Effect of Final Irrigation Materials and Techniques on Apical Sealing Ability of Bioceramic Sealer

Asteria Monika, Diatri Nari Ratih**, Wignyo Hadriyanto***

**Conservative Dentistry Specialty Program, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta.*
***Departement of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta.*

Correspondence: asteria.monika@mail.ugm.ac.id

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ABSTRACT

Background: *One crucial function of root canal irrigation is eliminating smear layer, thus increasing apical sealing ability of endodontic sealers. This study was conducted to compare the effect of final irrigation using 17% EDTA, novel silver citrate, and 0.2% chitosan nanoparticles agitated manually, sonically, and ultrasonically on apical sealing ability of bioceramic sealers in the apical third.*
Method: *Forty-five mandibular premolars were prepared and randomly divided into three experimental groups (n=15). Group 1 used 17% EDTA as the final irrigation, 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 used manual agitation, subgroup B used sonic agitation, and subgroup C used ultrasonic agitation. All teeth were obturated using single cone and bioceramic sealers. Observations were made using 120x magnification SEM. The sealer's apical sealing ability was measured by comparing cross-sectional area of obturation area with cross-sectional area of preparation area in the apical third.*
Result: *Results of two-way ANOVA showed that there was a significant difference in final irrigation material ($p<0.05$), but there was no significant difference in technique and interaction of final irrigation material and technique ($p>0.05$).*
Conclusion: *Irrigation materials affected the apical sealing ability of bioceramic sealers in the apical third, and .0.2% chitosan nanoparticles produced significantly higher apical sealing ability among other groups. There is no difference in effect of final irrigation technique and the interaction of materials and final irrigation technique on apical sealing ability of bioceramic sealers in the apical third.*

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INTRODUCTION

Root canal treatment is performed to heal and prevent the occurrence of periradicular periodontitis, as well as to help retain the tooth in the mouth, ensuring it remains functional and aesthetically pleasing naturally.[1] The goal of root canal treatment is to shape, clean, and disinfect the root canal chemomechanically, followed by obturation of the root canal.[2] Endodontic instrumentation leaves a microscopic debris layer on the surface of the root canal known as the smear layer.[3] This smear layer consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components, and irrigation residues.[4] The smear layer reduces the apical sealing ability of root canal obturation, negatively affecting the treatment outcome.[5] The use of irrigation materials is necessary to kill microorganisms and clean both the organic and inorganic components of the smear layer present in all parts of the root canal. This process enhances the bond between the sealer and the root canal walls and reduces microleakage.[6]

Sodium hypochlorite (NaOCl) is a broad-spectrum antibacterial agent effective in dissolving organic tissue but cannot dissolve the inorganic components of the smear layer.[7] The effectiveness of NaOCl against the smear layer can be increased when used with chelating agents such as Ethylenediaminetetraacetic acid (EDTA) solution. A 17% EDTA solution dissolves the inorganic part of the smear layer and is considered the gold standard as a final irrigation material to remove the smear layer.[8] It reacts with calcium ions in dentin to form calcium chelation, thereby dissolving the inorganic tissue of the smear layer.[9] However, prolonged exposure to EDTA can alter dentin, leading to disturbances in mechanical integrity, erosion, and changes in the chemical and physical properties of the root canal dentin structure.[10] Previous research notes that 17% EDTA has minimal antibacterial effect.[11] Novel silver citrate, a new irrigation material available on the market, consists of silver ions produced electrolytically in citric acid. This solution has been tested as a material for root canal irrigation and has advantages due to the presence of silver compounds with antibacterial properties long used as medical disinfectants.[12] The citric acid in this solution is a weak organic acid with chelating properties comparable to those of a 17% EDTA solution.[13] Final irrigation with novel silver citrate for 1 minute is more effective in removing the smear layer in the apical third compared to final irrigation with 17% EDTA.[14]

