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Original article

Effect of 17% ethylenediaminetetraacetic acid and silver citrate on sealer resin penetration in the apical third

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ABSTRACT

Background: *Endodontic sealers limit bacteria growth and clean the smear layer of the root canal. Biocompatible irrigants silver citrate and ethylenediaminetetraacetic acid (EDTA) have a chelating agent that increases sealer penetration in dentinal tubules.* ***Purpose:*** *This study aims to investigate the final irrigation difference in epoxy resin and bioceramic sealer penetration into dentinal tubules at the apical third.* ***Methods:*** *A total of 30 extracted mandibular premolars were split into six groups; three received epoxy resin sealer and three received bioceramic resin with aquadest, silver citrate (BioAKT) or EDTA 17% irrigation. A confocal laser scanning microscope estimated sealer penetration in dentinal tubules. For quantitative data analysis, Olympus Fluoview ver.4.2a was used.* ***Results:*** *Silver citrate final irrigation with bioceramic resin sealer had the highest dentinal tubular penetration (24%; 1,431 µm), followed by EDTA 17% (20%; 1,202 µm), aquadest (16.3%; 969 µm), EDTA 17% with epoxy resin (15.8%; 938 µm, 14%; 803 µm), and distilled water (10%; 584 µm). Significant differences existed in all groups (p = 0.001). Epoxy resin sealer penetration into dentinal tubules was similar between final irrigants (p = 0.257) and bioceramic resin groups (p = 0.658).* ***Conclusion:*** *Silver citrate (BioAKT), a bioceramic resin sealer-based final irrigation solution, penetrates dentinal tubules better for endodontic therapy.*

Keywords: *endodontic irrigation material; EDTA; medicine; silver citrate; sealer*
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INTRODUCTION

Root canal treatment aims to prevent bacterial invasion and infection of the pulp and root canals. Root canal treatment is done through chemomechanical preparation procedures on the root canal, including the endodontic triad (Access, Clean and Shape, Obturation), cleaning, and shaping.[1] The use of irrigation materials plays a role in supporting the success of root canal treatment, including disinfecting the root canal, removing instrument debris, and dissolving organic and inorganic components from the smear layer to clean the dentin surface so the root canal can be obturated.[2] Nevertheless, forming the smear layer during the instrumentation process acts as an interface that hinders the bonding mechanism of the filling substance.[3]

The root canal smear layer comprises organic and inorganic components containing necrotic tissue and microorganisms. Its presence favors the adhesion and colonization of microorganisms and inhibits the disinfectant and action of medicaments on the root canals of teeth.[4] Also, the smear layer prevents the adaptation of sealers (cement lining the walls of the root canals) and root canal filling materials to the dentin layer in the root canal walls.[5] Irrigating agents are required to rinse debris, kill microorganisms, and clean organic and inorganic components of the smear layer contained in all parts of the root canal and parts not reached by instrumentation.[6]

The requirements for an ideal irrigation material are that it is capable of dissolving vital tissues, eliminating bacteria, not irritating extra-radicular tissues, and being non-toxic, non-antigenic, and non-carcinogenic.[7] In addition, an ideal irrigation material has a long-term effect, does not harm the physical properties of dentin, does not affect the sealer bond, can deactivate bacterial endotoxins, does not give color to the tooth structure, acts as a lubricant, removes the smear layer, and is easy to use/store.[8]

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Endodontics often uses 17% ethylenediaminetetraacetic acid (EDTA) for root canal therapy. As a chelating agent, it helps remove inorganic smear layer elements from the root canal system. This improves system permeability and disinfection. Silver citrate's antibacterial properties have been studied for their potential to aid root canal disinfection.[9] Epoxy-based and bioceramic resin sealers fill and seal the root canal space after cleaning and shaping. For a long time, endodontics has used epoxy resin sealers, which seal well but are technique-sensitive. Recent years have seen bioceramic sealers become popular. The benefits include their biocompatibility, capacity to connect with dentin and gutta-percha, and ability to speed healing.[10]

The dental root's apical third is the portion nearest the terminal point. Endodontic therapies depend on the apical third to seal and prevent bacteria re-entry.[11] Root canal therapy works best when this area is meticulously cleaned, contoured, and sealed. Sealers and disinfectants must be tested for efficacy to ensure therapeutic success, especially in the apical third.[12]

Silver citrate (BioAkt) is a new irrigation liquid containing silver citrate dihydrate particles dispersed in an aqueous solution, with strong biocidal properties upon contact with many microorganisms.[13] Tonini et al.[14] reported that administering final irrigation using silver citrate (BioAkt) for 1 minute removed the smear layer in the apical third more effectively than final irrigation with EDTA 17%. Based on theory and facts, previous research explains that silver citrate (BioAKT) as a bioceramic sealer has improved sealer penetration in endodontic therapy.[15] This study has evaluated the use of silver citrate (BioAkt) as a final irrigant to increase sealer penetration into dentinal tubules.

