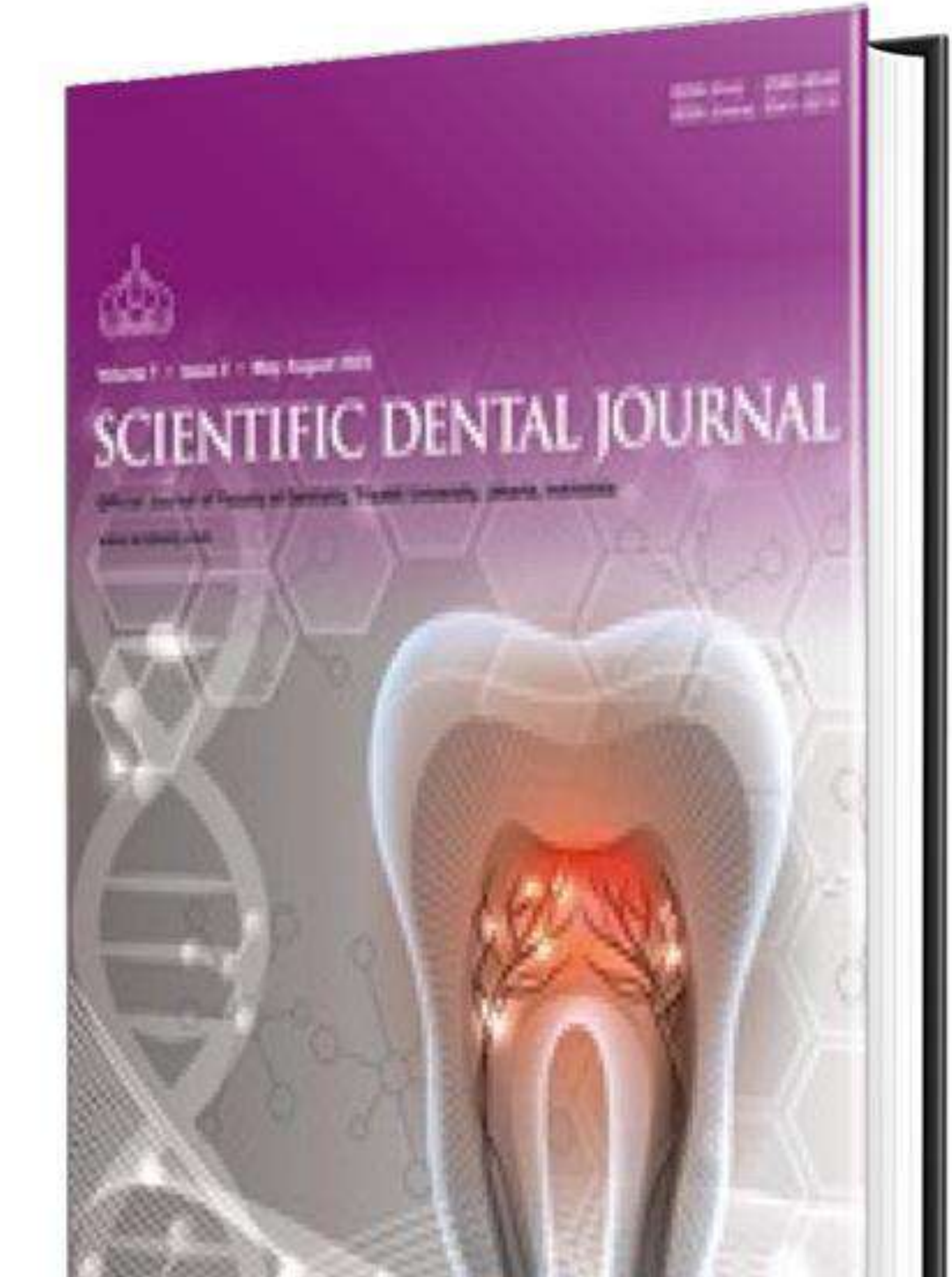
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The Capability of Three Final Irrigation Materials on apical leakage of Bioceramic Sealer Obturation after Calcium Hydroxide Cleaning

