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ORIGINAL ARTICLE

Evaluation of the Cleanliness and Sealer Penetration of the Root Canal System Following Final Irrigation Using Chelating Agents

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ABSTRACT

Objective: In root canal treatment, final root canal irrigation plays an essential role in removing smear layers, especially inorganic components, in order to achieve hermetic obturation of the root canal system. This study aimed to evaluate the root canal cleanliness and sealer penetration of the root canal system following final irrigation using chelating agents, namely nano-chitosan, which was compared to EDTA and novel silver citrate (NSC).

Methods: This study used 90 premolars, which were divided into two evaluations: root canal cleanliness and sealer penetration. Teeth were assigned into three groups. Group 1: EDTA, group 2: NSC, and group 3: nano-chitosan. Scanning Electron Microscope (SEM) was used to evaluate root canal cleanliness, and a stereo microscope was utilized to evaluate sealer penetration. The root canal cleanliness data were analysed using the Chi-Square test, whereas sealer penetration data were analysed with a two-way ANOVA and LSD Post-Hoc test with a significance level of 95%.

Results: In the root canal cleanliness evaluation, all three final irrigation solutions showed similar cleanliness of the root canals ($p>0.05$). In the evaluation of sealer penetration, nano-chitosan produced the highest sealer penetration compared to EDTA and NSC ($p<0.05$).

Conclusion: All final irrigations produced the same root canal cleanliness; however, nano-chitosan final irrigation solution generated the longest sealer penetration than EDTA and NSC.

Keywords: Final irrigation solutions, nano-chitosan, root canal cleanliness, sealer penetration

HIGHLIGHTS

- As a chelating agent, nano-chitosan can be suggested as the final irrigation in the future to replace EDTA and novel silver citrate.
- Nano-chitosan generated the same cleanliness, but the highest sealer penetration compared to EDTA and novel silver citrate.

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INTRODUCTION

Chemical root canal cleaning using an irrigation solution is one of the steps of biomechanical preparation that has a crucial effect on the success of root canal treatment. Root canal irrigation aims to clean the root canal from necrotic tissue remains and eliminate microorganisms and their products (1).

The goal of the final irrigation is to eradicate the smear layer. Hence, sealer penetration as a root canal obturation material can be increased, which, in turn, produces the maximum apical sealing ability of the root canal system and reduces apical leakage. This condition can avoid failure of endodontic treatment (2). For this reason, developing a final irrigation solution with chelating properties is necessary. This chelating property can dissolve inorganic material from the smear layer, which layer comes from rotary or manual endodontic instruments used for biomechanical preparations) (3). One of the crucial requirements for a final root canal irrigation solution is that it must be antimicrobial and produce the optimal root canal obturation.

Ethylenediamine tetra-acetic acid (EDTA) is one of the final irrigation solution frequently used in the clinic. EDTA has the properties of a chelating agent that dissolves the smear layer, particularly its inorganic portion. The action mechanism of EDTA is to bind calcium ions from dentine, causing decalcification of the dentine, especially the peritubular area, making the dentine easier to instrument (4). However, EDTA has drawbacks such as no antimicrobial properties and causes erosion of root canal dentine (3). Because of the disadvantages of EDTA, other final irrigation solutions need to be investigated that eradicate the smear layer and have antibacterial properties but no influence on the dentine structures (5).

Novel silver citrate (NSC) is one of the new irrigation solutions launched today. The advantage of NSC is the presence of a silver compound that has antibacterial properties and has long been used as a medical disinfection agent (6). The citric acid in this solution is a vulnerable organic acid with chelating agents equal to EDTA (7). Until now, there has not been much study on NSC being used as a final irrigation solution. Although, several studies have been conducted to study NSC (5–7).

Lately, chitosan has been frequently utilized in the area of medical field. Chitosan is a natural polysaccharide with the molecular formula ($C6H11NO4$), which can be obtained from chitin, which is deacetylated from crustaceans (such as shrimp shells). Chitosan has antimicrobial, biocompatible, non-toxic, and biodegradable properties and has the potential as a final irrigation solution due to its chelating properties (8). Several studies that have been carried out previously reported that 0.2% nano-chitosan can be used as final irrigation and had the same effect on smear layer removal and had a higher microhardness and minor dentine superficial roughness than 17% EDTA (9, 10).

