Effect Of Final Irrigation Materials And Techniques On Sealer Penetration Depth Into Dentinal Tubule Of The Apical Third Root Canal

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ABSTRACT

Background: *The smear layer may interfere with the penetration of the root canal sealer. Final irrigation is known to improve chelating agents' ability in smear layer removal. This study aimed to compare the effect of various materials and final irrigation techniques on the penetration depth of bioceramic silers in the apical third of the tooth.*
Method: *This study used 45 premolars that were prepared with a rotary instrument and then randomly divided into three experimental groups (n=15). Group 1 used 17% EDTA as the final irrigation material, group 2 used Novel Silver Citrate, and group 3 used 0.2% nanoparticle chitosan. Each group was divided into three subgroups (n=5), with subgroup A using manual agitation technique, subgroup B using sonic agitation, and subgroup C using ultrasonic agitation. The teeth were then obturated with the single cone technique using a bioceramic sealer. Teeth then cut vertically for observation and measurement sealer penetration using a stereo microscope with a magnification of 30x followed by observation using SEM at 1000x magnification. data where analyzed using a two-way ANOVA followed by an LSD Post Hoc test, with a significance level of 95%.*
Result: *Two-way ANOVA showed significant differences in materials, techniques, and material-technique interactions (p<0,05).*
Conclusion: *This study concludes that there is an effect of material, technique, and material-technique interaction of final irrigation on the penetration of bioceramic sealer into the dentinal tubules, Combination of 0.2% chitosan nanoparticles and sonic agitation as material and final irrigation technique resulted in the most longest penetration of bioceramic sealer into dentinal tubules.*

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INTRODUCTION

The final outcome of root canal treatment depends on the success in eliminating bacteria from the root canal and preventing the re-growth of these bacteria.[1] Biomechanical preparation consists of mechanical instrumentation of the root canal and irrigation with antibacterial materials. The stages of root canal instrumentation and irrigation are carried out together, precisely, and adequately. Biomechanical preparation aims to shape the ideal root canal and disinfect the entire root canal system.[2]

Root canal preparation procedures result in the formation of a smear layer. The smear layer can interfere with the antibacterial effects of intracanal medicaments and the adaptation of root canal sealers.[3] Eliminating the smear layer reduces bacterial retention during root canal filling. Additionally, removing the smear layer can improve the quality of root canal obturation. This is related to the potential increase in the sealer's ability to penetrate dentinal tubules.[4] Adequate sealer penetration into dentinal tubules allows endodontic sealers to eliminate remaining bacteria in dentinal tubules and enhance sealer adaptation to dentin walls.[5] Bioceramic-based sealers can also form chemical and physical bonds with dentin and root canal filling materials.[35] The potential bonding between bioceramic sealers and dentin may occur due to several factors, including the diffusion of sealer particles into the dentinal tubules (tubular diffusion) to create mechanical bonds with the dentin walls. Furthermore, the infiltration of mineral content from the sealer into the intertubular dentin can lead to the formation of a mineral infiltration zone resulting from collagen fiber denaturation due to the high alkalinity of the bioceramic sealer. Additionally, the reaction of phosphate with calcium silicate hydrogel and calcium hydroxide, produced through the reaction of calcium silicate in the presence of dentin moisture, results in the formation of hydroxyapatite along the mineral infiltration zone, which enhances the bonding between the bioceramic sealer and the dentin walls.[36]

Irrigation solutions with chelating properties are used to remove the smear layer, which may hinder sealer penetration into dentinal tubules.[6] The most commonly used chelating agent for removing the smear layer in root canals is the inorganic acid ethylenediaminetetraacetic acid (EDTA). In addition to good biocompatibility, EDTA can dissolve inorganic tissue. Ethylenediaminetetraacetic acid is usually used at a concentration of 17% and can remove the smear layer when in direct contact with the root canal wall.[7] However, the neutral pH of EDTA makes its chelating effect less potent in eliminating the smear layer in the apical third area of the tooth root.[3]

