

Effect of Contact Times of Chitosan Nanoparticle as a Final Irrigation Solution on Microhardness and Surface Roughness of Root Canal Dentin

Diatri N Ratih¹, Fransisca D Rosalia², Margareta Rinastiti³, Nofa MN Kaswati⁴

ABSTRACT

Aim and objective: The aim and objective of this study was to investigate the contact time effect of 1 minute and 3 minutes of 0.5% chitosan nanoparticle as a final irrigation solution on microhardness and surface roughness of root canal dentin.

Materials and methods: This study was divided into two experiments, namely microhardness and surface roughness. Both experiments used 56 mandibular premolars, which assigned to 28 teeth of each study. Twenty-eight teeth were then divided randomly into two groups of 14 teeth. Group I was final irrigated using 17% ethylenediaminetetraacetic acid (EDTA) and group II with 0.5% chitosan nanoparticle. Each group was further assigned into two groups of seven each according to the contact time, group I, 1-minute and group II, 3-minute contact time. The microhardness test was carried out using the Vickers microhardness tester while the surface hardness test using the surface roughness measuring instrument. The data from each study were statically analyzed using two-way ANOVA followed by the least significant difference (LSD) test with a significance level of 95%.

Results: Contact time of 1 minute using 0.5% chitosan nanoparticles produced the greatest microhardness and lowest surface roughness of root canal dentin, while contact time of 3 minutes using 17% EDTA had the lowest microhardness and highest surface roughness ($p < 0.05$).

Conclusion: The contact time of 1 minute using 0.5% chitosan nanoparticle caused the least effect on the microhardness and surface roughness.

Clinical significance: The findings of this study revealed that contact time of 1 minute using 0.5% chitosan nanoparticle caused the least effect on both microhardness and surface roughness. Thus, the contact time of 1 minute is suggested to be employed when using 0.5% chitosan nanoparticle for the final irrigation solution in the clinic.

Keywords: Chitosan nanoparticle, Contact time, Final irrigation solution, Microhardness, Surface roughness.

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INTRODUCTION

The objective of root canal therapy is removal of microorganisms present in the root canal and to avoid reinfection.¹ It includes biomechanical preparation, cleaning by irrigation solution and intracanal medicament (disinfectant), as well as obturation of the root canal to obtain hermetical condition.²

Biomechanical preparation of the root canal system generates a smear layer, which contains organic and inorganic constituents. This smear layer must be eliminated from the root canal; thus, the irrigation solution can penetrate dentinal tubules.³ The requirements of an ideal irrigation solution include antibacterial property, aid in root canal debridement, low toxicity, and ability to dissolve necrotic tissue, debris, and smear layer.¹

Sodium hypochlorite (NaOCl) at concentrations ranging between 2.5% and 5% is frequently used as a solution for irrigation due to the antibacterial property. Although NaOCl can eliminate organic tissues, it cannot eradicate inorganic tissues.⁴ Thus, NaOCl alone cannot be used as a final irrigation solution; however, it can be used as a final irrigant in combination with other irrigation solutions such as ethylenediaminetetraacetic acid (EDTA).⁵

Currently, EDTA is the gold standard for the final irrigation solution. The advantage of EDTA includes chelation capacity, resulting in chelating the inorganic component of the smear layer.⁶ Nevertheless, the extended contact time of EDTA might cause excessive removal of peritubular and intertubular dentin inducing dentin erosion.⁵

^{1,3}Department of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

²Specialist Study Program of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

⁴Nanotech Indonesia, Serpong, Indonesia

Corresponding Author: Diatri N Ratih, Department of Conservative Dentistry, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia, Phone: +62274515307, e-mail: diatri@ugm.ac.id

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This dentin erosion might affect the root canal dentin properties, such as microhardness and surface roughness. Consequently, declining microhardness could induce easily fracture of teeth following root canal therapy. In contrast, increasing surface roughness may reduce the adhesion of the obturation sealer to the root canal wall, resulting in microleakage.^{6,7} Additionally, the disadvantage of using EDTA as a final irrigation solution is that it has no antibacterial property.⁸ Thus, the requirement as a final irrigating solution should only expose the dentin collagen and avoid the

compromised effect on the dentin structure insignificantly.⁹ Since EDTA has several disadvantages as a final irrigation solution, many studies have been conducting to search for a new final irrigation solution that has the chelation ability but not compromise the root canal dentin.