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Abstract

Background: Obturation is an important step in root canal treatment. Obturation has to result in a tight root canal closure and eliminate all apical and coronal connection pathways. Calcium hydroxide intracanal medicament residue left behind may interfere with the endodontic sealer penetration, and cause an apical leakage. Chitosan acetate 0,2% has been shown to have antibacterial effects and can remove the smear layer. **Objective:** To determine the effectiveness of different types of final irrigation on the apical seal of bioceramic sealer obturation after calcium hydroxide intracanal medicament cleaning. **Method:** The study is performed on a sample of lower first premolar teeth that have been prepared and filled with calcium hydroxide intracanal medicament paste. One week later, calcium hydroxide removal is performed with NaOCl. The samples were divided into 3 groups based on the final irrigation material prior to obturation: group 1 was irrigated with 2.5% NaOCl, group 2 with 17% EDTA, and group 3 with 0.2% chitosan acetate. All samples were obturated using the single cone technique and bioceramic sealer and then immersed in Indiana ink. Apical leakage was measured based on the deepest penetration of the dye into the root canal. **Results:** The smallest apical leakage value was the final irrigation group with 0.2% Chitosan Acetate, and the apical leakage value of the 2.5% NaOCl group was the same as 17% EDTA, but there was no statistically significant difference between the three study groups. **Conclusion:** There is no difference in the effectiveness of final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of bioceramic sealer obturation after calcium hydroxide removal. Chitosan-acetate 0.2% can be used as an alternative as a final irrigation material for root canals after calcium hydroxide intracanal medicament, in obturation using a bioceramic sealer.

Keywords: Chitosan acetate, EDTA, Bioceramic sealer, Ca(OH)2, Apical Leakage

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Background

The primary goal of endodontic treatment is to remove as much bacteria from the root canal system as possible before establishing a setting in which the remaining organisms are unable to survive.[1] Obturation is an important phase in root canal therapy.

It should remove all apical and coronal connection paths and create a tight root canal seal.[2] Poorly hermetic obturation of the root canal is the most common cause of root canal therapy failure.

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The apical of the root canal must be sealed tightly to prevent harmful bacteria from percolating into and out of it.[2] Periapical disease is mainly caused by microorganisms that remain in the root canal. Although mechanical and chemical root canal cleaning methods have improved over time, no method has yet been able to eliminate all bacteria from the root canal system. As a result, when pulp necrosis or root canal re-treatment occurs, intracanal medications are applied to inactivate the bacteria that remain in the root canal system.[1,3]

Calcium hydroxide (Ca(OH)2) is a commonly used material as an intracanal medicament in endodontic treatment due to its biological ability, antimicrobial activity, and ability to inactivate bacterial endotoxins.[1,3] However, numerous studies on the removal of intracanal medications that contained Ca(OH)2 using chemical and mechanical methods have shown that there is no effective method of completely removing calcium hydroxide from the root canal system. Incomplete Ca (OH)2 removal could affect a root canal treatment's long-term prognosis, which could also significantly impact bonding and sealing.[3]

Obturation of the root canal aims to stop root canal reinfection by forming an adequate seal against bacteria reinfection and their toxin. Solid core material and sealer were the two types of materials employed for canal obturation. Various kinds of sealer base materials exist, such as sealers based on silicone, methacrylate, calcium hydroxide, glass ionomer cement, epoxy resin, and zinc oxide eugenol (ZnOE). Manufacturers claim that these sealers are insoluble, radiopaque, aluminum-free, and water-hardenable. The hydrophilic nature of this particular sealer gives it the ability to slightly expand after hardening, enhancing sealing against the root canal wall.[3] However, previous studies have shown that the number of microleakages in canals filled with bioceramic materials and resins increases with the presence of Ca(OH)2 intracanal medicament left behind.[1,3]

The most common technique for removing calcium hydroxide is using a primary apical file combined with irrigation using a 2.5-5% sodium hypochlorite (NaOCl) solution followed by 17% ethylenediaminetetraacetic acid (EDTA).[4,5] NaOCl has the advantage of being antibacterial, proteolytic, and able to dissolve organic tissue. However, it cannot remove inorganic tissue from the smear layer on the root canal wall. In addition, it is toxic to tissues, corrosive, and has a strong odor.6 EDTA is frequently used in the clinic and as the gold standard for final irrigation solutions due to its ability to bind to dentine calcium ions, and dissolve inorganic components of the smear layer. However, prolonged application time of EDTA on the dentine wall may cause erosion and reduce dentine microhardness. In addition, EDTA does not have antibacterial properties.[2,7,8]

