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# Apical Sealing Ability of Epoxy Resin Sealer after Application of Final Irrigation Using EDTA and Chitosan Nanoparticle with Different Contact Time

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***Abstract : Background: One crucial function of root canal irrigation is to eliminate the smear layer, thus increasing root canal sealer penetration. Good sealer penetration improves apical sealing.***
***Aim: To determine the differences of apical sealing after final irrigation using 17% EDTA and 0.2% chitosan nanoparticles (CNP) with different contact times.***
***Method: Thirty premolars were prepared using a rotary file and irrigated with 2.5% NaOCl and saline. Teeth were divided into two groups of 15 each randomly (A: 17% EDTA and B: 0.2% CNP) and were further divided into three groups of 5 each according to contact time. Root canals were obturated with epoxy resin sealer and single gutta-percha cone. The specimen was placed into a tube containing 2% methylene blue (MB) and centrifuged (3000 rpm, 3 minutes). The penetration of MB from apical to coronal was observed under a stereomicroscope (10x magnification). Data were analyzed by two-way ANOVA and LSD test (95% significance level).***
***Result: There were significant differences in mean apical leakage based on final irrigation and contact time (p <0.05); however, no interaction occurred between irrigation and contact time (p> 0.05). High apical leakage shows low apical sealing.***
***Conclusion: 0.2% CNP final irrigation produced greater apical sealing; contact time of 5 minutes of final irrigation solution produced greater apical sealing ability compared to 3 minutes and 1 minute.***
***Keywords - final irrigation, apical sealing, chitosan nanoparticles, EDTA, epoxy resin sealer***

## I. INTRODUCTION

Successful root canal treatment depends on cleaning, shaping, and disinfection through chemomechanical preparation of root canal in order to remove pulp tissue, dentin debris, and infective microorganisms[1][2]. During shaping procedures, the smear layer is formed on instrumented root canal walls. The presence of this smear layer may delay the effect of disinfectants and may also interfere with the adaptation and penetration of root canal sealers [3].

Ethylenediaminetetraacetic Acid (EDTA) in 17% concentration is usually used as the gold standard for the removal of the smear layer[4]. One factor that influences the effectiveness of EDTA to remove the smear layer is contact time. Research by Niu et al. [5] showed that a 1-minute contact time of 17% EDTA on to root canal dentin is enough to remove the smear layer in apical one-third of the root canal and to open the dentinal tubule orifices. Scelza et al.[6] mentioned that a 3-minute contact time of 17% EDTA showed the most significant number of open dentinal tubules compared to 10 minutes and 15 minutes. Andriukaitiene et al.[7] in another study showed that 17% EDTA application for 5 minutes produced the highest bond strength of sealer in the apical third and lowest bacterial leakage compared to methacrylate-based and MTA based sealer. Prolonged contact time of EDTA can induce excessive erosion of peritubular and intertubular dentin[8]. This condition is the major drawback of EDTA as a final irrigation solution. Additionally, EDTA has no antibacterial property.

Chitosan is a natural polysaccharide acquired by the deacetylation of chitin from shells of Crustaceans. It has beneficial properties, such as biocompatibility, biodegradability, bioadhesion, and chelating capacity for different metal particles[9]. Various research has been carried out regarding the contact time of chitosan as a final irrigation solution. Silva et al. investigated the chelating ability of chitosan to remove smear layer. The formation of complexes of chitosan and the metal ion is due to the mechanism of adsorption, ion exchange, and

chelation[10][11]. Chitosan modification into nanoparticle size would increase its efficiency as root canal irrigant due to better adsorption and penetration ability, especially in root canal complexities and dentinal tubules[12][35][36].

Several researchers indicated the most suitable concentration of chitosan as root canal irrigation is 0.2% while contact time may vary. Pimenta et al.[13] showed that 0.2% concentration of chitosan with 5 minutes application time was the most viable combination for use on root canal dentin. While a study by Silva et al.[11] showed that 0.2% chitosan for 3 min removed the smear layer adequately and caused less erosion than 17% EDTA. Another study was carried out by Kesim et al.[14] indicated that 0.2% chitosan for 1 minute showed similar effects on the percentage of epoxy resin sealer penetration with 17% EDTA and 10% citric acid.