Chitosan is a naturally occurring cationic polysaccharide composed of glucosamine and N-acetylglucosamine units linked by β(1-4) glycosidic bonds, obtained from the alkaline deacetylation of chitin.[15] Chitosan has antimicrobial, biocompatible, non-toxic, and biodegradable properties.[16] Research by Silva et al. states that using 0.2% chitosan for 3 minutes effectively removes the smear layer with minimal erosion effects, making 0.2% chitosan a potential alternative to EDTA.[17] Ratih et al. reported that 0.2% chitosan has the same effect on smear layer removal as 17% EDTA, with higher microhardness and lower dentin surface roughness compared to 17% EDTA.[18] Kusumo et al. demonstrated that irrigation with 0.2% chitosan nanoparticles for 5 minutes improves the apical sealing ability of epoxy resin sealer better than shorter contact times of 3 minutes and 1 minute.[19]

The agitation technique involves activating the root canal irrigant using an instrument inserted into the canal and then moved.[9] This technique aims to help distribute the irrigant throughout the root canal to reach all areas.[20] DiSpirito et al. (2022) state that maximizing the use of irrigants in cleaning root canals involves delivering the irrigant as effectively as possible throughout the root canal system.[22] Currently, sonic and ultrasonic agitation techniques are commonly used. Agitation techniques in endodontic treatment have

improved treatment quality, including higher smear layer removal from the root canal walls compared to conventional irrigation.[1]

The success of root canal treatment requires thorough obturation of the root canal and achieving optimal apical sealing ability.[1] Obturation is performed after the cleaning and preparation of the root canal are completed, with the aim of creating a fluid-tight seal along the entire root canal system from the orifice to the apical end.[2] A good combination of sealer and gutta-percha as the core material can achieve optimal obturation.[22] Good apical sealing abilty is essential for the sealer material to avoid factors that can cause post-treatment failure.[23] Factors affecting apical sealing ability include root canal anatomy, apical diameter, mechanical and chemical preparation, final irrigation, and the type of sealer used.[24] Assessment of the apical sealing ability of the root canal sealer is performed using a Scanning Electron Microscope (SEM), followed by measuring the ratio of the cross-sectional area of the obturation to the cross-sectional area of the root canal, multiplied by 100%.[25]

Bioceramic sealers are currently a preferred choice in endodontic treatment.[26] These sealers have favorable physicochemical and biological properties. They exhibit high biocompatibility with tissues, possess antibacterial activity, and contain calcium phosphate, which can enhance the apical sealing ability of the dentin walls of the root canal and support apical healing.[27,28]

The apical third region typically has a narrower anatomy compared to the coronal third, with a curved shape and complex anatomy, often featuring root canal branches, making it the most challenging part for root canal treatment.[4] Mechanical instrumentation alone is not sufficient to clean the complex anatomy of the root canal, especially in the apical third area. Mechanical preparation must be supported by irrigation materials that can clean the smear layer and have chemically active antibacterial properties.[29]

Currently, 17% EDTA and novel silver citrate are available chelating agents on the market, while 0.2% chitosan nanoparticles are still in development. Although still in development, 0.2% chitosan nanoparticles have shown several advantages as chelating agents compared to 17% EDTA and novel silver citrate.[30] Agitation irrigation techniques are used to enhance the effectiveness of irrigants.[20] This study will compare the effects of final irrigation using 17% EDTA, novel silver citrate, and 0.2% chitosan nanoparticles, agitated manually, sonically, and ultrasonically, on the apical sealing ability of bioceramic sealer in the apical third of the root canal.

RESEARCH METHOD

This research has been approved by the Ethics and Advocacy Unit of the Faculty of Dentistry, Universitas Gadjah Mada, with reference number 52/UNI/KEP/FKG-RSGM/EC/2023. This in vitro study utilized forty-five single-rooted mandibular premolars, with the inclusion criteria of intact crown, single straight root, and a single root canal. Exclusion criteria for the sample include the presence of cracks, resorption, and calcification in the root. The root canal diameter was controlled by preparing the root canal using the crown-down technique with rotary files up to M3P3 (30.04) file.