The development of irrigation solutions has led to improved chelating abilities and the addition of antibacterial effects of the final irrigation solution. Solutions containing silver citrate have become commonly used irrigation solutions. Root canal irrigation materials are made from silver ions (0.003%) electrolytically mixed with citric acid (4.846%). Novel silver citrate irrigation solutions have been tested as biomaterials for root canal disinfection and cleaning. Novel silver citrate is reported to be a biocompatible materials. The chelating effect of novel silver citrate is derived from the citric acid content, while its bactericidal ability depends on silver ions. This composition results in the dual function of novel silver citrate during endodontic treatment.[9] Although novel silver citrate is a new irrigation material with several advantages, the toxicity of silver ions needs to be noted and considered.[37] In addition to using silver and citric acid-based irrigation solutions, chitosan is now being used as a material in endodontics. Chitosan is a natural polysaccharide obtained from the deacetylation of chitin. Chitin is commonly found in the extracellular matrix of crustaceans.[10] Chitosan nanoparticles have also been used in endodontics due to their biological properties such as biocompatibility, biodegradability, bioadhesion, and low toxicity.[11] A 0.2% chitosan nanoparticle irrigation solution has the ability to remove the smear layer and open dentinal tubules without causing excessive dentin erosion compared to 17% EDTA.[12] The chelating ability of chitosan is known to come from its ability to chelate metal ions. Chitosan polymers are hydrophilic and have hydroxyl and free amino groups, making them cationic. This allows for ionic interactions with calcium ions in the smear layer in the chelation mechanism of chitosan, which makes the smear layer easily soluble.[13]

Root canal irrigation with a needle without agitation is the most common technique used to flow irrigation fluid into the root canal. However, in complex root canals, irrigation fluid is difficult to reach all parts of the root canal. Agitation techniques using sonic and ultrasonic instruments are now used to enhance chemical debridement by forming micro-acoustic waves, which are rapid and circular movements of irrigation fluid around vibrating files, resulting in acoustic cavitation during ultrasonic agitation. These acoustic waves also generate irrigation fluid flow to the entire complex root canal area.[14] Sonically agitated irrigation uses waves with frequencies of 1–6 kHz. Sonically agitated techniques use instruments consisting of handpieces with polymer tips. Polymer tips tend to be safe and do not cut the root canal walls.[15] Ultrasonically agitated techniques use files oscillating at ultrasonic frequencies of 25-30 kHz. Ultrasonic oscillations will activate irrigation fluid in the root canal system by inducing acoustic flow and cavitation.[16]

This study aims to investigate the effect of final irrigation with 0.2% chitosan nanoparticle solution compared to NSC and 17% EDTA, which are sonically and ultrasonically activated, on the penetration of bioceramic sealers into dentinal tubules.