Root canal sealers are needed to seal the space between the dentinal wall and obturation core material and also to fill the voids and irregularities of root canal [15]. Epoxy resin sealers have been widely used because of their advantages, including low solubility, small expansion, good apical sealing, and adhesion to dentin; therefore, epoxy resin sealer is used as a "Gold Standard" sealer[16]. Epoxy resin sealers have better penetration into micro- irregularities due to its creep capacity and long setting time, which increases the mechanical interlocking between sealer and root dentin[17]. This penetration ability improves the mechanical retention of the material and potentially reduces leakage [18].

Irrigation chelating agents remove the smear layer, which affects the adhesion and sealing ability of root canal sealers to dentin[19]. Removal of the smear layer encourages the creation of a good apical seal to prevent microleakage and enhances the bond strength of resin-based sealers[20]. Apical leakage can be assessed using several methods, linear measurement of dye penetration is the one such method which is most common, relatively easy, and fast to gauge the microleakage of the sealers [21].

## II. MATERIAL AND METHOD

This study was approved by the institutional Ethics Committee no. 00201/KKEP/FKG-UGM/EC/2019. This in vitro study used thirty mandibular premolars, which were decoronated and leaving 13 mm of roots. K-File# 20 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted until the tip of the file was visible on the apical foramen to obtain the same apical foramen diameter. Root canals were prepared using rotary files (Protaper Next, Dentsply, Maillefer) up to X3 file with 12 mm working length. Root canal irrigation using 2.5% sodium hypochlorite (Golden Falcon, Dubai, UAE) and sterile saline 3mL each was performed at each change of instrument. Root canal irrigation was carried out using a 30G single side vented needle (OneMed, Indonesia).

Chitosan solution was obtained by dissolving 0.2 gram of chitosan nanoparticles (Nanotech Herbal Indonesia, Tangerang, Indonesia) in a 1% acetic acid solution with 100ml volume. The solution was then placed into a Beaker glass and stirred for 30 minutes using a magnetic stirrer speed of 500 rpm until homogeneous. 0.2% chitosan nanoparticle solution has a pH of 3.2.

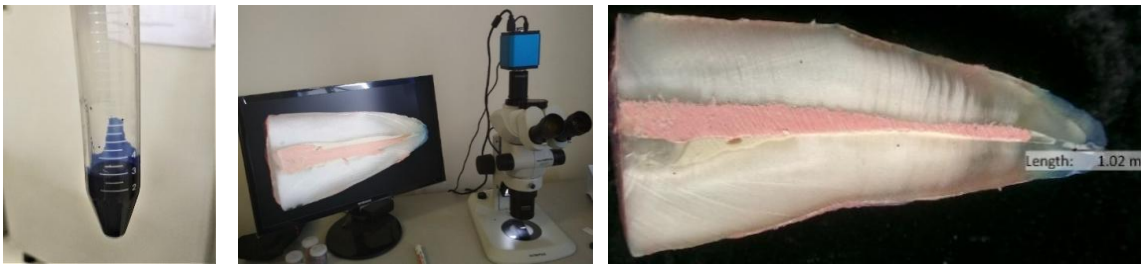
The prepared specimens were randomly divided into two groups of 15 each based on final irrigation solution [group 1: 17% EDTA (Pulpdent, Watertown, MA, USA) and group 2: 0.2% chitosan nanoparticles], the specimens then further divided into three sub-groups of 5 each according to contact time (group A: 1 minute, group B: 3 minutes, and group 3: 5 minutes). Following the application of the final irrigation solution, root canals were then dried with paper points.