This study aims to develop nano-chitosan as a final irrigation solution and compare it to other irrigation solutions employed in clinics, namely EDTA, the current golden standard for final irrigation solutions, and a new final irrigation solution, NSC. The null hypothesis was that root canal cleanliness and sealer penetration using EDTA, NSC, and nano-chitosan as the final irrigation solutions were not different.

MATERIALS AND METHODS

This study was approved by the Universitas Gadjah Mada, Faculty of Dentistry-Prof. Soedomo Dental Hospital Ethics Committee (no: 49/UNI/KEP/FKG-RSGM/EC/2023, date: 27/04/2023) and accomplished in agreement with The Declaration of Helsinki.

Sample Preparation

This study used ninety intact, single-root, straight mandibular premolars removed because of orthodontic treatment, with initial fie # 20 (Dentsply Maillefer, Ballaigues, Switzerland). This study was allocated into two investigations: the influence of final irrigation solutions using EDTA, NSC, and nano-chitosan on cleanliness and root canal sealer penetration of tooth root canal system. Each evaluation used 45 teeth. Teeth were cut at the cemento-enamel junction (CEJ) to attain 12 mm- root length. The working lengths were subtracted 1 mm from the lengths of the roots. The apical root of the tooth was covered with sticky wax to simulate clinical conditions.

All root canals were extirpated by using a barbed broach (Dentsply Maillefer, Ballaigues, Switzerland). Then, root canals were instrumented using a crown-down method with rotary files (Protaper Universal, Dentsply Maillefer, Ballaigues, Switzerland) up to file # F3. After every instrument was changed, before and after applying final irrigation solutions the root canal was irrigated using 2.5% NaOCl (Golden Falcon, Dubai, UAE) for 1 minute using 2 mL volume. The root canal was then rinsed with distilled water for 1 minute with 2 mL volume.

In each investigation, the roots were allocated into three groups randomly, each consisting of 15 samples: group 1: final irrigation with EDTA ((Pulpdent, Watertown, MA, USA), group 2: final irrigation with NSC (BioAKT endo, New Tech Solution, Brescia BS, Italy), and group 3: final irrigation with nano-chitosan. Each final irrigation solution was delivered to the root canal system using a single-vented irrigation needle (side vented 30G, M3, United Dental, Shanghai, China). The needle was introduced 1 mm shorter than the working length, and the volume of the irrigation solution was 5 mL. The final irrigation solution was inside the root canal system for 3 minutes. After applying final irrigations, all root canals were irrigated using distilled water for 1 minute with 2 mL volume, and dried root canal with sterile #30 paper points.

Preparation of Nano-chitosan Irrigation Solution

A magnetic stirrer stirred the 50 mL chitosan solution (Sigma Aldrich, St Louis, MO, USA), and Tripolyphosphate (TPP) solution with a volume ratio 5:1 was added slowly to the chitosan solution. Stirring continued for 1 hour. The 0.2% nano-chitosan irrigation was obtained by diffusing chitosan in 1% acetic acid. A 100 mL chitosan solution was stirred for 30 minutes using a magnetic stirrer at 500 rpm until homogeneous. To verify that chitosan was in nanoparticles, a particle size analyser test using Cordouan Technologies Particle Size Analyser (Zurich, Switzerland) at room temperature was used (8).

Evaluation of Root Canal Cleanliness

After the root canal was finally irrigated according to each group, as mentioned above, longitudinal grooves were created on the bucco-lingual sides of every root using a low-speed diamond disk (Horico, Berlin, Germany). Each root was then split using a chisel and was cut at the apical third (4 mm distance from the root apex). The apical third of the root canals were then mounted on a metal plate, coated with gold on the surface, and observed with SEM (JSM IT-700, JEOL, Tokyo, Japan) with 5000X magnification.