In recent years, the study of chitosan to be utilized in dentistry has broadly occurred because of its many beneficial characteristics, such as biocompatibility, biodegradation, bioadhesion, antibacterial property, and nontoxicity.¹⁰ Chitosan is a biopolymer derived from the alkaline deacetylation of chitin, which is an element created from seashells of crustaceans and has antibacterial action as well as chelating properties.¹¹ Chitosan is available in large quantities since it can be obtained from the food industry's recycled waste product.¹² Former researchers stated the capacity of chitosan to induce smear layer removal from the root canal dentin.^{13,14}

Previous finding stated that chitosan could eliminate the smear layer adequately in 0.2% concentration for 3 minutes and lead to dentin erosion that is lower than EDTA.¹⁵ Other investigators indicated that 17% EDTA and 0.5 and 0.2% chitosan nanoparticles did not show a significant difference to remove the smear layer. The 0.5 and 0.2% chitosan nanoparticle were lower than 17% EDTA in removing calcium.⁶ However, at present, there are no available solutions that fulfill all the ideal requirements of the final irrigation. Chitosan nanoparticles, which is a promising solution for final irrigation, need to be investigated further, particularly the appropriate contact time of using chitosan nanoparticle as final irrigation. Studies of the contact time effect when using final irrigation on microhardness and surface roughness are also scarce in the literature. Therefore, this study aimed to determine the contact time effect of 0.5% chitosan nanoparticles as a final irrigation solution on microhardness and surface roughness of root canal dentin.

MATERIALS AND METHODS

The research protocol was permitted by the Institutional Ethics Committee No.00175/KKEP/FKG-UGM/EC/2019. This study used 56 intact mandibular premolars extracted for orthodontic reasons. A total of 28 teeth were employed for a microhardness experiment, and 28 others for surface roughness experiment. The remaining tissues and debris were removed, then stored in a sterile saline solution to avoid the teeth from dehydration. The teeth of each experiment were distributed randomly by two groups of 14 each. Group I was final irrigated using 17% EDTA and group II using 0.5% chitosan nanoparticle. Each group was then ascribed into two groups of seven each according to the contact time: group I, 1 minute and group II, 3 minutes.

The solution of 0.5% chitosan nanoparticle was created by diluting 0.5 g of chitosan powder (NHI, Tangerang, Indonesia) in 1% acetic acid (volume of 100 mL). Chitosan was produced from shrimp exoskeleton, which has a deacetylation degree >75%, using a method named ionic glass and polyanion tripolyphosphate (TPP) as a cross-linker.¹⁶ The mixing was agitated using a magnetic agitator for 2 hours to attain a standardized solution.

All teeth for both experiments were cut utilizing a diamond disc at the cement-enamel junction, resulting in the root length of 13 mm. The crown-down technique was performed with rotary files (Protaper Universal, Dentsply Maillefer, Ballaigues, Switzerland), up to F3 file at 250 rpm, to prepare root canals. To irrigate the root canal during preparation and after employing each file, continuous irrigation technique was used with 2.5% NaOCl solution (Golden Falcon, Dubai, UAE).

Furthermore, a sterile 30-gauge needle was introduced approximately 2 mm of working length to deliver the final irrigation solution. Finally, distilled water with a volume of 5 mL was used for flushing root canals; then the canals were desiccated using absorbent points #30.