A 0.2% chitosan nanoparticles solution was prepared by dissolving chitosan in a 1% acetic acid solution, followed by homogenization using a magnetic stirrer at 500 rpm in a room temperature for 1 hour. The 50 ml chitosan solution was then transferred to a glass beaker and stirred with a magnetic stirrer. Tri Poly Phosphate (TPP) solution in a 5:1 volume ratio was slowly added to the chitosan solution, and stirring continued for 1 hour until homogenous.

The teeth were coronally sectioned using a diamond bur wheel attached to a low-speed handpiece, leaving a 12 mm root. Root canals were prepared using the crown-down technique with rotary files driven by an endomotor, working length adjusted according to the file sequence, and finishing with a 30.04 file. After each instrument change, the root canal was irrigated with 5 ml of 2.5% NaOCl and rinsed with 5 ml of saline for 30 seconds. The apical foramen of each tooth was sealed with a wax block to prevent irrigation solution from flowing through the apical foramen.

Sample preparation was performed following the protocol previously described by Maranantan et al. (2025).[31] Briefly, final irrigation was performed with each irrigation material in 5 ml volumes, applied using a syringe with a single-vented irrigation needle positioned 1 mm from the apex.

Root canals were then agitated according to their respective groups for 30 seconds with each irrigation material (Figure 1) and rinsed with 5 ml of saline for 30 seconds. The root canals were dried with five paper points.

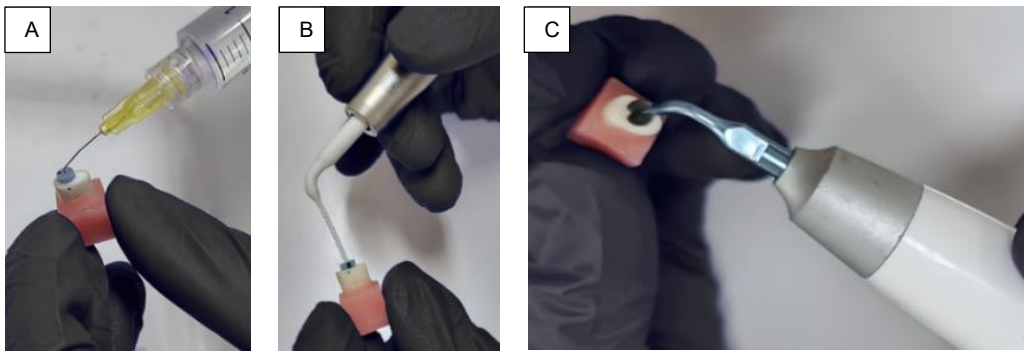

Figure 1. Root canal irrigation. **(A)** Manual agitation technique, **(B)** Sonic agitation technique, **(C)** Ultrasonic agitation technique.

The teeth were obturated using the single cone technique and bioceramic sealer. After obturation, the samples were immersed in saline solution and stored in an incubator at a temperature of 37°C with 100% humidity for seven days. After seven days, the samples were dried at room temperature, measured using a sliding calliper, and horizontally cut at the apical third of the tooth root using a diamond disc. The samples were mounted on metal plates, coated with gold for electrical conductivity on the surface, and observed under Scanning Electron Microscope (SEM) at a magnification of 120 times. Evaluation of the apical sealing ability was conducted using the formula by Mulyawati et al.[25], which compares the cross-sectional area of obturation with the cross-sectional area of the root canal at the apical third of the tooth (Figure 2). The obtained data were analyzed using a two-way ANOVA and LSD test with a significance level of 95%.