Three calibrated, blinded examiners have assessed the SEM figures. The Kappa test was performed to verify the conformity

TABLE 1. Scoring of the root canal cleanliness, mean and standard deviation of the penetration depth of bio-ceramic sealer into tubules dentine

Scoring	Root canal cleanliness (%)				Sealer penetration (µm)
	1	2	3	4	
EDTA	0 (0)	3 (16.7)	2 (66.7)	0 (0)	215.2±19.1
NSC	0 (0)	4 (22.4)	1 (33.3)	0 (0)	439.8±42.1
Nano-chitosan	3 (12.5)	2 (11.1)	0 (0)	0 (0)	498.0±23.9

EDTA: Ethylenediamine tetra-acetic acid

between the examiners (Kappa ≥ 0,88). Root canal cleanliness was established by the presence or absence of a smear layer in root canal by scoring as follows: score 1: No smear layer and debris at all, with all tubules cleaned and opened; score 2: A few areas were covered by a smear layer and debris, with most tubules cleaned and opened; score 3: Smear layer and debris cover almost all the surfaces, with few tubules opened; score 4: Smear layer and debris covering the entire surfaces (11).

Evaluation of Root Canal Sealer Penetration

After the root canal was finally irrigated according to each group, as mentioned above, a single-cone method with a gutta-percha cone (Dentsply Maillefer, Ballaigues, Switzerland) and bio-ceramic sealer (Ceraseal, Metabiomed, South Korea) mixed with 0.1% rhodamine B (Sigma Aldrich, St Louis, MO, USA) until homogenous. Rhodamine B was used in this study to assist microscopic observations (5). The samples were then stored in an incubator for seven days at a temperature of 37°C until the sealer was set completely (12).

Afterward, the samples were sectioned into two parts vertically on the bucco-lingual surfaces using a diamond disc bur (Horico, Berlin, Germany), and then were cut at the apical third (4 mm from the root apex). The apical third of each root was observed using a stereo microscope at 30X magnification. The Optilab Viewer 4 software (Informer Technologies, Inc.) was utilized to measure the penetration depth of sealers. Each sample's observation area was divided into four regions, and the furthest sealer penetration to the dentine tubules was calculated (13).

Statistical Analysis

The data obtained from the cleanliness evaluation were analysed with the Chi-Square test (a significance level of 95%). The data obtained from the investigation of root canal sealer penetration were analysed with a two-way ANOVA and a post-hoc test with the Least Significant Difference (LSD) (a significance level of 95%). Version 25 of IBM SPSS Statistics Software (IBM Corp., Armonk, NY, United States) was utilized to analyse the data.

RESULTS

The results of the investigation of the cleanliness and sealer penetration of the root canal system can be seen in Table 1. The root canal cleanliness evaluation exhibited that EDTA, NSC, and nano-chitosan final irrigation solutions produced similar cleanliness in the apical third of the root canal ($p>0.05$). Representative SEM images of the root canal's cleanliness are exhibited in Figure 1.

Evaluating root canal sealer penetration demonstrated that final irrigation using nano-chitosan produced the highest sealer penetration depth into the dentinal tubules ($p<0.05$) compared to NSC and EDTA. It also shows that EDTA generated the lowest sealer penetration depth into the dentinal tubules ($p<0.05$). The representative stereo microscope images of the sealer penetration are demonstrated in Figure 2.

DISCUSSION

This study indicates a similar influence of final irrigation solutions, namely EDTA, NSC, and nano-chitosan, on root canal cleanliness; however, there is different influence on sealer penetration. Therefore, the null hypothesis was partially rejected in this study. Although three final irrigation solutions generated the same cleanliness, each solution used in this study has a different mechanism for eliminating the root canal system's smear layer. EDTA, with a concentration of 17%, has been widely employed in the clinic for final irrigation solutions in root canal treatment. EDTA has chelating properties and can attach to many metals; therefore, it can remove the inorganic elements of smear layers and debris after root canal instrumentation. EDTA comprises four carboxylic groups and two amine groups. EDTA reacts with calcium ions in dentine and forms easily soluble calcium chelates. Many factors contribute to the effectiveness of final irrigation solu-