Specimens were obturated using gutta-percha #X3 (ProTaper, Dentsply Maillefer) and epoxy resin sealer (AH Plus, Dentsply, DeTrey, Konstanz, Germany). The gutta-perchas were cut 2 millimeters below the orifice, followed by filling using composite resin. Specimens were stored in a 37 °C incubator with 100% humidity for seven days. Furthermore, all samples were covered with nail polish and sticky wax except 1 mm from the apex. Specimens then were put into a 15 mL centrifuge tube containing 2% methylene blue with the apical part heading toward tube opening (Picture 1a) and centrifuged for 3 minutes at 3000 rpm. Nail polish and sticky wax then were removed, followed by a copious rinse of water. Specimens were cut longitudinally into halves using a diamond disc and observed using stereomicroscope (10x magnification) (Picture 1b). The penetration of methylene blue dye was measured by observing penetration of methylene blue solution from apical to the coronal direction (in millimeters) using Image Raster v3 software (Optilab) (Picture 1c). In this study exhibited that the shortest methylene blue penetration indicated the best apical sealing ability. The data obtained were analyzed by two-way ANOVA and LSD test with a significance level of 95%.

## III. RESULTS

Apical sealing ability was measured based on the penetration of 2% methylene blue from apical to the coronal direction. The mean and standard deviation of apical sealing ability can be seen in Table 1. The lowest sealing ability was found in the group with 17% EDTA for 1 minute (5.79 ± 0.82 mm), and the greatest sealing ability was shown in the group using 0.2% chitosan nanoparticles for 5 minutes (0.68 ± 0.21 mm). The analyzes using two-way ANOVA can be seen in Table 2. There was a significant difference in apical sealing ability between final irrigation type groups and among contact times ($p < 0.05$). However, no interaction occurred

between contact time and type of final irrigation solution (p> 0.05). Post Hoc test with Least Significant Difference (LSD) was done to determine differences between groups of final irrigation solution type and contact times. The LSD test results (Table 3) showed that the final irrigation of 17% EDTA with a contact time of 1 minute, 3 minutes and 5 minutes was significantly different from the final irrigation of chitosan nanoparticles with a contact time of 1 minute, 3 minutes and 5 minutes (p <0.05), except for three comparison groups, namely between EDTA 3 minutes and chitosan 1 minute, between EDTA 5 minutes and chitosan 3 minutes, and between chitosan 3 minutes and chitosan 5 minutes(p>0.05).




**(a)** **(b)** **(c)**

**Picture 1a**. The sample was placed inside a tube containing 2% methylene blue before centrifugation, **1b**. Sample observation using stereomicroscope (10x magnification), **1c**. Dye penetration measurement

**Table 1.** The mean and standard deviation of the apical sealing ability of epoxy resin sealer after application of final irrigation using 17% EDTA and 0.2% chitosan nanoparticle with different contact time (in millimeters)

| Contact Time | EDTA | Chitosan |
|---|---|---|
| **1 minute** | 5.79 ± 0.82 | 3.29 ± 0.99 |
| **3 minutes** | 3.38 ± 0.79 | 1.37 ± 0.44 |
| **5 minutes** | 1.68 ± 0.34 | 0.68 ± 0.21 |

**Table 2.** A two-way ANOVA of the apical sealing ability of epoxy resin sealer after application of final irrigation using 17% EDTA and 0.2% chitosan nanoparticle with different contact time

| Source of Varians | Sum | Degree of freedom | Mean square | F | P |
|---|---|---|---|---|---|
| **Irrigation** | 25.172 | 1 | 25.172 | 56.589 | 0.000* |
| **Contact time** | 58.010 | 2 | 29.005 | 65.207 | 0.000* |
| **Irrigation and time** | 2.914 | 2 | 1.457 | 3.275 | 0.055 |

**Table 3.** The Post Hoc LSD test of the apical sealing ability of epoxy resin sealer after application of final irrigation using 17% EDTA and 0.2% chitosan nanoparticle with different contact time