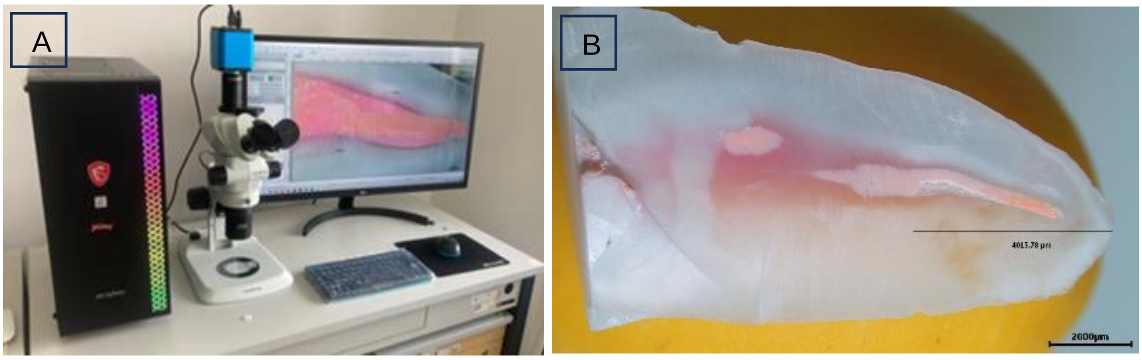
RESEARCH METHOD

This research obtained Ethical Clearance with number 49/UN1/KEP/FKG-RSGM/EC/2023 from the Research Ethics Commission of the Faculty of Dentistry-RSGM Prof. Soedomo, Universitas Gadjah Mada Yogyakarta. This is an in vitro research. In this research have two independent variables which is type of final irrigation materials used: 17% EDTA, Novel Silver Citrate, 0.2% chitosan nanoparticles. And the methods of final irrigation used: manual agitation, sonic agitation, ultrasonic agitation. This research was conducted to examine the penetration length of bioceramic sealer into the dentinal tubules of the apical third of the tooth The penetration of root canal sealer refers to the extent to which bioceramic-based sealer enters the dentinal tubules. The measurement involves determining the longest penetration of the bioceramic sealer into the dentinal tubules from the root canal wall to the furthest point of sealer penetration. The length of sealer penetration is measured in microns (µ). The depth of sealer penetration is calculated using OptiLab Viewer 4. Research start with forty-five mandibular premolar teeth that met the requirements were confirmed radiographically to prove they had a single and straight root canal. The teeth were sectioned using a wheel diamond disc bur attached to a low-speed handpiece. The teeth were cut coronally, leaving a 12 mm root. The cut teeth were embedded in red resin blocks. A #10 K-file was inserted into the root canal until its tip was visible at the apical foramen, and the working length (WL) was set 1 mm shorter than this length.[11] Root canal preparation was performed using the crown-down technique with rotary files (M3 Pro-Gold, United Dental) according to the WL, ending with a size 30/.04 file. Root canal preparation was done using rotary files and an endomotor sequentially according to the working length. After each instrument change, the root canal was irrigated with 3 ml of 2.5% NaOCl and 3 ml of saline. The samples were then randomly divided into three experimental groups (n=15) with group 1 using 17%

EDTA as the final irrigation material, group 2 using Novel Silver Citrate, and group 3 using 0.2% Chitosan nanoparticles. Each group was divided into three subgroups (n=5), with subgroup A using manual agitation technique, subgroup B using sonic agitation, and subgroup C using ultrasonic agitation. The root canals were then dried with 5 paper points. Root canal obturation was performed using the single-cone technique. A M3PG3 gutta-percha cone (M3, United Dental, Shanghai, China) marked at the working length (12 mm) was inserted into the prepared root canal until tug back was achieved. Bioceramic-based sealer (Ceraseal, Metabiomed, South Korea) mixed with 0.1% rhodamine b (Sigma Aldrich, Germany) until homogenized to aid microscopic observation.[17] The sealer was then injected 0.2 grams into the root canal using a syringe. The sealer was spread on the root canal walls using a #25 lentulo spiral marked at the working length with a rubber stop.

Gutta-percha coated with sealer in the apical third was then inserted into the root canal and pumped in and out to ensure all root canal wall surfaces were coated with sealer. Gutta-percha was cut 1 mm coronally from the apical direction to provide space for temporary filling and condensed using a heated heat carrier plugger. The depth of gutta-percha cutting was checked using a periodontal probe. Excess sealer was removed with a cotton pellet, and the cavity was sealed with a temporary filling. The tooth samples were placed in an incubator for 7 days at 37°C to allow the sealer to set completely.[11]

The samples were then vertically sectioned into two pieces using a diamond disc bur (Horico, Germany). The root canal sections were rinsed with 17% EDTA and then distilled water for 15 seconds.[34] The sample sections were observed with a stereo microscope at 12x magnification. The apical third was measured with a ruler using OptilabViewer 4 software and marked at 4 mm from the apex (Figure 1).



(a) (b)

Figure 1. Measurement of sealer penetration length. (A) Application of OptiLab Viewer 4 with Stereo Microscope (Olympus SZX7®, Japan). (B) Measurement of observation area 4mm from the apex. Black arrows indicate observed sealer penetration, further observed samples at 30x magnification.

Furthermore, at 30x magnification, the observation area is divided into 4 sections, and observation as well as measurement of the depth of sealer penetration from the root canal wall to the furthest sealer penetration in the dentinal tubules are performed (Figure 2). The results of sealer penetration depth are then recorded and tabulated.[18]

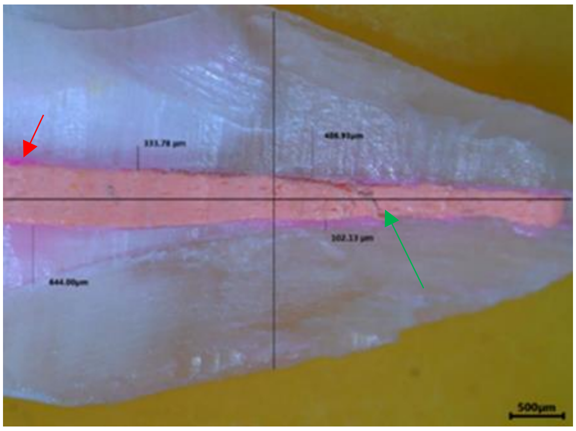


Figure 2. Measurement of the length of bioceramic sealer penetration into dentinal tubules at 30x magnification. The red arrow indicates the depth of sealer penetration, The green arrow indicates gutta-percha.