Microhardness Study

After root canals' final irrigation solution was applied following the above-mentioned protocol, 28 teeth for the microhardness experiment were then split in the buccolingual direction with a diamond cutting saw (Buehler Ltd., Evanston, IL, USA), and then attached in self-curing acrylic leaving the dentin lumen exposed. A Vickers hardness tester (Shimadzu HMV-2, Shimadzu Corporation, Kyoto, Japan) at 100-g load and dwell time of 5 seconds was used to test the microhardness of each sample. Three separate indentations were created at each canal: one in the center and two others were in the periphery of the apical third level. The indentations were observed through the monitor linked to the microhardness tester. The results of indentations were averaged to obtain the ultimate value of microhardness (in VHN) of each sample. A two-way ANOVA and least significant difference (LSD) test were employed to analyze the data obtained with a 95% significance level.

Surface Roughness Study

Following 28 root canals for the surface roughness study, final irrigation solution was applied following the above-mentioned protocol; roots were then perpendicularly cut in a buccolingual direction to obtain mesial and distal segments. To make the sample stable in its location, it was placed and screwed on the surface of a plane table. A digital roughness tester (SR 300, Taylor Hobson, Leicester, England) was used to test the surface roughness. The needle was positioned on the surface of lumen dentin, and three tracings of a different location were performed. The roughness values of samples were recorded and viewed numerically on the apparatus monitor as Ra (μm). Data were statistically analyzed using a two-way ANOVA and LSD test with a 95% significance level.

RESULTS

The microhardness study (Table 1) showed that the contact time of 1 minute using 0.5% chitosan nanoparticle produced the greatest microhardness of root canal dentin, whereas the contact time of 3 minutes using 17% EDTA had the lowest microhardness ($p < 0.05$). However, no significant difference of microhardness occurred between 1-minute contact time of 17% EDTA and 3-minute contact time of 0.5% chitosan nanoparticle, and between 1-minute and 3-minute contact time of 0.5% chitosan nanoparticle ($p > 0.05$).

The study of surface roughness (Table 2) revealed that a 1-minute contact time of 0.5% chitosan nanoparticle produced the lowest surface roughness, and the highest was 3-minute contact time with 17% EDTA ($p < 0.05$). Conversely, there was no significant difference in surface roughness between 1-minute contact time

Table 1: Mean and standard deviation of the contact time effect of final irrigation solutions on the microhardness of root canal dentin (in VHN)

Final irrigation	17% EDTA	0.5% chitosan nanoparticle
Contact time		
1 minute	110.92 \pm 19.12 ^a	137.97 \pm 18.29 ^b
3 minutes	75.97 \pm 7.36 ^c	127.68 \pm 17.31 ^{a,b}

^{a-c}Dissimilar letters reveal that statistically significant differences occurred. EDTA, ethylenediaminetetraacetic acid; VHN, Vicker hardnesses

Table 2: Mean and standard deviation of the contact time effect of final irrigation solutions on the surface roughness of root canal dentin (in μm)

Final irrigation	17% EDTA	0.5% chitosan nanoparticle
Contact time		
1 minute	1.27 \pm 0.23 ^a	0.85 \pm 0.11 ^b
3 minutes	2.41 \pm 0.09 ^c	0.93 \pm 0.26 ^{a,b}

^{a-c}Dissimilar letters show that there were statistically significant differences. EDTA, ethylenediaminetetraacetic acid

using 17% EDTA and 3-minute contact time with 0.5% chitosan nanoparticle, and between 1-minute and 3-minute contact time of 0.5% chitosan nanoparticle ($p > 0.05$).

