

RESEARCH ARTICLE

Apical sealing ability of chitosan nanoparticles in epoxy-resin-based endodontic sealer

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ABSTRACT

A good sealing ability of apical part of root canal system is needed to prevent microleakage thus resulting long-lasting successful treatment. Chitosan nanoparticles as nanofillers could be added to epoxy resin sealer to increase adaptation to dentinal wall. The purpose of this study was to evaluate the apical sealing ability of chitosan nanoparticles addition to epoxy-resin-based sealer. Thirty mandibular premolars were used in this study. The root canals were prepared using rotary files up to #30/0.09. The canal was irrigated with 2.5% NaOCl and 17% EDTA, then was rinsed with sterile water. All teeth were randomly divided into 3 groups (n=10). Group I was obturated with gutta-percha, Group II was obturated with gutta-percha and epoxy resin sealer, group III was obturated with gutta-percha and epoxy resin sealer that was added with chitosan nanoparticles. All specimens were stored in an incubator for 7 days at 37 °C. After that, specimens were tested with centrifuging dye penetration method with methylene blue 2% solution. The specimens were longitudinally sectioned, observed under stereomicroscope (8x magnification) and measured in millimeters (mm). The data were analyzed using one-way Anova. The results of one-way Anova showed that dye penetration in apical part of the root canal in all groups was statistically significant difference. The addition of chitosan nanoparticles to epoxy resin sealer increases the apical sealing ability of root canal obturation material.

Keywords: apical sealing ability; chitosan nanoparticles; epoxy-resin-based sealer

INTRODUCTION

Obturation is an important stage in root canal treatment and must produce a complete closure of the root canal and eliminate all pathways to the oral cavity.¹ The most common cause of root canal treatment failure is less hermetic obturation of the root canal.² The hermetically closure of apical parts of the root canal is needed to prevent the entry of bacteria and to guarantee the success of root canal treatment.³

Epoxy-resin-based sealer is widely used as root canal sealer because it has better sealing ability and good adhesion compared to zinc oxide eugenol-based sealer, glass ionomer cement and calcium hydroxide.⁴ The epoxy-resin-based sealer is able to make a monobloc. It can penetrate to dentine tubules, however the resin components

penetrate into dentinal tubules, while filler particles, which are too large to enter the dentine tubules, remain at the interface of the root canal wall and gutta percha.^{4,5} This causes a weak bond strength of the sealer to root canal wall.

Chitosan is a natural biopolymer, in the form of a linear polysaccharide composed of β- (1-4) -linked D-glucosamine and N-acetyl-D-glucosamine with random distribution. Chitosan is produced through the deacetylation process of chitin compounds, which are the main components in the shells of crustacean animals such as crabs and shrimp.⁶ The use of chitosan in the field of dentistry in recent years has been increasing.⁷ Chitosan nanoparticles is a new technology that is widely investigated in the field of endodontics, including the potential to improve mechanical and antimicrobial properties. In

addition, chitosan nanoparticles were investigated as an irrigation solution, medication, and as an additive to endodontic sealers.⁸

Chitosan nanoparticles is a potential material in endodontic fields, so that research needs to be done to evaluate and explore its potencies. The aim of this study was to evaluate the effect of the addition of chitosan nanoparticles on epoxy-resin-based sealer to the apical sealing ability of root canal obturation material.

MATERIALS AND METHODS

This study was approved by Ethics Commission Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia No.001550/KKEP/FGK-UGM/EC/2018. Experimental subjects consisted of 30 non-carious single-rooted, mandibular first premolars extracted for orthodontic treatment purpose- with fully developed apices were selected. Soft tissue debris and calculus were removed using ultrasonic scaler and stored in containers filled with sterile water. All samples were decoronated at the cement-enamel junction using diamond disc, leaving 14 mm root length. Canals were negotiated using K-file #15 (Dentsply Maillefer, Switzerland) until the tip was visible from apical foramen. Preparation were performed using crown down technique with rotary files (Protaper Universal, Dentsply). Working length was maintained at 13 mm and canals were prepared until F3 file, lubricated using 15% EDTA gel. Irrigants used between file sequence were 2 ml 2.5% NaOCl for 1 minute, 2 ml sterile aquadest, and 3 ml 17% EDTA solution.