MATERIALS AND METHODS

This research has passed ethical clearance No: 12/KEPK/USU/2022 from the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Thirty single-rooted caries-free premolars were extracted by the Department of Oral Surgery, Dentistry Faculty, Universitas Sumatera Utara, Medan, Indonesia. EDTA 17%, Sodium hypochlorite (NaOCl) 2.5%, silver citrate, and gutta-percha were used by Sigma Aldric products (Merck KGaA, Darmstadt, Germany) as well as confocal laser scanning microscopy (CLSM) (Nikon, Natori, Miyagi Prefecture, Japan).

The 30 teeth were divided into six treatment groups: Group A (EDTA 17% irrigation with epoxy resin), Group B (EDTA 17% with bioceramic), Group C (silver citrate with epoxy resin), Group D (silver citrate with bioceramics), Group E (aquadest with epoxy resin), and Group F (aquadest with bioceramics). The dental sample was cleaned of external debris and remaining soft tissue and then immersed in saline before treatment. The crowns were cut transversely 2.0 mm occlusal beyond the buccal cementoenamel junction by diamond disks, and the residual roots with an average length of 15.0 ± 1.0 mm were retained.[16]

In the first stage, irrigation was carried out with 3 mL of NaOCl 2.5% for 1 minute using an irrigation needle (one-side-vented 30 G) and then measured with K-file #10.[17] Reciprocating files painstakingly constructed root canals. Initially, high-speed dental burs provided direct access to the canal orifice, ensuring sufficient visualization. A #10 or #15 K-file was gently inserted into the canal to check its patency. The working length was measured from a coronal reference point to the canal preparation and filling stop. Electronic apex locators and/or periapical radiography with a file in situ were used for this. This measure made access more accessible and helped carry equipment. Using stainless steel K- and H-files in the step-back technique was the standard canal instrumentation method. Occasionally, reamers were rotated to extend canals. Finally, canal preparation used NiTi systems like WaveOne Gold and Reciproc, which reciprocate instead of rotate. Next, the needle was bent and inserted loosely into the root canal. The hand was moved up and down continuously during root canal irrigation to produce agitation. Agitation was achieved with ultrasonic for 3 1 minute with 2 mL of NaOCl 2.5%, then rinsed with aquadest.

The root canals were then irrigated with 5 ml of EDTA 17% solution, left for 1 minute (according to the sample group), then rinsed with 2 mL of distilled water. The root canals were dried using paper points, based on a sample group, using a single cone technique. The root canals were then filled with gutta-percha cone and sealer (epoxy resin and bioceramic). The gutta-percha was then smeared with a sealer mixed with fluorescent rhodamine B to a concentration of 0.1%. Once inserted into the root canal, the excess sealer was removed, and the gutta-percha was cut with a hot instrument. After the root canal filling was completed, the samples were stored in a container lined with aluminum foil and incubated at 37°C with 100% humidity for seven days.[18] Then, the examination was conducted with CLSM.

The 30 incubated teeth were cut horizontally at a distance of 4 mm from the apex with a thickness of 2 mm using a disk bur under cold water to prevent heat from friction. The surface was polished using sandpaper under running water to remove dentin debris generated during cutting. Analysis of the CLSM image began by placing a tooth sample on a coverslip measuring 24 60 mm, which was then examined using CLSM with a magnification of 100 and a wavelength of 543 nm. The image was focused on the target area, rhodamine dye was selected from the dye list in the CLSM software, the scanning process was carried out, and the image acquisition control was adjusted to obtain the relevant image. To see the brightness in the image, the HV setting was changed, similarly with the offset to set the background to black. The resulting image appeared on the computer monitor screen, which was then analyzed using the Olympus Fluoview ver.4.2a software. At the initial stage, the image was circled with the existing