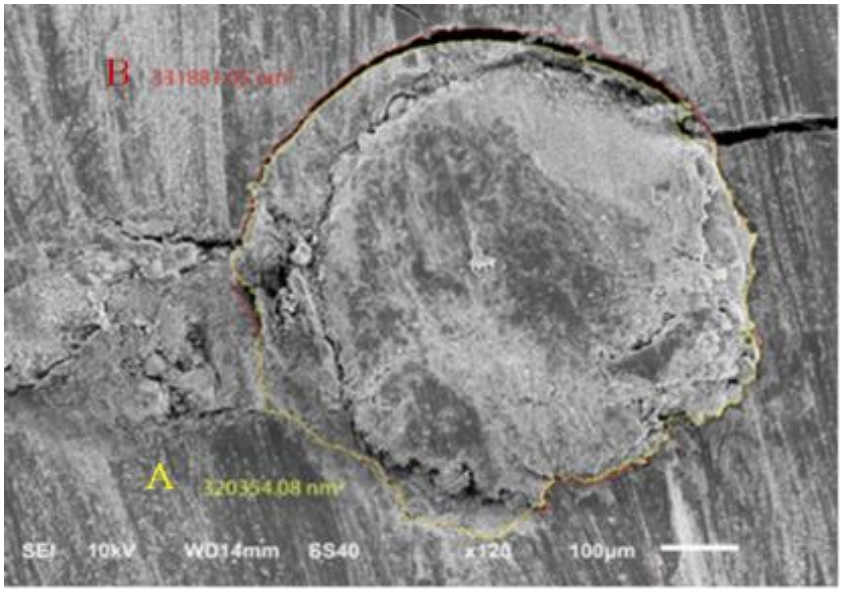

Figure 2. (A) Cross-sectional area of obturation area, **(B)** Cross-sectional area of root canal.

RESULTS

The apical sealing ability of the bioceramic sealer was observed using a Scanning Electron Microscope (SEM) at a magnification of 120x on the cross-section of the root canal cut at the apical one-third with a horizontal cutting direction (Figure 3).