Prepared specimens were randomly divided into 3 groups (each group consist of 10 teeth) for single cone obturation. Samples from Group I was obturated using gutta percha without sealer, Group II were obturated using gutta-percha points (Dentsply Maillefer, Switzerland) with epoxy resin sealer (AH 26, Dentsply, Switzerland), and Group III were obturated using gutta-percha points with epoxy resin-chitosan nanoparticles sealer. Epoxy resin-chitosan nanoparticles sealer was obtained by mixing powder with chitosan nanoparticles: epoxy resin sealer ratio 15 mg: 100 mg. Powder was mixed with 1 unit (57.5 mg) epoxy resin

using stainless steel spatula. Sealer was applied into root canal using lentulo (Dentsply Maillefer, Switzerland). After obturation, all samples were stored over moist cloth in containers and incubated (Sanyo, Japan) for 7 days. After 7 days, all samples were dried in room temperature, and divided into 2 sub-groups: A and B (each sub-groups consist of 15 teeth), coated with clear nail polish twice and uncoated, respectively). Apical portion of groups IA, IIA, and IIIA were uncoated

All specimens from group IA, IIA, and IIIA (n=15) were put into 12 ml centrifuge tubes and 2% methylene blue solution (pH 7) were poured into tubes, soaking all root specimens. Specimens were centrifuged at 3000 rpm for 3 minutes. All specimens were rinsed under running water until clean and cut in halves longitudinally using diamond disc for observation using 8x magnification stereo microscope (Olympus, Japan). Dye penetration was measured in millimeters from apical point to the most coronal part of dye using software program (AutoCad2018). Specimens of group IB, IIB, and IIIB (n=15) were sectioned transversally at the apical third, polished using abrasive sand papers, and mounted on stubs and coated with palladium gold. All specimens from group B were observed under scanning electron microscope (SEM) with varied magnifications.

Measurement data from dye penetration method (n=15) was analyzed using one way ANOVA, with 95% confidence level. Statistical analysis used SPSS 17 software. Results from SEM observation were used as supporting data to observe interface morphology between root canal wall and sealer.

RESULTS

Sealing ability in the apical region was tested using 2% methylene blue dye penetration method. Normality and homogeneity tests showed data was distributed normally and homogeny. Table 1 showed means, standard deviations, and significance from one way anova. Dye penetration means in apical region in epoxy resin – chitosan nanoparticle group was smaller (2.01 ± 0.44 mm) compared to other groups, and there was significant difference

($p < 0.05$) among 3 experimental groups. Table 2 showed significant differences ($p < 0.05$) in sealing ability from post hoc LSD among 3 experimental groups, between gutta percha obturation and gutta percha-epoxy resin sealer groups, gutta percha and gutta percha-epoxy resin with chitosan nanoparticles sealer groups, and gutta percha-epoxy resin sealer and gutta percha-epoxy resin with chitosan nanoparticles sealer.

Figure 1 showed the SEM of apical one third of all groups. The gutta percha without sealer group showed wide gaps at the root canal dentin wall and sealer interface (Figure 1A). The group of gutta percha and epoxy-resin-based sealer showed less gaps at the interface, compared to the first group,

but there was still gaps in the interface (Figure 1B). The gutta percha and epoxy-resin-based sealer with chitosan nanoparticles showed more adaptation and less gaps at the interface than the other two groups (Figure 1C). However, none of them showed complete apical sealing.

Table 1. Means, standard deviations (SD) of dye penetration measurements (in mm), and p value of one-way ANOVA from three experimental groups

Obturation material	n	Mean \pm SD	p
Gutta percha	5	14.00 \pm 0.00	0.000
Gutta percha, epoxy resin sealer	5	2.99 \pm 0.65	
Gutta percha, epoxy resin sealer, chitosan nanoparticles	5	2.01 \pm 0.44	

Table 2. Post-hoc LSD analysis of dye penetration from three experimental groups

Groups		Mean differences	Convidence interval		p
			Minimum	Maximum	
Gutta percha	Gutta percha, siler resin epoksi	11.010	10.38	11.63	0.000*
Gutta percha	Gutta percha, siler resin epoksi + kitosan	11.986	11.35	12.61	0.000*
Gutta percha, siler resin epoksi	Gutta percha, siler resin epoksi + kitosan	0.976	0.348	1.603	0.005*

