The effect of oligosaccharide chitosan irrigating agent on penetration of two type endodontic sealers into the dentinal tubules: a confocal laser scanning microscopy study

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ABSTRACT

Introduction: Irrigation during root canal treatment is one of the important factors that play a role in determining the quality of sealer penetration into the dentinal tubules, especially in the apical third of the tooth root. This is inseparable from the selection of the used irrigation agent. Currently, oligosaccharide chitosan has been developed which is easier to manipulate as an alternative irrigation agent. This study aimed to analyze the effect of the chitosan oligosaccharide irrigating agent on the penetration of two types of endodontic sealers into the dentinal tubules. **Methods:** This study used 60 mandibular premolars which were randomly divided into 3 groups (n=3). Group 1 was irrigated with 2% chitosan oligosaccharides; Group 2 with a combination of 2.5% NaOCl and 0.2% chitosan nanoparticles; and group 3 was irrigated with a combination of 2.5% NaOCl and 17% EDTA. At the time of obturation, each group was divided into two groups, for sealer treatment using CeraSeal Bioceramic and AH Plus sealer. The sealer's penetration depth was measured using a confocal laser scanning microscope. **Results:** The highest mean on the penetration depth of the sealer was seen in the irrigation group using chitosan oligosaccharide solution with CeraSeal bioceramic sealer (2575,151 AU) ($p>0.05$). **Conclusion:** There is an effect of 2% oligosaccharide chitosan solution on sealer penetration into dentinal tubules. CeraSeal bioceramic sealer showed better penetration than AH Plus sealer.

Keywords: oligosaccharide chitosan; ceraseal bioceramic sealer; AH plus sealer; confocal laser scanning microscope

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INTRODUCTION

Root canal treatment is a treatment that aims to relieve pain, eliminate microorganisms from the root canal, and prevent the recurrence of infection.[1] There are three main principles when performing root canal treatment or commonly referred to as triad endodontic treatment, namely biomechanical preparation which includes cleaning and formation of the canal (shaping),

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disinfection or irrigation of the root canal (cleaning), and obturation of the canal.[1,2] Sealers on the obturation stage are used to increase the sealing and mechanical interlocking strength to prevent re-infection of the dentinal tubules and root canals.[3] Factors that affect the penetration ability of the sealer include dentin permeability, removal of the smear layer, filling technique, and the physical properties of the sealer material.[4] The presence of a smear layer on the root canal wall especially in the apical third can reduce dentin permeability.

The reduced dentinal permeability prevents sealer adaptation into the dentinal tubules. The smear layer that is attached to the root canal wall can also interfere with the binding between the main filler and the root canal wall.[3,4] Therefore, the chemical cleaning step is very important to remove the remaining organic and inorganic tissue so that it can improve the quality of the root canal filling.[5]

Until now, root canal treatment that uses sodium hypochlorite (NaOCl) is still considered as the gold standard for root canal irrigation agent.[1,5] However, NaOCl is reported to exhibit cytotoxicity, allergic reactions, and unpleasant taste, and has the potential to cause severe irritation if exposed to the mucosa.[6] Due to the nature of NaOCl which is only able to dissolve organic tissue, Ethylenediaminetetraacetic acid (EDTA) concentrate 17% is often used as a flushing agent. However, the use of EDTA can cause erosion of the peritubular dentin, thereby affecting the hardness and permeability of dentin in the root canal wall.[2]Chitosan $(C6H11NO4)n$ is a natural derivative of polysaccharide obtained from the deacetylation of chitin which has biocompatibility, biodegradability, and bioadhesion, lacks toxicity, a broad antimicrobial effect, and the ability to chelation.[5]

The study of Ayu and Trimurni[7], which used chitosan as an irrigant, looked at the effect of high molecular chitosan with concentrations of 0.1% and 0.2% used as irrigation material and a combination of 2.5% NaOCl and 17% EDTA, SEM image showed that chitosan 0.2% is cleaner in removing the smear layer in the apical 1/3 compared to other groups.[7] Ernani, et al. compared the fracture resistance of teeth after root canal treatment, reporting that the group irrigated with 2.5% NaOCl plus 0.2% chitosan had the highest fracture resistance compared to the other groups. This shows that 0.2% chitosan solution affects the pressure distribution, because chitosan has a D-glucose structure so it has the ability to strengthen dentin on the root canal wall. However, one of the major limitations behind chitosan is its insolubility at neutral pH with high viscosity, thus making it difficult to manipulate.[10]