| Comparison groups | | LSD | P |
|---|---|---|---|
| **17% EDTA-1 Min** | **17% EDTA-3 Min** | 2.40600 | 0.000* |
| | **17% EDTA-5 Min** | 4.10800 | 0.000* |
| | **0.2% Chitosan-1 Min** | 2.49200 | 0.000* |
| | **0.2% Chitosan-3 Min** | 4.41400 | 0.000* |
| | **0.2% Chitosan-5 Min** | 5.10400 | 0.000* |
| **17% EDTA-3 Min** | **17% EDTA-5 Min** | 1.70200 | 0.000* |
| | **0.2% Chitosan-1 Min** | 0.086 | 0.84 |
| | **0.2% Chitosan-3 Min** | 2.00800 | 0.000* |
| | **0.2% Chitosan-5 Min** | 2.69800 | 0.000* |
| **17% EDTA-5 Min** | **0.2% Chitosan-1 Min** | -1.61600 | 0.001* |
| | **0.2% Chitosan-3 Min** | 0.306 | 0.475 |
| | **0.2% Chitosan-5 Min** | 0.99600 | 0.027* |
| **0.2% Chitosan-1 Min** | **0.2% Chitosan-3 Min** | 1.92200 | 0.000* |
| | **0.2% Chitosan-5 Min** | 2.61200 | 0.000* |
| **0.2% Chitosan-3 Min** | **0.2% Chitosan-5 Min** | 0.69 | 0.115 |

## IV. DISCUSSION

This research was conducted to determine differences in the apical sealing ability of root canal sealer in the use of 17% EDTA and 0,2% chitosan nanoparticles (CNP) as a final irrigation solution with a contact time of 1 minute, 3 minutes and 5 minutes. The results of this study showed that the type of final irrigation solution affected the sealing ability of obturation using an epoxy resin sealer. The use of a 0.2% CNP irrigation solution produced a higher apical sealing than the 17% EDTA; thus, the research hypothesis was proven. The apical sealing ability of epoxy resin sealer is related to the presence of a smear layer. Mulay et al.[22] reported that epoxy resin sealer has better wettability when complete removal of smear has been achieved through the application of chelating irrigation. This phenomenon could be due to the intimate contact of sealer with the dentin surface leading to the penetration of the sealer in the dentinal tubules. According to Nunes et al.[23] the presence of the smear layer affects the adhesion of root canal sealers negatively because it is able to form an interface between the sealing material and dentin, hindering or impeding sealer penetration into the dentinal tubules.

The higher apical sealing ability by using 0.2% CNP irrigation is related to the superiority of chitosan in removing the smear layer compared to 17% EDTA. The results of this study is in accordance with previous research by Darrag [24], which stated that the application of 1 ml 0.2% CNP irrigation for 3 minutes produced the lowest smear layer at three regions of root canal compared to 17% EDTA, 10% citric acid, and Tetracycline Citric Acid and Detergent Mixture (MTAD). A study by Kamble et al.[25] mentioned that 0.2% of chitosan irrigation was more efficient in removing the smear layer than 17% EDTA with 5 minutes application time. Silva et al.[10] also concluded that the application of 5 ml 0.2% chitosan solution for 3 minutes as final irrigation was the most efficient in removing the smear layer compared to other irrigation materials (15% EDTA solution, 1% acetic acid, and 10% citric acid).

Epoxy resin sealers are known to have good penetration into root canal micro-irregularities due to their creep capacity and long setting time, which increase the mechanical interlocking between sealer and root canal dentin[17]. The removal of the smear layer on the root canal wall facilitates the penetration of sealer into dentinal tubules, thereby increasing the adhesion of sealer to root canal dentin [26]. A study by Kesim [14] stated that using the chelating solution (0.2% chitosan, 17% EDTA, and 10% citric acid) produced a higher epoxy resin sealer penetration in the coronal third of root canal due to greater smear layer removal. In addition, Bayram et al.[27] suggested that the use of 0.2% chitosan for final irrigation increased the bond strength of several root canal sealers, including epoxy resin sealers.

This present study also revealed that the contact time of the final irrigation solution produced a significant difference in the apical sealing ability of epoxy resin sealer. Apical leakage decreased as contact time increased, and more smear layers were removed. This condition is in accordance with a previous study [28] that the quantity of smear layer removal by a material depends on the length of exposure time. Dechichi and Moura[29] stated that the final irrigation solution needs to be applied with a larger volume, hence allowing longer contact time with root canal walls. Aksel and Serper[30] also stated that irrigation solutions required sufficient working time to work effectively.