The samples were then observed again using a scanning electron microscope. The samples were mounted on aluminum plates and coated with gold. Observation was conducted using a Scanning Electron Microscope (JSM IT-700, Jeol, Tokyo, Japan). SEM initially magnified the samples at 20x to determine the area with the deepest sealer penetration. Then, the depth of sealer penetration into the tubules from the root canal wall was directly assessed quantitatively at a magnification of 1000x.[19] The data obtained from this research were ratio data, specifically the depth of penetration of bioceramic-based sealer in the 9 treatment groups. Normality test (Shapiro-Wilk) was conducted to determine the normal distribution of data for each group, and homogeneity test (Levene) was performed to assess variance homogeneity. Subsequently, a two-way ANOVA test was conducted with a confidence level of 95% ($\alpha=0.05$). The results of the ANOVA test showed a significant difference; thus, it was followed by the post hoc Least Significant Difference (LSD) test.

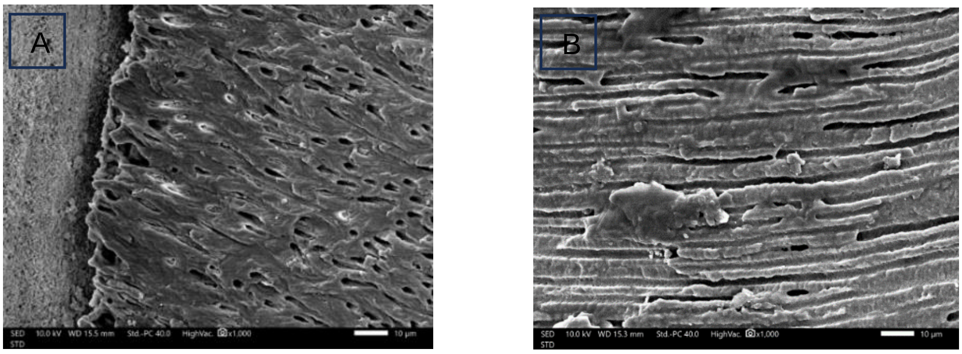
RESULTS

The depth of sealer penetration was observed using a stereo microscope at 30x magnification, on vertical sections of premolar teeth with a single root canal. Images were captured using a stereo microscope camera. In this study, sealer penetration appeared as pink lines within the dentinal tubules. The depth of penetration was measured in micrometers using the OptiLab Viewer 4 application. Additionally, the quality of sealer penetration into the dentinal tubules was also observed using a scanning electron microscope (SEM). The measurement results of sealer penetration depth are presented in Table 1.

Group	Manual Agitation	Sonic	Ultrasonic
EDTA 17%	215.2 ±19,1 µm	613,6 ±20,3 µm	516,2 ±26,1 µm
Novel silver citrate	439,8 ± 42,1 µm	866,6 ± 32,42 µm	713,8±2 5,67 µm
Chitosan Nanoparticle 0,2%	498,0± 23,9 µm	1320,4± 32,4 µm	1128,2± 64,4 µm

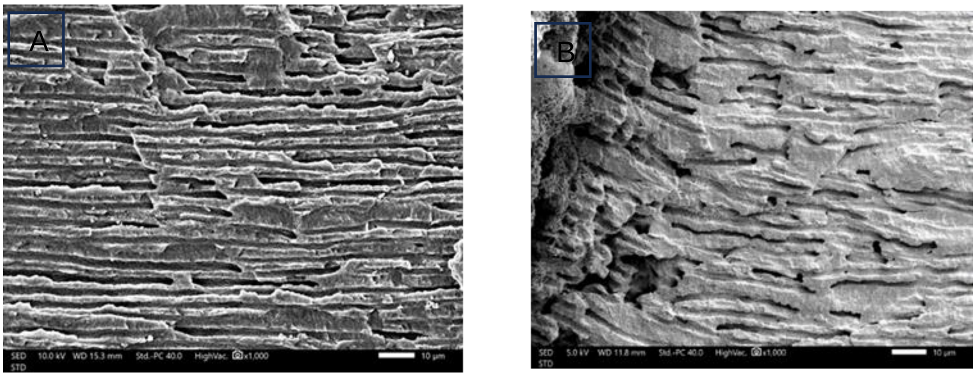
Table 1. Mean length of bioceramic sealer penetration into dentin tubules after irrigation with different materials and techniques.