DISCUSSION

Previous studies have reported that the smear layers are formed during manual or mechanical instrumentation on the root canal wall. The smear layers could be removed by chelating agents such as EDTA and chitosan.^{17,18} The results of the present study revealed chelation capability of chitosan and EDTA in dissolving inorganic parts of the smear layer that consequently affect characteristic microhardness and surface roughness of dentin, which are concurrent with the findings of previous studies.⁶ Several factors may influence the efficacy of chelating agents; however, contact time plays a crucial factor in affecting the physicochemical property of root canal dentin.^{17,19} This phenomenon confirmed that the contact time of the final irrigation solution, which has chelation capacity, at a specific time to root canal dentin, alters root canal dentin in both chemical and physical aspects, resulting in the decline of microhardness and the enhancement of surface roughness.^{7,20}

This condition can be elucidated due to the effect of the chelating substance in the demineralizing of root canal dentin. The loss of calcium ion caused by chelating action leads to the alteration in the ratio of Ca/P in the dentin structure.²¹ Besides, this chelating effect could change the topography of the root canal surface by producing irregularity on the dentin surface.^{7,22} Therefore, this ratio alteration influence microhardness and surface roughness, by decreasing in the former and increasing in the latter. However, this chelating action on root canal dentin may be an advantage in the clinic, as it not only assists instrumentation of narrow or blocked root canals²³ but also improves the sealers' bonding to the wall of the root canal that necessitates the roughness of the dentin surface wall for sealer retention.²⁴

This present study also showed that microhardness of root canal dentin was greater, and surface roughness was lower in 1-minute than 3-minute contact time using 0.5% chitosan nanoparticle and 17% EDTA. Consequently, this study finding is in concurrence with the previous research, which exhibited that extended contact time of more than 1 minute of EDTA and chitosan produced more erosive effect resulting in affecting of microhardness and surface roughness of the dentin. However, the concentration of chitosan used was different than the previous findings.^{6,14} When there is an increase in the contact time of 17% EDTA irrigation solution, there is an increase in the loss of calcium ion on root canal dentin.²⁵ This result sheds light on the fact that EDTA is a strong chelating agent. If a strong chelating agent is applied for a prolonged time to root canal dentin, it may not only eliminate the smear layer but also considerably remove the peritubular and intertubular dentin.²⁶ Subsequently, the dentinal tubules' diameter might increase, and dentin structure

strength could decrease, along with the topography of the root canal dentin surface that may change.²⁰ If excessive surface roughness occurs, it could cause a lack of adaptation of sealers to root canal dentin. It allows bacterial adhesion resulting in plaque establishment in the root canal.²⁷

The chitosan chelating effect to root canal dentin may be described in three phases: adsorption mechanism, ion exchange, and chelation.²⁸ Hydrophilic characteristics of chitosan cause this solution to be absorbed rapidly to the root surface wall; hence, ionic contact between calcium in dentin and chelating agent may occur.²⁹ The 0.5% chitosan nanoparticles with a contact time of 1 minute and 3 minutes produce the same effect on the microhardness and surface roughness. This probably indicates that chitosan is a weak chelating agent, consequently affecting a smaller amount of the root canal dentin surface compared to EDTA.^{26,30} In addition, if the root canal surface is exposed to chitosan, it can immobilize the covalent connection of chitosan to the collagen of dentin, producing remineralization of demineralized dentin. This condition can be explained that the phosphate groups may draw calcium ions to generate crystal nucleation to form a calcium-phosphate layer.¹³ Conversely, EDTA is unable to remineralize the demineralized dentin.³¹ Thus, this phenomenon may explain why the chitosan solution produces higher microhardness and smaller surface roughness than EDTA regardless of contact time employed.

CONCLUSION

Within the limitation of this study, it was concluded that the contact time of 1 minute using 0.5% chitosan nanoparticle caused the least effect on the microhardness and surface roughness.

CLINICAL SIGNIFICANCE

The findings of this study revealed that contact time of 1 minute using 0.5% chitosan nanoparticle causes the least effect on both microhardness and surface roughness. Thus, the contact time of 1 minute is suggested to be employed when using 0.5% chitosan nanoparticle for the final irrigation solution in the clinic.

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