This study also demonstrated that no significant differences occurred using the final irrigation of EDTA 3 minutes and CNP 1 minute; EDTA 5 minutes and CNP 3 minutes; and CNP 3 minutes and CNP 5 minutes. These results indicated that the final irrigation of 17% EDTA for 3 minutes and 0.2% CNP for 1 minute produced similar apical sealing ability, as well as 17% EDTA for 5 minutes and 0.2% CNP for 3 minutes. It can be elucidated that CNP required shorter contact time to remove the smear layer than EDTA due to its efficiency. Additionally, Mittal et al.[31] stated that EDTA is effective at a neutral pH; the exchange of calcium from dentin by hydrogen would decrease pH and leads to a decrease in the efficacy of EDTA. Previous studies reported that 0.2% chitosan is more efficient in removing smear layers than 17% EDTA [11] [24] [25]. The results of this study also showed that no significant difference of 0.2% CNP with 3 minutes and 5 minutes of contact time. This phenomenon is in accordance with the findings of Pimenta et al. [13], which stated that 0.2% concentration of chitosan with a 3 to 5 minutes contact time was the best combination of concentration and contact time to clean root canal dentin.

Concentration and contact time of chelation agents are important factors since a higher concentration of the solution and longer contact time are known to increase the surface roughness of dentin [11]. Shorter contact time is beneficial to minimize the erosion of dentin. Erosion of dentin occurs due to the demineralization of the peritubular and intertubular dentin as a result of the increasing diameter of the dentinal tubules and the changing of root canal surface. Severe erosion are characterized by loss of intertubular dentin [8]. Erosion of root canal dentin would affect the root canal obturation. Root dentin erosion not only interfered with the adaptation but also reduced the sealing ability of obturation material[32]. It is known that chitosan produces less erosion than EDTA[11]. Chitosan has beneficial properties because it can encourage the remineralization of root canal dentin when exposed by chelation material. Chitosan caused covalent immobilization on dentinal collagen that might induce the remineralization of the exposed and demineralized dentin structure because its functional phosphate

groups might bind to calcium ions to form a favorable surface for crystal nucleation, resulting in the formation of a calcium phosphate layer[12].

Chitosan mechanism is able to form complexes with metal ions from the smear layer through absorption, ion exchange, and chelation and plays a role in attracting calcium ions from dentin. The hydrophilic nature of chitosan facilitates adsorption on the root canal wall [25]. Chitosan has hydroxyl and free amino groups; hence it is cationic, which plays a role in ionic interactions between calcium ions from dentin with chelation material [24]. Silva et al.[11] and Del Carpio et al. [12] stated that two theories attempt to explain the chelation mechanism by chitosan. The first theory is the bridge model which explains that there are two or more amino groups of chitosan attached to metal ions simultaneously. The second theory is the pendant model, which demonstrates that an amino group that plays a role in causing attachment to metal ions, and subsequently, the metal ions are connected with other amino groups. Chitosan used in this study was nano-sized to obtain better adsorption and penetration ability into the dentinal tubules to increase smear layer removal efficiency[12]. Acetic acid with a concentration of 1% was used as a solvent in this study because chitosan nanoparticles cannot be dissolved in water. This acetic acid had no effect on the ability to remove the smear layer. This finding was proven by Silva et al. [10], which stated that a 0.2% chitosan solution promoted superior cleaning of root canal walls compare to 1% acetic acid as root canal irrigation solution.

This study used a dye penetration method using a centrifuging machine. The use of centrifugation is more reliable than passive immersion methods to show the distance of dye penetration. Centrifugation pressure can push dye solution from the apical to the coronal passing through empty spaces, which formed by trapped air in obturated root canal rather than relying solely on the capillary force of dye solution [33]. The obturation method used in this study was a single cone because this method is simple; therefore, it can reduce working time and minimizes the pressure against the root canal wall. The combination of gutta-percha with epoxy resin sealer produced uniform obturation material [34].

## V. CONCLUSION

Based on the results of the study, it can be concluded that the apical sealing ability of epoxy resin sealer is higher with the application of 0.2% chitosan nanoparticles compared to 17% EDTA as final irrigation solution. Contact time of final irrigant for 5 minutes resulted in the greater apical sealing ability of epoxy resin sealer compared to 3 minutes and 1 minute respectively.

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