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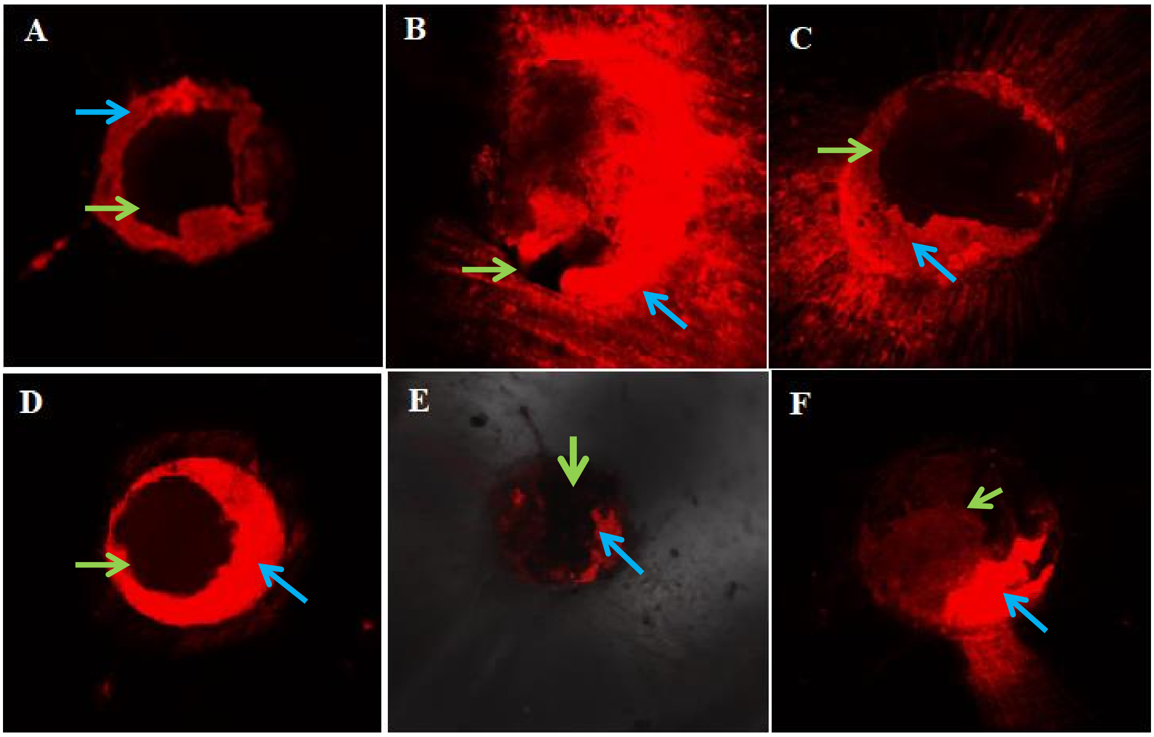

Figure 1. Representative CLSM depiction of sealer penetration into the dentinal tubules (blue arrow) and the boundary of the dentinal surface with the sealer (green arrow). (A) Final irrigation of 17% EDTA with epoxy resin sealer; (B) final irrigation of 17% EDTA with bioceramic sealer; (C) final irrigation of silver citrate (BioAKT) with epoxy resin sealer; (D) final irrigation of silver citrate (BioAKT) with a bioceramic resin sealer; (E) aquadest final irrigation with an epoxy resin sealer; (F) aquadest final irrigation with a bioceramic sealer (magnification 400).