*p value significant

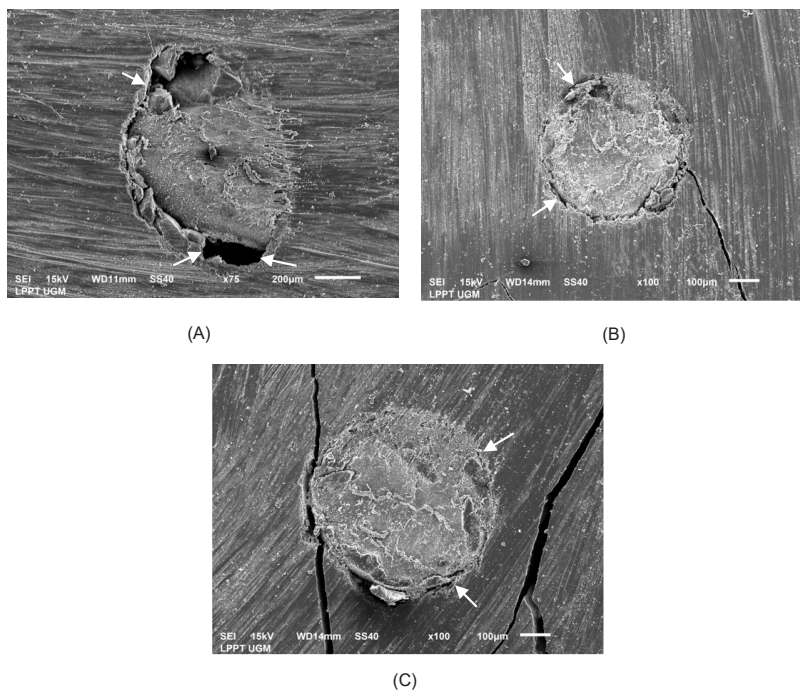


Figure 1. Scanning electron microscope of apical one third. Obturation with gutta percha without sealer (A) gutta percha and epoxy-resin-based sealer (B) and gutta percha and epoxy-resin based with addition of chitosan nanoparticles (C) The gutta percha and epoxy-resin-based sealer with chitosan nanoparticles (C) showed more adaptation and less gaps at the interface than the other two groups (white arrows).

DISCUSSION

The results of observations and dye penetration measurements in this study showed that in all groups of obturation material occurred apical microleakage. The results are in agreement with Ayer et al,⁹ which states that all root canal obturation materials allow marginal infiltration and are not impenetrable.

In the present study, dye penetration of gutta percha and epoxy-resin-based sealer with addition of chitosan nanoparticles group showed least penetration. Based on the One-way ANOVA analysis, there were statistically significant different in dye penetration of all groups, and also from the post-hoc LSD showed that all between groups were statistically significant difference. It means that the apical sealing ability of chitosan nanoparticles addition to epoxy-resin-based sealer is tighter and better compared to the other groups. The epoxy-resin-based sealer with chitosan nanoparticles has good adaptation to root canal wall. Good adaptation of sealer to the root canal wall is an important factor in obtaining sealing ability. Wetting ability and flow of sealer material affect the adaptation of sealer to the root canal wall.¹⁰ According to Bernardes et al,¹¹ the flow of sealer is influenced by the size of the filler particles, i.e. the smaller the particles, the greater the flow of sealer. The greater the flow of sealer, the more capable it is to penetrate on irregular surfaces and into the root canals.

Satheesh et al.¹² explained that chitosan nanoparticles bind intermolecularly with epoxy on epoxy resin. The polycationic properties of chitosan with amine groups (-NH₂) and hydroxyl groups (-OH) are known to be able to act as reaction sides in producing relatively homogeneous mixtures, therefore chitosan can be used as a filler in epoxy resins. The addition of nanofillers in epoxy-resin-based sealer increases the flow property of the sealer so that increase adaptation to root canal wall.

The epoxy-resin-based sealer with addition of chitosan nanoparticles in the present study has a greater apical sealing ability, this is probably caused by the hydrophilic property of chitosan nanoparticles. The epoxy-resin-based sealer that was used in this study was AH-26 which is hydrophobic. Chitosan has hydrophilic properties

that are resistant to humid conditions,¹³ while epoxy resin has hydrophobic properties, so that the addition of chitosan nanoparticles on epoxy resin sealer increase the bond strength of epoxy resin to the moist root canal wall.

In the present study, all specimens were prepared and then irrigated using EDTA 17%. The use of EDTA 17% aims to eliminate the smear layer so that the dentinal tubules are open, resulting in an irregular surface.¹⁴ According to Chau et al,¹⁵ wetting ability is influenced by surface roughness and hydrophobic/hydrophilic properties of particles. Dentin wall surfaces that have been prepared and irrigated using EDTA 17% can produce irregular surfaces. Hydrophobic particles will produce large contact angles on irregular surfaces, while hydrophilic particles have smaller contact angles.¹⁵ This causes the epoxy-resin-based sealer which is added with the hydrophilic chitosan nanoparticles has a greater wetting ability and increases adhesion to irregular root canal walls. Chitosan is hydrophilic, so it can be in close contact with root canal dentine and is adsorbed better into the root canal wall. Chitosan has a large number of hydroxyl and amino groups which make chitosan become cationic, allowing ionic interactions with dentine calcium ions. The amino group in chitosan can be protonated resulting in the withdrawal of other molecules for adsorption into the root canal dentine and being able to enter deeper into the dentinal tubules.¹⁶ The size of the nanoparticle chitosan produces a larger surface-volume proportion, so that the hydrophilic property of nanoparticle chitosan is increasing.¹⁷

In the present in vitro SEM study showed that epoxy-resin-based sealer with addition of chitosan nanoparticles had less gaps than epoxy-resin-based sealer without chitosan and gutta percha without sealer in apical areas of the root canal. The epoxy-resin-based sealer with addition of chitosan nanoparticles had better dentine wall-sealer interface compared to the other groups. Application of sealers with appropriate properties such as adhesion, adaptation and tubular penetration allow establishment of root canal sealing due to higher sealer interface with dentine wall and entombment of residual bacteria in dentine tubules.¹⁸

CONCLUSION

It can be concluded that the addition of chitosan nanoparticles to epoxy resin sealer increases the apical sealing ability of root canal obturation material.

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