Recently, degradation product of chitosan have been developed namely chitosan oligosaccharides which are soluble in water, easier to manipulate, and can dissolve the smear layer when used as an irrigation agent.[9,10] Chitosan oligosaccharides when compared with chitosan, have a low molecular weight and high solubility.[11]

The effect of adequate root canal irrigation is expected to be able to clean the root canal from microorganisms and smear layers that can interfere with the adaptation of the obturation material and sealer penetration. In the field of endodontics, a crucial step that can influence the long-term success of root canal therapy is the proper closure of the cleaned and shaped root canal system.[12] Several types of root canal sealers include bioceramic and resin based sealers. CeraSeal bioceramic sealer (Meta Biomed, South Korea) has a physical and chemical properties, has a good biocompatibility with tissue and contains calcium phosphate which can increase the density of the dentin wall.[3,13]

AH Plus® sealer (Dentsply, Maillefer, Ballaues, Switzerland) is a type of resin based sealer that has an antimicrobial effect and maintains a bond with the collagen network in the dentinal tubules, insoluble, radiopaque, and has low toxicity.[3]Based on this background, the aim of this study was to evaluate the extent to which bioceramic sealer and AH Plus sealer can penetrate the dentinal tubules in the apical third region after irrigating with chitosan oligosaccharide irrigant using a scanning confocal laser microscope .

METHODS

This research was a laboratory study with a post-test-only group design, namely by analyzing the penetration depth of root canal sealers after irrigation during root canal treatment procedures. This research was conducted for 5 months, starting with sample preparation, irrigation solution production, endodontic treatment, sample treatment, and samples cutting before analysis and examination with CLSM.

Sample preparation

A Sample of 60 premolars was cleaned from remaining tissue and adhering dirt. The sample was immersed in a saline solution before being treated, then all samples were crowned with a bur disc at about the cemento-enamel boundary. The samples were randomly divided into three groups of 20 teeth. Group 1 was irrigated with 2% chitosan oligosaccharides; Group 2 with a combination of 2.5% NaOCl and 0.2% chitosan nanoparticles; and Group 3 with a combination of 2.5% NaOCl and 17% EDTA. At the time of obturation, each group was divided into two, for sealer treatment using CeraSeal bioceramic sealer and AH Plus sealer.

Manufacture of irrigation materials

Chitosan oligosaccharides were obtained from the Center of Excellence for Chitosan and Advanced Materials (Centre of Excellence for Chitosan and Advanced Materials, University of North Sumatra, Medan, North Sumatra, Indonesia). The manufacture of oligosaccharide chitosan irrigation material is obtained by dissolving 2 grams of oligosaccharide chitosan powder in 100 ml of distilled water and stirred until homogeneous using a magnetic stirrer (Cimarcc Thermo Fisher, USA). The final product of this material is an irrigation solution of chitosan oligosaccharides with a concentration of 2%. A solution of 0.2% chitosan nanoparticles made from 0.2 grams of chitosan powder was dissolved in 100 ml of 1% acetic acid, then the solution was stirred until homogeneous with a magnetic stirrer for 2 hours. After being homogeneous, the solution was added 20 drops of sodium tripolyphosphate, stirred again with a magnetic stirrer for 30 minutes until the solution is homogeneous. Afterwards, the chitosan solution was put into an ultrasonic bath for 1 hour to break the chitosan particles into nanoparticles.

Endodontic treatment and sample treatment

Each sample is measured for working length before root canal preparation. Then, each treatment group is given an initial irrigation of 5 ml according to each treatment group's irrigating agent using a 30G needle syringe and one-side vented design with a depth of 1 mm shorter than the working length. Followed by root canal exploration using K-files #08, #10, and #15 until the root canal feels loose. Root canals are accessed using K -file #15 according to the working length. Then, every sample was prepared using a crown down technique using a rotary instrument with an endomotor (Saeshin, Korea) and file i3 gold (Denjoy, China). Preparation is carried out up to 25/.06 files according to the working length.