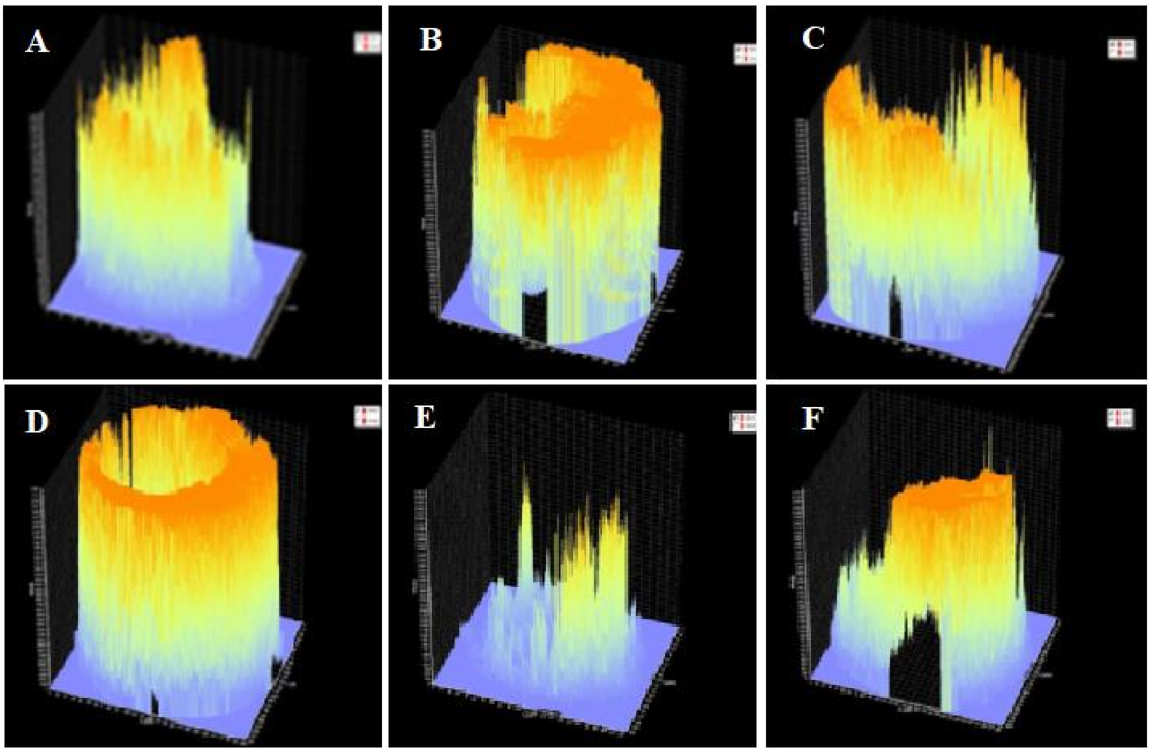

Figure 2. Graph visualization of dentin tubule intensity with CLSM. (A) Final irrigation of EDTA 17% with epoxy resin sealer; (B) final irrigation of EDTA 17% with bioceramic sealer; (C) final irrigation of silver citrate (BioAKT) with epoxy resin sealer; (D) final irrigation of silver citrate (BioAKT) with a bioceramic resin sealer; (E) aquadest final irrigation with an epoxy resin sealer; (F) aquadest final irrigation with a bioceramic sealer (magnification 400).

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tools in the software, and a measurement was chosen to see the intensity of expression of the sealer labeled with rhodamine. The analysis results were then generated automatically, giving the average value, standard deviation, and intensity profile graph.[19]

The data obtained were evaluated for normality with the Shapiro-Wilk and Levene tests to see the homogeneity of p š 0.05. Furthermore, an analysis of variance (ANOVA) was carried out for quantitative data on sealer penetration in dentinal tubules, followed by the least significance difference test with significance ($p < 0.05$) and Pearson correlation ($r = 1$) for a strong relationship. Statistical analysis was completed using Statistical Package for Social Science (SPSS) version 23.0 (IBM, Illinois, Chicago, US).

RESULTS

Figure 1 shows the penetration of the bioceramic sealer and the epoxy resin in the dentinal tubules. Based on the confirmation of Figure 2, the graph of dentin tubule intensity, the best sealer penetration was in group (D) Final irrigation with silver citrate (BioAKT) with bioceramic resin sealer, followed by Group B (final irrigation with EDTA 17% with bioceramic sealer) and Group C (final irrigation with silver citrate (BioAKT) with an epoxy resin sealer).