The average results for each sample group indicate that the Chitosan nanoparticles 0.2% - Sonic group has the highest mean depth of sealer penetration into the dentinal tubules compared to other material and treatment groups, followed by the Chitosan nanoparticles 0.2% - Ultrasonic and Novel silver citrate - Sonic groups. The EDTA 17% - Manual sample group has the shortest mean depth of sealer penetration into the dentinal tubules. The samples were then observed using a scanning electron microscope in 1000x magnification. (Figures 3 and 4).



(a) (b)

Figure 3. Observation of the depth of sealer penetration using SEM in the 17% EDTA group. (A) After manual irrigation, uneven sealer penetration into dentinal tubules is observed (red arrow).(B) After irrigation using sonic agitation technique, sealer is visible within dentinal tubules, and bonding between dentin and sealer is evident (yellow arrow).



(a) (b)

Figure 4. Observation of the depth of sealer penetration using SEM in the 0.2% Chitosan Nanoparticle group.(A) After manual irrigation, sealer penetration into dentinal tubules is observed.(B) After irrigation using sonic agitation technique, sealer fills the dentinal tubules, and bonding between dentin and sealer is evident.

Before conducting parametric testing using a two-way ANOVA, two conditions are required for the obtained data: homogeneity (equality of data variance) and normality (normal distribution of data). The Shapiro-Wilk test is conducted to determine and confirm the normality of the data. Based on the normality test of the data using the Shapiro-Wilk test, it is known that the data in each research group have a normal distribution. This is evident from the significance value of each group being greater than 0.05 (p>0.05). Furthermore, the equality of data variances (homogeneity) is tested using the Levene's test. From the Levene's test, it is found that the data distribution is homogeneous, as indicated by the value of p = 0.72 (Sig p>0.05). Since the data meet the requirements, further testing is conducted using a two-way ANOVA. The purpose of this two-way ANOVA test is to determine whether there is an influence from various tested criteria on the desired outcomes.

Table 2. The statistical analysis using two-way ANOVA shows the penetration of sealer into dentinal tubules after irrigation with different materials and techniques.

Variable	Degree of freedom	Square mean	F	P
Materials	2	740283,41	132,87	0,00*
Techniques	1	2260685,51	405,75	0,00*
Materials- Techniques	2	157293,81	28,23	0,00*

Explanation: * = significant difference if p < 0.05

The test results indicate significant differences in material, technique, as well as the interaction between material and technique, with a p-value of 0.000 (p < 0.05). This value suggests that there is an influence from both material, technique, and the interaction between material and technique. The value also indicates that the hypothesis is accepted. Subsequently, a post-hoc test using the Least Significant Difference (LSD) is conducted to determine the differences among each group.

Table 3. The results of the post-hoc test Least Significant Difference (LSD) show the penetration of silver into the dentinal tubules after irrigation with different materials and techniques.

Material-technique		Mean difference	Sig.
EDTA-Manual	EDTA-Sonic	-398,40	0,00*
	EDTA-Ultrasonic	-301,00	0,00*
	NSC-Manual	-224,60	0,00*
	NSC-Sonic	-651,40	0,00*
	NSC-Ultrasonic	-498,60	0,00*
	Chitosan-Manual	-282,80	0,00*
	Chitosan-Sonic	-1105,20	0,00*
	Chitosan-Ultrasonic	-913,00	0,00*
EDTA-SONIC	EDTA-Ultrasonic	97,40	0,00*
	NSC-Manual	173,80	0,00*
	NSC-Sonic	-253,00	0,00*
	NSC-Ultrasonic	-100,20	0,00*
	Chitosan-Manual	115,60	0,00*
	Chitosan-Sonic	-706,80	0,00*
	Chitosan-Ultrasonic	-514,60	0,00*