For each instrument change, 2 ml of irrigation was performed in each treatment group (Group 1: 2% chitosan oligosaccharide solution; Group 2 and 3: 2.5% NaOCl solution). Then, 5 ml of final irrigation was performed (Group 1: 2% chitosan oligosaccharide solution; Group 2: 0.2% chitosan nanoparticle solution; Group 3: EDTA 17%). After each irrigation, activation is carried out using an EndoActivator (Dentsply Sirona, USA) which aims to agitate the irrigation material to facilitate the removal of the smear layer into the dentinal tubules, this results in clean dentin tubules, so that good sealer penetration is obtained. Then, all samples were rinsed with saline and dried with paper points.

After drying, Master Apical Cone (MAC) was measured in accordance with the working length of the tooth sample. The main obturation material for all samples was a.06 gutta-percha taper, and the sealants used were AH Plus and CeraSeal bioceramic sealer. The sealer material was mixed with fluorescent dye rhodamine B to a concentration of 0.1%; mixing was carried out in the dark room. Groups 1,3 and 5 were obturated with a single cone technique and with a CeraSeal bioceramic sealer. Groups 2, 4 and 6 used the lateral condensation technique with a sealer using AH Plus.

After the endodontic treatment was completed, the samples were stored in a container lined with aluminum foil, and then incubated at 37^{o}C with 100% humidity for 7 days. Then, each sample was cut 4 mm from the apical with a thickness of 2 mm using a disc bur under the running water to prevent heat due to friction.

Sample examination using CLSM

Image analysis of CLSM was carried out at the Central Laboratory of Biological Sciences, University of Brawijaya. The sample was placed

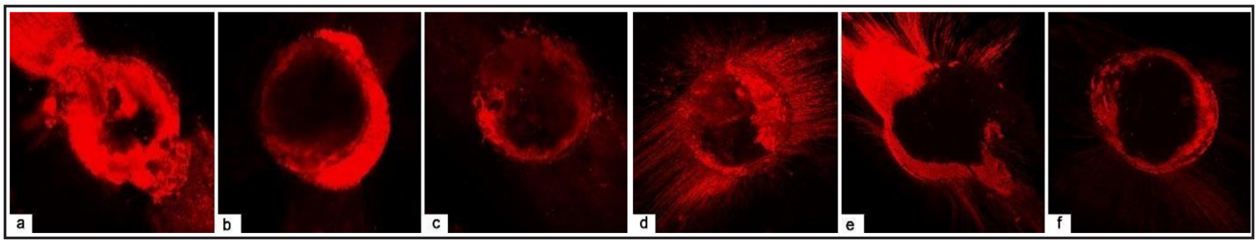

Figure 1. Representative picture of penetration sealer. (a,b,c) CeraSeal (d,e,f) AH Plus (a,d) irrigation with chitosan oligosaccharide solution (b,e) combination of 2.5% NaOCl irrigation and 0.2% chitosan nanoparticle solution (c,f)) combination of 2.5% NaOCl irrigation and 17% EDTA.

on a coverslip measuring 24 x 60 mm and then examined with Confocal Laser Scanning Microscopy (CLSM) - Olympus, Japan; with a magnification of 100x and a wavelength of 543 nm to focus the image. Then, Rhodamine dye on the dye list in the CLSM software was selected, and followed by a scanning process to carry out and adjust the image acquisition control. The High Voltage setting was adjusted to control the brightness of the image and the background was offset to black. The resulting image that appeared on the computer monitor screen was then analyzed using the Olympus Fluoview ver.4.2a software. Initially, the image was circled with the tools included in the software, then a measurement was chosen to see the intensity expression of the sealer that has been labeled with Rhodamine. Then, the analysis results automatically came out in the form of the average value and standard deviation, as well as the intensity profile graph.

RESULTS

The appearance of sealer penetration in each group that is analyzed under CLSM shows an overall difference. The sealer that has been mixed with Rhodamine B dye looks red under CLSM (Figure 1).These images were generated from CLSM and analyzed using the Olympus Fluoview ver.4.2a software.