In Figure 1 and Figure 2, Group A exhibits a uniformly red yet irregular pattern, indicating imperfect sealer penetration (Figure 1A). The intensity profile graph, shown in orange, is highlighted to represent the sealer's penetration intensity, with orange indicating high intensity and yellow indicating a decrease in intensity (Figure 2A). In Group B, the red color distribution is uneven (Figure 1B), and the intensity profile graph (Figure 2B) shows a slight orange coloration at the end of the line, reflecting a non-uniform sealer penetration with varying lengths. For Figure 1C, the red color spreads in one direction, leaving the opposite direction without any red. The intensity profile graph (Figure 2C) shows non-circular lines, with orange appearing on one side. In Group D, the sealer penetrated very well into the dentinal tubules, with a uniform red color around the tubules (Figure 1D). This picture is also visualized in its intensity profile graph (Figure 2D). For Group E, a one-sided red outline is more visible (Figure 1E). The intensity profile graph analysis depicts an orange color at the end of the uneven line (Figure 2E). For Group F, the red image is uneven (Figure 1F), and the intensity graph shows only a few lines (Figure 2F).

Table 1 shows the quantitative results of the penetration of epoxy resin-based sealers and bioceramics. The bioceramic-based group generally had better penetration into the dentinal tubules than the epoxy resin-based group. One-way ANOVA showed significant differences between

Table 1. Quantitative data on the penetration of epoxy resin and bioceramic sealers in dentinal tubules

Sealer	Variable groups	N	Penetration (μm)	S.Devt	Frequency (%)	Category	*p-value
Epoxy resin-based sealers	EDTA 17% (A)	5	803.54	162.12	14%	High	
	Silver citrate (BioAKT) (C)	5	938.74	275.14	15.8%	High	0.257
	Aquadest (E)	5	584.94	460.09	10%	Medium	
Bioceramic resin-based sealers	EDTA 17% (B)	5	1201.95	905.76	20%	Very high	
	Silver citrate (BioAKT) (D)	5	1431.49	782.17	24%	Very high	0.658
	Aquadest (F)	5	969.41	641.81	16.3%	High	
*p-value					0.011		

Table 2. Pearson correlation of epoxy and bioceramic resin sealers penetration in dentin tubules

Group	Correlations	Group B	Group C	Group D	Group E	Group F
Group A	Pearson correlation	0.401	-0.914	-0.644	-0.495	-0.080
	Sig. (2-tailed)	0.599	0.086	0.356	0.505	0.920
	N	4	4	4	4	4
Group B	Pearson correlation		-0.697	-0.077	0.002	-0.396
	Sig. (2-tailed)		0.303	0.923	0.998	0.604
	N		4	4	4	4
Group C	Pearson correlation			0.380	0.218	0.386
	Sig. (2-tailed)			0.620	0.782	0.614
	N			4	4	4
Group D	Pearson correlation				0.984*	-0.702
	Sig. (2-tailed)				0.016	0.298
	N				4	4
Group E	Pearson correlation					-0.816
	Sig. (2-tailed)					0.184
	N					4

*. Correlation is significant at the 0.05 level (2-tailed).

Note: Group A (EDTA 17% final irrigation with epoxy resin). Group B (EDTA 17% final irrigation with bioceramics). Group C (silver citrate last irrigation with epoxy resin). Group D (silver citrate final irrigation with bioceramic resin. Group E (final irrigation of distilled water with epoxy resin). Group F (final irrigation of aquadest with bioceramic).

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the groups based on the epoxy resin ($p < 0.05$; p: 0.011). Meanwhile, there was no significant difference between the bioceramic resin-based groups ($p > 0.05$; p: 0.683) as well as in the epoxy resin group ($p > 0.05$; p: 0.257).

Table 2 shows that the bioceramic resin sealer with silver citrate final irrigation showed a strong relationship ($r = 0.984$) and that it was significantly different ($p < 0.05$; p: 0.016) from the epoxy resin sealer group with aquadest final irrigation. It is indicated that the use of the type of sealer and the final irrigation solution strongly influence the penetration of the sealer in the dentinal tubules. The final irrigation of silver citrate with epoxy resin sealer had a weak relationship with the group of final irrigation of silver citrate with bioceramic resin sealer ($r = 0.380$), the group of final irrigation of distilled water with epoxy resin sealer ($r = 0.218$), and the group of final irrigation with aquadest with a bioceramic sealer ($r = 0.386$). There is no significant difference between these three groups ($p > 0.05$).