EDTA-Ultrasonic	NSC-Manual	76,40	0,00*
	NSC-Sonic	-350,40	0,00*
	NSC-Ultrasonic	-197,60	0,00*
	Chitosan-Manual	18,20	0,41
	Chitosan-Sonic	-804,20	0,00*
	Chitosan-Ultrasonic	-612,00	0,00*
NSC-Manual	NSC-Sonic	-426,80	0,00*
	NSC-Ultrasonic	-274,00	0,00*
	Chitosan-Manual	-58,20	0,01*
	Chitosan-Sonic	-880,60	0,00*
	Chitosan-Ultrasonic	-688,40	0,00*
NSC-Sonic	NSC-Ultrasonic	152,80	0,00*
	Chitosan-Manual	368,60	0,00*
	Chitosan-Sonic	-453,80	0,00*
	Chitosan-Ultrasonic	-261,60	0,00*
NSC-Ultrasonic	Chitosan-Manual	215,80	0,00*
	Chitosan-Sonic	-606,60	0,00*
	Chitosan-Ultrasonic	-414,40	0,00*
Chitosan-Manual	Chitosan-Sonic	-822,40	0,00*
	Chitosan-Ultrasonic	-630,20	0,00*
Chitosan-Sonic	Chitosan-Ultrasonic	192,20	0,00*

* = significant difference ($p < 0.05$)

Based on the post-hoc LSD test, it is known that there is a significant difference between each sample group, except between the EDTA-Ultrasonic group and the 0.2% Chitosan Nanoparticle-Manual group.

DISCUSSION

This study aims to determine the effect of several materials and final irrigation techniques on the depth of penetration of bioceramic sealer into dentinal tubules. The penetration of sealer into dentinal tubules can be influenced by various factors, including the removal of smear layer after root canal treatment, obturation technique, root canal and dentinal tubule anatomy, as well as the physical and chemical properties of the sealer material itself.[20]

The two-way ANOVA analysis in this study indicates significant differences in material, technique, and the interaction between material and technique of final irrigation on the depth of penetration of bioceramic sealer into dentinal tubules. Currently, chitosan is used as an irrigation material in the field of endodontics due to its ability to chelate calcium ions. Chitosan is a natural polysaccharide obtained from the deacetylation of chitin, which is commonly found in the extracellular matrix of crustaceans.[10] Chitosan nanoparticle 0.2% irrigation solution has the ability to remove smear layer and open dentinal tubules.[12] Chitosan polymer is hydrophilic and contains hydroxyl and free amino groups, making it cationic. This allows for ionic interaction with calcium ions in dentin through the chelation mechanism of chitosan.[13] Chitosan solution also has low surface tension.[21] The low surface tension will enable the chitosan nanoparticle solution to more easily enter the dentinal tubules.[22] With better elimination of the smear layer, the bioceramic sealer can penetrate into the dentin tubules without hindrance, allowing the sealer to penetrate deeper into the dentinal tubules..

The results of the post-hoc least significant difference (LSD) analysis of materials in this study show that 0.2% chitosan nanoparticles significantly produce longer penetration of bioceramic sealer compared to 17% EDTA and novel silver citrate. These results are consistent with previous research where chitosan as a final irrigation material demonstrated longer sealer penetration compared to the 17% EDTA and citric acid groups.[23] The penetration of bioceramic sealer into dentinal tubules after final irrigation with novel silver citrate in this study shows a significant difference compared to post-EDTA 17% irrigation, but it is not longer compared to post-0.2% chitosan nanoparticle irrigation.

Novel silver citrate as a final irrigation material has chelating abilities due to its citric acid content. Citric acid is an organic acid with strong chelating properties. Its chelating ability on inorganic tissues is due to the chelation of calcium ions at low pH conditions.[24] The effectiveness of a chelating agent depends significantly on its pH value.[25] Regarding the acid-base nature of a chelating agent, 17% EDTA has a lower ability to chelate calcium ions compared to novel silver citrate and chitosan nanoparticles because it has a neutral pH. Another study reported that novel silver citrate had better smear layer removal capabilities compared to EDTA, indicating that with more effective smear layer removal, novel silver citrate can achieve deeper sealer penetration into dentinal tubules compared to the EDTA 17% group.[24] These findings align with the results of this study. LSD analysis in this study shows that the penetration of sealer into dentinal tubules after irrigation with novel silver citrate is longer compared to post-EDTA 17% irrigation.