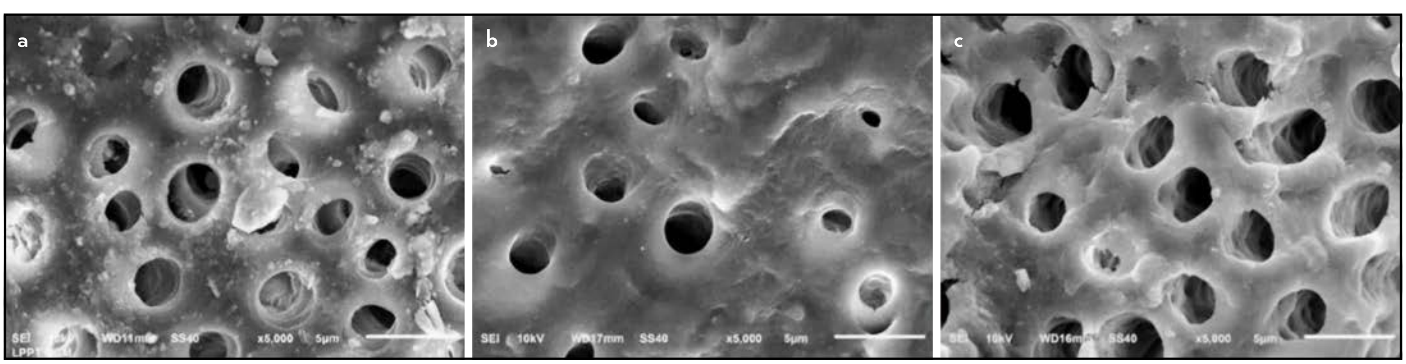

Figure 1. Representatives SEM images of cleanliness of root canal system of final irrigation solutions using EDTA (a), NSC (b), and nano-chitosan (c) at 5000x magnification

SEM: Scanning electron microscope, EDTA: Ethylenediamine tetra-acetic acid, NSC: Novel silver citrate

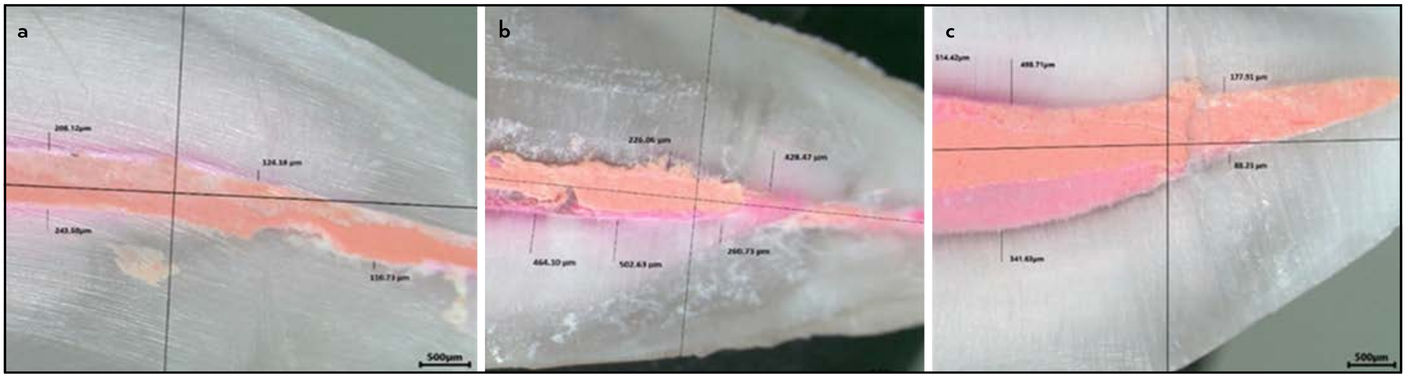

Figure 2. Representatives stereo microscope images of the sealer penetration into the dentinal tubules after final irrigation solutions using EDTA (a), NSC (b), and nano-chitosan (c) at 30x magnification

EDTA: Ethylenediamine tetra-acetic acid, NSC: Novel silver citrate

tions in removing smear layers, including time, concentration, temperature, and irrigation methods (14).