DISCUSSION

The main objective of this study was to compare silver citrate and EDTA 17% as a final irrigation solution in endodontic treatment in increasing the penetration of epoxy resin sealers and bioceramic resins. This study reported that silver citrate and EDTA 17% had a similar ability to increase sealer penetration into dentinal tubules, although silver citrate was better than EDTA 17%.[14] However, irrigation materials are one factor influencing the tendency to fracture.[20] Both are reported as final irrigants often used in root canal treatment. Both of these materials have antibacterial and chelating properties and can reduce the formation of a smear layer on the root canal wall.[21]

The results of Figure 1 align with the graph profile of the dentinal tubule intensity (Figure 2). Silver citrate (BioAKT) is reported as an agent for cleaning the smear layer.[14] It improves sealer penetration, which is good at the apical part of the root canal system, and has significant antibacterial properties and low cytocompatibility.[15] Silver citrate, marketed as BioAKT, possesses inherent antimicrobial properties, but its role in sealer penetration might be attributed to its unique interaction with dentinal tubules. The dentinal tubules, microscopic channels extending from the pulp to the periphery of the dentin, play a crucial role in sealer penetration.[14] The sealer must penetrate these tubules for optimal adhesion and deep sealing ability.[22] The bioceramic resin sealer itself might be incompatible with the modifications brought about by silver citrate. They are known for their bioactivity, biocompatibility, and ability to bond to dentin chemically, thus ensuring a tight seal.[23] When combined with the preparatory action of silver nitrate on dentinal tubules, a synergistic effect results in enhanced sealer penetration compared to the traditional use of 17% EDTA (Figure 1).

Jeong et al.[24] reported that chelators such as EDTA or citric acid were required to remove inorganic constituents from the smear layer. The disadvantages of EDTA are that it is less sensitive to bacteria, and the elimination of the smear layer is limited in the root canal area.[25] This is related to its ability to reduce properties that allow increased penetration of the resin sealer into the dentinal tubules.[24] This occurs due to increased surface tension of the root canal walls.[26] In vitro studies have reported that the surface tension value of citric acid was lower than that of EDTA.[27]

The success of sealer penetration into the dentinal tubules is also influenced by the accumulation of the smear layer or possibly due to the root canal anatomy in the apical third, which is very complex and has fewer dentinal tubules than the coronal part.[28] Utilization of silver ions (silver citrate) in aqueous solutions results in surface stability, reduces surface tension, enhances the antibacterial effect of the resolution, and reduces the biofilm's mechanical stability by destabilizing its cohesive strength.[15] It was reported that the surface tension of silver citrate was lower than that of EDTA 17%, so the flow into the dentinal tubules was more profound than that of EDTA 17%.[29] This stability allows for faster and fuller penetration of the sealer in the dentinal tubules.[14]

Silver citrate irrigating solution removed more smear layer than EDTA 17% and had a deeper sealer penetration (Figure 2). The results showed that the penetration depth of the bioceramic sealer reached 24% of the six treatment groups. These results indicate that silver citrate can also reduce the smear layer. The more effective an irrigation solution removes the smear layer, the deeper the sealer penetration.[30]

In addition to irrigation materials, sealers also play an essential role in reasonable flow rates. They will be better able to enter difficult-to-access areas such as lateral channels, fins, and isthmus.[31] The study used an epoxy resin sealer and a bioceramic sealer, where the epoxy resin sealer has good adhesion to dentin and low water solubility. Bioceramic sealers have good flow ability, and their ability to release Ca^{2+} ions is also better than other sealer materials.[32]

Al-Haddad et al.[33] reported that bioceramic sealers contain calcium phosphate silicate with a particle size (<1 m), chemical composition, and structure similar to hydroxy apatite in teeth, so it can help increase the sealer bond with the root canal wall. In addition, bioceramic sealers have better adaptation and higher sealer penetration into the dentinal tubules than AH Plus, especially in the apical third of the root canal.[34] The utilization of silver citrate as final irrigation was associated with the depth of penetration of the sealer in the dentinal tubules (Table 1). These results indicate that the silver citrate with a bioceramic sealer has good adhesive properties. Both materials can maintain surface tension changes, thereby facilitating and protecting the changes in dentinal tubules when the sealer penetrates the dentinal tubules (Figures 1D and 2D). This research did not examine the release of calcium ions, which is a factor that influences sealer penetration in the dentin tubules. In addition, chemical elements from the surface of the root

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canal walls after sealer penetration were not examined. In conclusion, silver citrate as a final irrigation solution has a better ability than EDTA 17% to help increase the penetration of bioceramic resin sealers in dentinal tubules in endodontic treatment.

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