EDTA 17% has been the standard chelating agent in endodontic treatment. It has the ability to remove smear layer post-endodontic treatment. In this study, the EDTA 17% solution resulted in shorter sealer penetration compared to novel silver citrate and 0.2% chitosan nanoparticles. EDTA 17% has a complex structure that allows it to bind to various metals. The molecular composition of EDTA 17% consists of four carboxylate groups and two amine groups. EDTA reacts with calcium ions in dentin and forms soluble calcium chelate. Many factors contribute to the effectiveness of EDTA solution, including time, concentration, temperature, and irrigation method.[25]

The activation method of the final irrigation material in this study can enhance the length of sealer penetration into dentinal tubules. The oscillating movement generated can enhance smear layer removal in root canals.[26] The post-hoc test results for techniques in this study show that final irrigation with sonic activation can enhance sealer penetration ability. Three irrigation techniques were used in this study: manual with a needle, sonic agitation, and ultrasonic agitation. Sonic agitation differs from ultrasonic agitation; sonic agitation operates at a lower frequency than ultrasonic agitation. Sonic agitation produces frequencies ranging from 1000 to 6000 Hz.[27] The lower vibration frequency in sonic agitation reduces the flow rate of irrigation fluid within the root canal. Additionally, the movement pattern of sonic instruments differs from ultrasonic instruments; in sonic agitation, the file moves with one node near the file base and one antinode at the file tip. Longitudinal movement occurs during sonic agitation, while in ultrasonic agitation, the file moves to create acoustic cavitation.[28]

Previous studies have reported that ultrasonic agitation can remove more dentin debris from root canals compared to sonic irrigation, and there is a positive correlation between speed and frequency resulting in higher efficiency of ultrasonic agitation compared to sonic agitation.[29] However, other studies have shown that sonic irrigation with a polymer tip activated using an air scaler at a frequency of 6000 Hz is more effective than ultrasonic agitation in eliminating smear layer. [30] These results are consistent with this study; post-hoc analysis shows that sonic agitation results in longer sealer penetration compared to manual irrigation and ultrasonic agitation.

In root canals that are not straight or narrow, maintaining contact of the ultrasonic tip with the root canal walls is challenging. Metal tips contacting the root canal walls during ultrasonic agitation procedures may damage the root canal surface. Accidental contact of the metal tip with the root canal walls during ultrasonic agitation can lead to smear layer deposition in that area. Smear layer presence in root canal walls can hinder final irrigation fluid and sealer penetration into dentinal tubules.[31] Sonic agitation has a polymer tip, which tends not to damage the root canal surface upon contact with the canal walls, especially when used in non-straight root canals, thereby preventing unwanted smear layer formation between the polymer tip and dentin walls.[32] Good smear layer removal by chitosan nanoparticles can be enhanced with sonic agitation during final irrigation procedures. Sonic agitation can increase the flow rate of chitosan nanoparticle solution in root canals, facilitating deeper penetration of chitosan into dentinal tubules. From the LSD analysis results of material-technique interactions in this study, it is known that 0.2% chitosan nanoparticles combined with sonic agitation produce the longest sealer penetration into dentinal tubules. Long sealer penetration into dentinal tubules increases the contact area between the sealer and dentin, which can enhance root canal system sealing post-obturation. Long sealer penetration into dentinal tubules allows endodontic sealer to bury remaining bacteria in dentinal tubules and improve sealer adaptation to dentin walls. Additionally, deep sealer penetration enhances root canal fracture resistance.[33]

A limitation of this study is in the sample cutting technique. Sample cutting with a diamond disc bur covers the observed dentinal tubule area with a fairly thick smear layer. The closure of dentinal tubules can interfere with observation and measurement of the depth of sealer penetration into dentinal tubules. Ideally, cutting should use a microtome capable of cutting hard tissues and under flowing water to minimize debris and smear layer presence.

CONCLUSION

it can be concluded that chitosan nanoparticle 0.2% produces the longest penetration of bioceramic sealer into dentinal tubules. Sonic agitation technique creates the longest penetration of bioceramic sealer into dentinal tubules. Combination of chitosan nanoparticle 0.2% and sonic agitation generates the longest penetration of bioceramic sealer into dentinal tubules. In the future has potential to be as final irrigation solution.

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