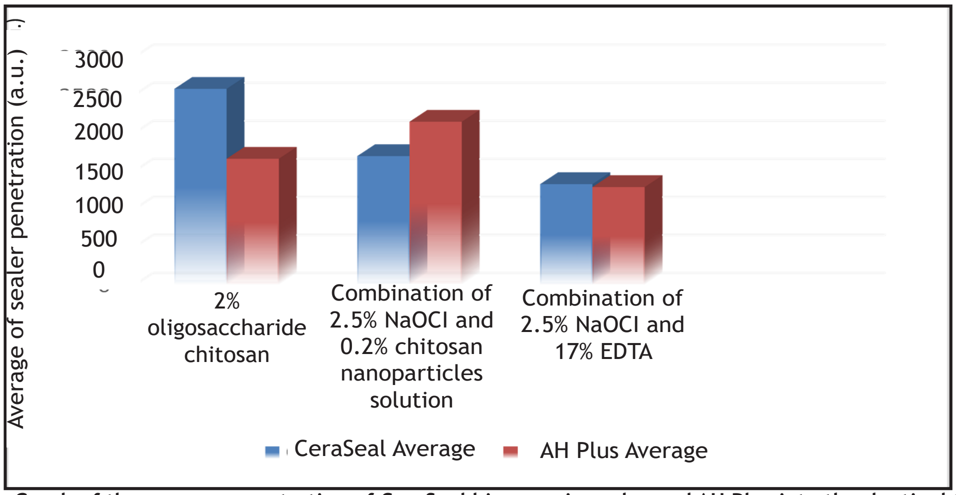

Figure 2. Graph of the average penetration of CeraSeal bioceramic sealer and AH Plus into the dentinal tubules

Table 1. One-way ANOVA test result

Sealer	Group	Average± SD (a.u.)*	p-value	Description
CeraSeal	2% oligosaccharide chitosan solution	2575.151 ± 793.390	0.001	There is a difference
	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	1691.317 ± 741.494		
	Combination of 2.5% NaOCl and 17% EDTA	1322.563 ± 306.579		
AH Plus	2% oligosaccharide chitosan solution	1656,533 ± 222,841	0.000	There is a difference
	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	2142,847 ± 138,340		
	Combination of 2.5% NaOCl and 17% EDTA	1283.920 ± 617,297		

**a.u.: Arbitrary Unit*

The average depth and standard deviation of CeraSeal bioceramic and AH Plus sealer penetration in each sample will be obtained from the CLSM analysis results. The graph of the mean and standard deviation of the penetration of CeraSeal bioceramic sealer into the dentinal tubules shows that the irrigant with 2% oligosaccharide chitosan solution occupies the highest position, followed by the combination of 2.5% NaOCl irrigant and 0.2% chitosan nanoparticle solution, and the lowest is a combination of 2.5% NaOCl and 17% EDTA. On the other hand, the penetration depth of the AH Plus sealer is highest in 2.5% NaOCl irrigant and 0.2% chitosan nanoparticle solution, then followed by 2% chitosan oligosaccharide solution in second place, and 2.5% NaOCl irrigant and 17% EDTA in third place showing the lowest penetration depth same as CeraSeal penetration.

The normality and homogeneity test showed that the data was normally distributed and homogenous, respectively. Therefore, the data in this study were analyzed using ANOVA test to see the significant difference between all irrigants on the penetration of CeraSeal and AH Plus sealers. The results of the ANOVA test can be seen in table 1. From the results of the One-way ANOVA test, a p-value <0.05 was obtained for both CeraSeal and AH Plus, which means that there is a significant difference between all of the irrigants on the penetration of CeraSeal and AH Plus into the dentinal tubules. Then, a follow-up test was carried out with the Post Hoc LSD test to see the difference between irrigation materials using the same sealer. The results of the Post Hoc LSD test can be seen in table 2.

