mm, it was observed that ES AH Plus® obtained a mean of 173.05 ± 48.37 ; Roekoseal®, a mean of 186.28 ± 71.8 ; and Apexit Plus®, a mean of 155.40 ± 60.29 (Table 1).

Table 1. Comparison of the penetration (μm) of three endodontic sealers into dentinal tubules with scanning electron microscopy.

Sealer	At 3 mm		At 7 mm		p ^b
	Mean	SD	Mean	SD	
AH Plus	127.14	54.55	173.05	48.37	0.040*
Roekoseal®	179.22	43.71	186.28	71.80	0.774
Apexit Plus®	132.61	59.24	155.40	60.29	0.360
p ^a	0.041*		0.468		

^a Statistical significance of ANOVA test.

^b Statistical significance of Student's t test.

* Statistically significant ($p < 0.05$); ANOVA test ($p = 0.04$).

Comparing the three ES, significant differences were only found at 3 mm ($p = 0.04$), with Roekoseal® ES obtaining the highest values. When each of the sealers was evaluated separately, it was observed that ES AH Plus® showed greater penetration at 7 mm than at 3 mm. This difference was statistically significant

($p = 0.04$). When the sealant groups were analyzed in pairs, no significant differences were found.

DISCUSSION

The scanning electron microscopy method was used for this study because it is one of the main and reliable

instruments that could best describe the image of the ES on the tooth surface *in vitro* (11, 16, 17), which, in turn, makes it possible to visualize images with a 3D effect, take microphotographs at high magnifications ranging from 3x to 150,000x, and observe the samples in longitudinal or transverse sections. This evaluation was performed with transverse sections at 3 mm and 7 mm from the root apex (11, 18).

Mamootil and Messer (19), Okşan et al. (20) and Bernardes et al. (21) state that a variety of factors, such as removal of the mud layer, dentin permeability (number and diameter of dentinal tubules), canal dimension, and the impact of physical and chemical characteristics of the ES, such as fluidity, affect the depth at which ES penetrate the dentinal tubules.

Zhou et al. (22) evaluated different ES: silicone-based, epoxy resin-based, mineral trioxide aggregate (MTA), zinc oxide-eugenol and bioceramic, and found greater flowability in the silicone-based ES, with statistically significant differences. Ordinola-Zapata et al. (10) evaluated the depth of penetration in the dentinal tubules of the following ES: silicone-based, calcium hydroxide-based and resin-based, obtaining results with no significant differences when comparing silicone-based ES and resin-based ES (10).

Cobankara et al. (23) observed the penetration and sealing ability of four ES: AH Plus® based resin, silicone-based Roekoseal®, glass ionomer-based Ketac Endo and zinc oxide-eugenol-based Sultan, using the lateral compaction technique; after 21 days of obturation, they observed better results for Roekoseal®, followed by AH Plus®, Ketac Endo and Sultan, with the latter showing less sealing and greater filtration in each of the samples.

In this study, the polydimethylsiloxane silicone-based Roekoseal® ES showed greater penetration into the dentinal tubules. Conversely, Balguerie et al. (11) obtained unfavorable results for Roekoseal®. This may be related to the hot gutta-percha filling technique used in that study. The manufacturer of Roekoseal® states that with heat sealing techniques, the working time is reduced (24). An increase in temperature could explain the lower penetration of ES at the level of the dentinal tubules and present a granular appearance in the scanning electron microscope. In our study, the manufacturer's recommendations were followed, which is to use Roekoseal® in cold gutta-percha filling technique. The greater penetration into the dentinal tubules may be due to the high fluidity of Roekoseal®, as mentioned by other authors and the manufacturer (24, 25).

Faira-Júnior et al. (26) evaluated the flowability of five ES, having as a result that the calcium hydroxide-based ES showed higher flowability compared to the epoxy resin-based ES. The author mentions that it could be because the formula of the epoxy resin sealer was modified in its composition by the manufacturers, substituting methamine with TCD-diamine. In this study, the AH Plus® epoxy resin-based ES showed greater penetration into the dentinal tubules compared to Apexit Plus®, which is a calcium hydroxide-based ES. Most importantly, although ES AH Plus® showed a lower penetration at 3 mm from the root apex, it had a notable improvement at 7 mm. Bernardes et al. (21) evaluated the flowability of three ES (Sealer 26, AH Plus® and MTA Obtura), reporting a higher flowability for the AH Plus® ES, which could be due to the concentration of epoxy resin it contains, unlike the other ES used (21).

Chandra et al. (27) showed that there was no significant difference in the depth of penetration in the apical third between RoekoSeal® and AH Plus® ES. In this study, a lower penetration was found for the three ES evaluated at the apical 3 mm, which is like the studies of Balguerie et al. (11), Bassem et al. (15), Teixeira et al. (28) and Paqué et al. (29), who agree that the poorest penetration of sealers into the dentinal tubules occurs in the apical third. This can be explained by the inefficient entry of irrigant into this region of the root canal, by the reduced diameter and number of dentinal tubules in this area, and by the greater presence of tubular sclerosis.

Siqueira et al. (14) evaluated the flowability and antibacterial effect of several ES, such as Kerr Pulp Canal Sealer™ EWT, Grossman®, ThermaSeal, Sealer 26 and AH Plus®, and found that AH Plus® and Kerr Pulp Canal Sealer™ have superior flowability values, and that all of them showed some antimicrobial activity, thus suggesting that these ES have potential to aid in the microbial control of the root canal system (14). Kwak et al. (30) also report that ES neutralize the nutrient pathway of microorganisms, and that they can exert an effect against residual bacteria in the dentinal tubules, thus preventing bacterial reinfection and isolation of residual stimuli in the root canal.

Versiani et al. (31) report that the penetration capacity of ES increases when the smear layer is previously removed. Kokkas et al. (12) evaluated the influence of the smear layer on the penetration of ES into the dentinal tubules, using AH Plus®, Apexit Plus® and Roth 811, obtaining favorable results for AH Plus® and Apexit Plus®. This study followed the same final

irrigation protocol for the removal of the smear layer used by Kokkas et al. (12) to favor the penetration of the ES used.

One of the limitations of the study could be the standardization of the samples with respect to the age of patients at the time the extractions were performed, since they could have differences in the size and density of the dentinal tubules.

The penetration of ES into the dentinal tubules provides positive effects in our root canal treatment, such as a larger contact surface between dentin and ES, micromechanical retentions, a hermetic seal that prevents any bacterial filtration, as it achieves a blockage of residual microorganisms in the dentinal tubules.

CONCLUSIONS

All three ES studied showed penetration into the dentinal tubules. The ES that showed the best penetration was Roekoseal®, with the best result at 3 mm.

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