Due to NSC's citric acid content, this solution has the ability to eradicate the smear layer. The citric acid in the NSC is an organic acid that can dissolve the inorganic material of smear layers in the root canal system. Hence, it can be performed as a chelating agent (15).

The previous investigators reported that 0.2% nano-chitosan could eliminate the smear layer and open the dentinal tubules (3, 9, 15). Chitosan polymer is hydrophilic and has free hydroxyl and amino groups, which makes it cationic. This condition allows ionic interactions with calcium ions in root canal dentine, causing the chelation mechanism of nano-chitosan (16). In addition, nano-chitosan has low surface tension, which might induce this solution to enter the dentinal tubules more easily (17). The hydrophilic property of nano-chitosan enhances this solution, which is straightforwardly adsorbed into the root canal walls, effectively eliminating smear layers from the root canal system (18, 19).

The present study showed that sealer penetration after final irrigation utilizing 17% EDTA was significantly shorter than NCS and 0.2% nano-chitosan. This condition might be due to EDTA having potent chelation properties. Therefore, the application of a potent agent that has chelation properties for an extended period to root canal dentine could not only eradicate the smear layer but also substantially enhance the loss of calcium ions on the root canal dentine, leading to reduced microhardness and surface roughness of the root canal system (20). Consequently, the root canal topography alteration generated low push-out bond strength of the sealer (21). This phenomenon probably also occurred since chitosan and NSC have vulnerable chelation properties, resulting in lower root canal erosion compared to EDTA, leading to higher sealer penetration in this study (22).

In addition, nano-chitosan inhibits covalent bonding to dentine collagen, probable attracting calcium ions to form a calcium phosphate layer, thereby causing demineralized dentine to remineralize (23). On the contrary, EDTA and NSC cannot demineralize dentine to create remineralization (24). This condition might elucidate why chitosan yields the highest sealer penetration compared to EDTA and NSC (25). A similar study by Najafzadeh et al. (26) also demonstrated that nano-chitosan induced the least root canal dentine erosion which produced highest bioceramic sealer penetration compared with EDTA.

However, it is important to note that sealer penetration into the dentinal tubules can be influenced by various factors, including the smear layer elimination, obturation technique, the root canal, and dentinal tubules anatomy, as well as the sealer material used (23, 24). This study used bioceramic-based sealers because these sealers are superior to epoxy resin-based sealers; particularly, bioceramic-based sealers can penetrate deeper into the dentinal tubules compared to epoxy resin-based sealers (27). This phenomenon occurred due to bioceramic sealers having a tiny particle size of around one micron. Apart from that, this sealer is hydrophilic, highly alkaline (28), has good biocompatibility, has a bacteriostatic effect, and is able to form chemical bonds with the root canal dentine wall (27, 29). This recent study employed a stereo microscope at 30X magnification, equipped by The Optilab Viewer 4 software was adequate to measure the penetration depth of sealers, although other studies used confocal laser scanning microscope to assess of the penetration of an endodontic sealer into dentinal tubules (25).

This study possesses several drawbacks. First, factors such as root canal anatomy, dentinal tubule patency, and apical diameter of each sample may vary. Therefore, these factors may affect the results of this study. However, the employment of the constant conditions allowed equivalent results among the final irrigation solutions used in this study. Second, the cutting instrument of each sample, which was using a diamond disc bur, created the area of the dentinal tubules to be observed as covered with a thick smear layer. The closure of the dentinal tubules can interfere with the observation and measurement of the sealer penetration into the dentinal tubules. Cutting instruments should use a microtome that can cut hard tissue under running water to minimize the presence of debris and smear layers.

CONCLUSION

Despite the limitations of this study, it can be concluded that EDTA, NSC, and nano-chitosan have the same effect on the cleanliness of the root canal system. Nevertheless, nano-chitosan produces the longest sealer penetration of the root canal system compared to EDTA and NSC final irrigation solutions.

Disclosures

Ethics Committee Approval: The study was approved by the Universitas Gadjah Mada, Faculty of Dentistry-Prof. Soedomo Dental Hospital Ethics Committee (no: 49/UNI/KEP/FKG-RSGM/EC/2023, date: 27/04/2023).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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