Based on the results of the Post Hoc LSD test, it can be seen that there are differences in each irrigant in the penetration of CeraSeal and AH Plus, except for the combination of 2.5% NaOCl irrigant and 0.2% chitosan nanoparticle solution with a combination of 2.5% NaOCl irrigant and 17% EDTA against CeraSeal penetration

Table 2. LSD post hoc test results

Sealer	Treatment group		p-value	Description
CeraSeal	2% oligosaccharide chitosan solution	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	0.003	There is a difference
		Combination of 2.5% NaOCl and 17% EDTA	0.000	There is a difference
	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	2% Oligosaccharide chitosan solution	0.003	There is a difference
		Combination of 2.5% NaOCl and 17% EDTA	0.258	No difference
	Combination of 2.5% NaOCl and 17% EDTA	2% Oligosaccharide chitosan solution	0.000	There is a difference
		Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	0.258	No difference
AH Plus	2% oligosaccharide chitosan solution	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	0.009	There is a difference
		Combination of 2.5% NaOCl and17% EDTA	0.041	There is a difference
	Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	2% oligosaccharide chitosan solution	0.009	There is a difference
		Combination of 2.5% NaOCl and 17% EDTA	0.000	There is a difference
	Combination of 2.5% NaOCl and 17% EDTA	2% oligosaccharide chitosan solution	0.041	There is a difference
		Combination of 2.5% NaOCl and 0.2% chitosan nanoparticles solution	0.000	There is a difference

DISCUSSION

The process of cleaning and shaping the root canal will determine the level of disinfection and the ability of the obturation material to fill the canals in the root canal. The use of irrigation material is expected to be able to clean the root canal from the smear layer and smear plug, increase the adaptation of the obturation material to the dentin wall of the root canal, and penetrate the sealer further into the dentinal tubules.[14] The results of this study indicate that 2% chitosan oligosaccharide solution irrigation has an effect on increasing sealer penetration into dentinal tubules, both in bioceramic sealers and resin-based sealers.

Irrigation material with 2% oligosaccharide chitosan solution resulted in the highest penetration in the Ceraseal bioceramic sealer, while in the AH Plus sealer the 2% oligosaccharide chitosan solution took second position after the combination of 2.5% NaOCl and 0.2% chitosan nanoparticle solution. This is because the depth of penetration of the sealer is influenced by many factors, especially the cleanliness of the smear layer and the nature of the sealer used. The smear layer present in the root canal will reduce the adhesion and penetration of the sealer, so the more the smear layer is removed, the better the penetration of the sealer and adaptation of the obturation material with increasing the permeability of dentin.[9]

Solubility of chitosan oligosaccharides is influenced by molecular weight and the degree of deacetylation. The more acetyl groups released or the greater quantity of free amide active groups (-NH2) present in the depolymerized chitosan molecule, will increase the degree of deacetylation of chitosan.[14] The increase in the degree of deacetylation has an effect on reducing the molecular weight of chitosan which causes a lower viscosity so that chitosan oligosaccharides have a higher solubility in neutral solutions.[15] This leads to the advantages of chitosan oligosaccharides when used as irrigation materials, which can more easily enter the dentinal tubules in the apical third to dissolve the smear layer and smear plug through the chelating effect of chitosan oligosaccharides. It is known that the efficiency of chelating agents can be influenced by several factors including application time, concentration and volume of irrigation solution.[17]

The combination of 2.5% NaOCl and 17% EDTA irrigation materials resulted in the lowest penetration of both sealers. This is probably due to the low molecular weight NaOCl which is able to penetrate the collagen that is covered by the apatite matrix, causing loss of organic matter from the mineralized dentin. When EDTA is used, the chelating nature of EDTA can dissolve the inorganic components of dentin resulting in the exposure of the dentin structure that was previously degraded by NaOCl. As a result of the damaged dentin structure, the density and adaptation of the sealer material into the dentinal tubules was reduced and the hybrid layer on the AH Plus sealer was damaged.[1] not only dissolves inorganic structures in the smear layer, EDTA also dissolves the calcium hydroxyapatite matrix of dentin which can cause dentin collagen structure denaturation, thereby preventing optimal penetration of the sealer.[3]

In addition, the chelating ability of chitosan is also better than EDTA in the apical third region, due to the 0.2% chitosan nanoparticle solution's smaller particle size and the high solubility of chitosan oligosaccharides, the consequent adsorption and penetration impact was farther into the dentinal tubules, as a result there will be a lot of smear layer and smear plug dissolved.[5] Hosseini, et al.,[17] reported that the penetration of chitosan nanoparticles into the root canal was more effective than EDTA and NaOCl.[17] In CeraSeal bioceramic sealer, another possible cause is the sealer bond with dentin is reduced when the bioceramic sealer release calcium during setting.