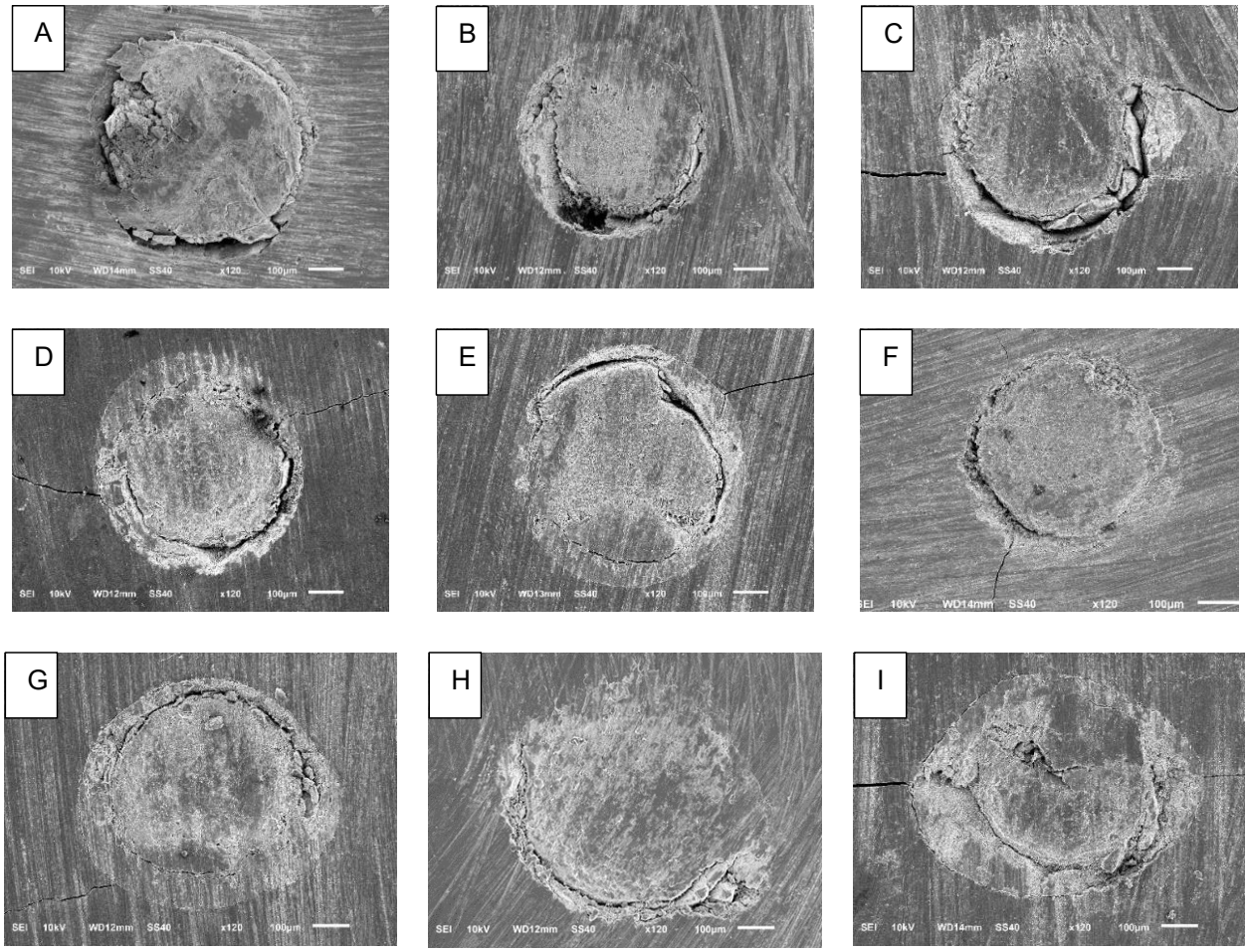

Figure 3. Result of apical sealing abilty of bioceramic sealers in irrigated root canals : **(A)** 17% EDTA – manual agitation, **(B)** 17% EDTA – sonic agitation, **(C)** 17% EDTA – ultrasonic agitation, **(D)** Novel silver citrate – manual agitation, **(E)** Novel silver citrate – sonic agitation, **(F)** Novel silver citrate – ultrasonic agitation, **(G)** 0.2% Chitosan nanoparticles – manual agitation, **(H)** 0.2% Chitosan nanoparticles – sonic agitation, **(I)** 0.2% Chitosan nanoparticles – ultrasonic agitation.

Subsequently, the measurement of the cross-sectional area of the obturation was compared to the cross-sectional area of the root canal and multiplied by 100%.[25] The measurement of apical sealing ability was conducted using ImageJ software in micrometer units. The average and standard deviation of the apical sealing ability of the bioceramic sealer are presented in Table 1. The lowest sealing ability was found in the 17% EDTA manual agitation group (97.36 ± 0.56), while the highest apical sealing ability was observed in the 0,2% chitosan nanoparticle sonically agitation group (99.72 ± 0.29).

Table 1. The average of apical sealing ability bioceramic sealers in the apical third of root canal after irrigation with different materials and techniques.

Treatment Group	Manual Agitation	Sonic Agitation	Ultrasonic Agitation
17% EDTA	97.36 ± 0.56	98.15± 0.86	98.09 ± 0.86
Novel silver citrate	98.20 ± 0.92	98.68± 0.64	98.48± 0.55
0.2% Chitosan nanoparticles	99.20± 0.41	99.72± 0.29	99.28± 0.49

The result of the normality test conducted using the Shapiro-Wilk test revealed normal data (P> 0.05) and homogeneous variance (Levene's test showed P > 0.05). The analysis using a two-way ANOVA can be seen in Table 2. There was a significant difference in the material groups (P < 0.05), but no significant difference was observed in the technique groups and their combination. Post Hoc testing with the Least Significant Difference (LSD) was conducted to identify differences between the types of final irrigation materials. The LSD test results (Table 3) indicate significant differences in mean values (P < 0.05) among all material groups: 17% EDTA with novel silver citrate, 17% EDTA with 0.2% chitosan nanoparticles, and novel silver citrate with 0.2% chitosan nanoparticles.