EDTA has a chelating effect that binds calcium, residual EDTA can bind the calcium so that it will reduce the bond strength of the bioceramic sealer to the dentin walls of the root canals.[19] However, statistically there was no significant difference between the combination of 2.5% NaOCl irrigant with 0.2% chitosan nanoparticle solution and the combination of 2.5% NaOCl irrigant with 17% EDTA solution on the penetration of bioceramic sealer. This is in line with the research conducted by Ayu and Trimurni which showed that there was no significant difference between the combination of 2.5% NaOCl and 0.2% chitosan in its ability to remove the smear layer when compared to the combination of 2.5% NAOCL and EDTA. 17%.[7]

The nature of the sealer that is used also plays an important role in addition to the ability of the irrigating material to remove the smear layer, where a sealer with a good flow rate makes it possible to fill difficult-to-access areas such as lateral root canals, fins, and isthmus. Bioceramic sealer has excellent flowability and ability to release Ca^{2+} ions and is also better than other sealer materials. Bioceramic sealer contains calcium phosphate silicate that has very small particles (<1 m) that is hydrophilic and this sealer can enter the lateral root canals and has a chemical composition and structure similar to the structure of hydroxyapatite in teeth, thereby increasing the bond between sealer with root canal walls.[13,18] This means that bioceramic *sealer* can induce bioactive activity when in contact with the tissue, namely the interaction of calcium hydroxide produced by the hydration process of tricalcium silicate and phosphate in the tissue fluid to form a mineral infiltration zone.[19,20]

Sealer AH Plus is hydrophobic, radiopaque, and has a high binding capacity and flowability.[3] AH Plus works on dentin through the bonds that form a hybrid layer on the dentin collagen fibrils. However, the hydrophobic nature of AH Plus can prevent bonding, especially in the apical third, because the area is not completely dry. The particle size of AH Plus which is 1.5-8 μm makes it difficult to enter the very small dentinal tubules. In addition, the AH Plus sealer undergoes shrinkage during hardening, thereby facilitating microleakage between the sealer material and the dentinal surface.[13,21] This theory is in line with the research conducted by Asawaworarit et al., which stated that the adaptability in the apical third of the AH Plus sealer was lower than the EndoSequence BC sealer.[22] In this study it is also proven that the penetration of bioceramic sealer was higher than AH Plus.

In this study, the irrigation technique was carried out using a positive-pressure irrigation system. This study used an irrigation needle with a 30 G one-side vented design followed by activation using an EndoActivator. The positive pressure irrigation system has a disadvantage of its less effectiveness in delivering irrigating material to the apical third of the root canal. So it is necessary to activate the EndoActivator to increase the effectiveness of the irrigating agent, especially in the apical third because it increases infiltration into the smallest canal area, so that the irrigant can work optimally.[1] At the time of root canal obturation, obturation material is expected to provide adequate closure of all communication pathways of the root canal system with the periradicular tissue.[21]

Obturation in this study used a single cone technique on the bioceramic sealer and the lateral condensation technique on the AH Plus sealer. Other factors that can affect the depth of penetration of the sealer are dentinal permeability, the number and size of dentinal tubules, and the anatomy of the root canal system.[23] These factors also affect differences in the results of the penetration of each irrigating material of the bioceramic sealer and AH Plus sealer. Irrigation material should meet several requirements to become an ideal irrigation material, because this research is limited to knowing the effect of chitosan oligosaccharide irrigant on the penetration of root canal sealers, it is necessary to do further research such as evaluating the antibacterial effect of chitosan oligosaccharide irrigant on bacteria of the root canal, and examining the cleanliness of the dentin surface of the root canal after irrigation with oligosaccharide chitosan. In addition, it is also necessary to research the combination of irrigation between oligosaccharide chitosan irrigant and other irrigation materials to see the optimal effect so that it meets the requirements as an ideal irrigation material.

CONCLUSION

The 2% oligosaccharide chitosan irrigant shows good properties in increasing the penetration of bioceramic sealers and resin-containing sealers into the dentinal tubules, and that oligosaccharide chitosan can be developed as a root canal irrigant. Bioceramic sealer has better dentinal tubule penetration than AH Plus due to its smaller particle size. It also has hydrophilic nature which can diffuse into the dentinal tubules up to the apical third, and contains calcium silicate which is similar to apatite crystals in teeth.

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