Table 2. Statistical analysis results of two-way ANOVA on the apical sealing ability of bioceramic sealer at the apical third of the root canal after irrigation with different materials and techniques.

	Sum of squares	Df	Mean square	F	P
Material	17.046	2	8.523	19.968	0.000*
Technique	2.444	2	1.222	2.862	0.070
Material * Technique	0.568	4	0.142	0.333	0.854
Total	437171.299	45			

*Significantly different (P <0.05)

Table 3. Post Hoc Least Significant Difference test results on the apical sealing ability of bioceramic sealer at the apical third of the root canal after irrigation with different materials.

Materials	LSD	Sig.

17% EDTA – novel silver citrate	-0.5873	0.019*
17% EDTA – 0.2% chitosan nanoparticles	-1.4961	0.000*
Novel silver citrate– 0.2% chitosan nanoparticles	-0.9088	0.001*

*Significantly different (P <0.05)

DISCUSSION

This study aimed to investigate the influence of final irrigation using 17% EDTA, novel silver citrate, and 0.2% chitosan nanoparticles agitated manually, sonically, and ultrasonically on the apical sealing ability of bioceramic sealer in the apical one-third of the root canal. The apical sealing ability of bioceramic sealer is affected by the removal of the smear layer in the root canal. Previous research by Ulusoy et al. indicated that a clean root canal wall without a smear layer enhances the apical sealing ability of the root canal sealer.[32] The removal of the smear layer not only improves the ability of the bioceramic sealer to achieve apical sealing ability but also enhances its adhesion strength to dentin.[33]

The results of this study show a difference in the effect of irrigation materials on the apical sealing ability of bioceramic sealers in the apical third of the root canal, with 0.2% chitosan nanoparticles showing the highest average apical sealing ability. The 0.2% chitosan nanoparticles are effective as calcium ion binders, which are the inorganic components of dentin, thus capable of dissolving the smear layer. The nanosize of chitosan enhances its hydrophilic properties. These hydrophilic properties allow the chitosan nanoparticles to adsorb to the root canal surface walls and remove the smear layer. Additionally, 0.2% chitosan nanoparticles are a weak acid irrigation material, giving them higher chelating ability compared to neutral EDTA. Clean root canal walls free from the smear layer will increase the apical sealing ability of the sealer in the apical third of the root canal.

This finding is consistent with research by Silva et al. which found that low-concentration chitosan can remove the smear layer from the dentin surface.[17] Erlagista also reported that 0.2% chitosan nanoparticles provide the highest root canal cleanliness from the smear layer compared to 17% EDTA and novel silver citrate.[30] The hydrophilic nature of chitosan polymer supports close contact with the root canal dentin, allowing the nanoparticles to easily adsorb to the root canal walls. The nanoparticle size of chitosan affects the irrigation solution's ability to penetrate deeper into the dentinal tubules of the root canal. The chitosan nanoparticle solution also contains free hydroxyl and amino groups, which lead to ionic interactions with dentin calcium ions.[34] The amino groups in chitosan bind to calcium ions in the dentin, thus removing the inorganic substances in the smear layer.[34] Research by Hosseini et al. and Hong also noted that irrigation materials with good wetting ability, such as 0.2% chitosan nanoparticles, can penetrate deeper and are more effective in cleaning the smear layer.[11, 36]

In this study, novel silver citrate showed a significant difference in the average apical sealing ability of bioceramic sealers in the apical third, being higher than 17% EDTA but still lower than 0.2% chitosan nanoparticles. This is because EDTA is neutral, resulting in lower chelating ability. This finding is consistent with research by Alyahya et al. which stated that an important factor affecting the effectiveness of chelating agents is their pH value.[37] Novel silver citrate is an acidic chelating agent due to its citric acid content, an organic acid with strong chelating properties against inorganic tissue by binding calcium ions at low pH conditions.[38] Research by Tonini et al. also indicated that novel silver citrate is more effective in eliminating the smear layer compared to 17% EDTA and enhances tubular sealer penetration, thereby increasing apical sealing ability of bioceramic sealer in the apical third of the root canal.[13]

The statistical results of the study did not show any significant differences between the average groups of agitation techniques. Sonic agitation techniques have a lower frequency, resulting in lower intracanal waves and, consequently, lower smear layer cleaning ability compared to ultrasonic agitation techniques. Ultrasonic agitation techniques, which use steel tips, have a higher smear layer cleaning ability due to their strength but can cause scratches on the dentin, leading to debris accumulation in the scratched areas.

Sonic irrigation operates at lower frequencies (1-8 kHz) and produces higher amplitudes. By vibrating the tip both automatically and manually, short vertical movements at the tip end occur, activating the fluid and generating intracanal waves that hit the root canal walls to remove debris and the smear layer.[39] Sonic instruments have advantages over ultrasonic instruments because sonic tips are made of polymer material that does not damage the root canal walls.[40]

Ultrasonic technology generates high-frequency waves (25-30 kHz) with low amplitude. Energy is transmitted from the oscillating file to the irrigation in the root canal through ultrasonic waves. Ultrasonic agitation techniques work with the acoustic microstreaming effect, causing the smear layer and debris adhering to the root canal walls to be released. A drawback of ultrasonic instruments is that their steel tips, although non-cutting, are harder than dentin, potentially damaging the root canal walls and causing debris accumulation on the scratched surfaces.[41]

The lack of influence in the agitation technique groups is consistent with research by Choudhary et al. which found no significant effect of sonic and ultrasonic agitation techniques on the cleanliness of root canal debris.[42] Although the sonic agitation technique showed better results in cleaning root canal debris compared to ultrasonic techniques, the difference was not statistically significant.

In this study, the combination of 0.2% chitosan nanoparticles and sonic irrigation technique resulted in the highest average apical sealing ability compared to other groups, but there was no statistically significant difference. This may be due to the fact that the samples used in this study were premolar teeth with straight roots. The working length of the single-vented needle, sonic irrigation tip, and ultrasonic irrigation tip were controlled to the same size, resulting in similar root canal anatomy. Consequently, the apical sealing ability of bioceramic sealers showed no significant difference in influence.

Various factors, such as root canal anatomy, apical diameter, mechanical and chemical preparation, and final irrigation, can affect the apical sealing ability of sealers in the root canal.[24] Research by DiSpirito et al. stated that to maximize the effectiveness of irrigation materials in cleaning the root canal, it is important to deliver the irrigation material as thoroughly as possible into the root canal system.[21] The bond between bioceramic sealer and dentin can occur due to several factors, including the diffusion of sealer particles into the dentinal tubules to produce a mechanical bond with the dentin wall. Infiltration of the sealer mineral content into the intertubular dentin can result in the formation of a mineral infiltration zone resulting after denaturation of collagen fibers due to the strong basicity of the bioceramic sealer. The reaction of phosphate with calcium silicate hydrogel and calcium hydroxide, which is produced through the reaction of calcium silicate with the presence of dentin moisture, also results in the formation of hydroxyapatite along the mineral infiltration zone, which will create a bond between the bioceramic sealer and the dentin wall.[43] Therefore, in this study, the combination of materials and final irrigation techniques did not show any significant difference because the irrigation material could be evenly distributed with each technique due to the straight root canal anatomy and controlled working length of the irrigation tips.

CONCLUSION

Based on the results of the study, it can be concluded that irrigation materials have an effect on the apical sealing ability of bioceramic sealer in the apical one-third of the root canal, and 0.2% chitosan nanoparticles demonstrate the highest apical sealing ability. There is no significant effect from the final irrigation technique and the combination between irrigation material and technique on the apical sealing ability of bioceramic sealer in the apical one-third of the root canal.

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