Recently, chitosan has been widely researched and utilized in dentistry due to its biocompatibility, biodegradability, bioadhesion, and non-toxic properties. Chitosan is a polysaccharide from chitin, which is obtained from the shells of crustaceans and shrimp through a process called deacetylation.[2,9] Chitosan has been used in various fields, such as food, cosmetics, biomedicine, and pharmaceuticals. The use of chitosan as an irrigator in the field of dental research is particularly interesting because of the substance's ability to bind (chelate) various metal ions, such as the treatment of dentine tubule infections, direct pulp capping, and in tissue regeneration in pulp injuries.10–13 Previous studies have shown that chitosan has high antibacterial properties against Enterococcus faecalis and Candida albicans. Chitosan has a chelation ability, so it can dissolve the smear layer. [2,14,15]

Root canal irrigation with chitosan acetate on the dentine surface can remove the smear layer and open the dentine tubules with little erosion of the peritubular dentine.[16] Chitosan is an effective chelation material with little change to the root dentine and can be considered a less invasive substitute than 17% EDTA.[17] Previous study showed that the apical seal and bond strength of epoxy sealer after final irrigation using 0.5% chitosan nanoparticle solution was created by dissolving 0.5 grams of chitosan powder in 1% acetic acid was equivalent to final irrigation with 17% EDTA. An application time of 3 min resulted in better apical sealing and push-out bond strength than an application time of 1 min.

Previous studies have shown that microleakage in canals obturated with epoxy and bioceramic resins increases in root canals previously treated with Ca(OH)2 intracanal medicament. With the increasing use of large tapering file systems and single cone gutta percha obturation techniques using bioceramic sealer, it is necessary to study the effectiveness of final irrigation with chitosan-acetate on the apical sealing of bioceramic sealer obturation after calcium hydroxide intracanal medicament removal.

Material and Methods

This study is a laboratory experimental with post-treatment with control carried out in the Microbiology Center of Research and Education (MiCore) laboratory at the Teaching Dental and Oral Hospital (RSGM-P), Faculty of Dentistry, Universitas Trisakti, Indonesia from November to December 2022. This study used a sample of mandibular premolar teeth after extraction of orthodontic treatment with a total of thirty-four teeth taken by random sampling from forty teeth that have been collected. The thirty teeth became the experimental sample group for the final irrigation with each group having ten samples. These samples have been calculated using Lwanga and

Lemeshow's formula concerning the bioceramic sealer apical seal study using the clearing tooth technique that was previously carried out.19 The four teeth that served as the control group in this study were divided into positive and negative control groups.

The sample inclusion criteria in this study were teeth that were perfectly formed, with: no caries, no calcification, had never had prior root canal treatment, had straight roots, completed grown roots, had the apical size of no more than #20. The sample exclusion criteria were teeth that had more than one root canal and apical foramen, open apex or immature teeth, internal/external resorption, caries/cracks/fractures on the root surface, and/or root canal curvature of more than 10°.[20]

Preparation of 0,2% chitosan acetate

Chitosan acetate (CA) was prepared from shrimp shell chitosan (CHIMULTIGUNA Indonesia, medical grade DDA 96%). The acetic acid solution was prepared with 99 ml of distilled water and 1 ml of 100% acetic acid to obtain a 1% acetic acid solution. A total of 0.2 grams of chitosan was then added to 100 mL of the 1% acetic acid solution and stirred using a magnetic stirrer for 2 hours to obtain 0.2% CA.[21]

Preparation of specimens

The collected teeth, according to the inclusion criteria, were randomly selected, and 34 teeth were used in this study. The selected teeth were crowned (decorated) using a diamond disk or a round-end taper to obtain the root length. K-file #10 was inserted into the root canal until it was visible at the main apical foramen, and the working length was determined by subtracting 1 mm from this point.

Root canals were prepared using ProTaper Universal (Dentsply, Maillefer) to F3 (size 30/.06). At each file change, the root canal was apically patency with K-file #10 and the root canal was irrigated with 2 mL of 2.5% NaOCl. The final rinse used 1 mL of 5% NaOCl for 60 seconds and 1 mL of 17% EDTA for 60 seconds. Irrigation with a working length of 12 mm, which is 2 mm from the apical foramen with in-out movements, ended with 5 mL of distilled water flushing. The specimen was dried using a paper point and given a calcium hydroxide intracanal medicament.[20,22]

Calcium hydroxide application

In this study, 32 tooth samples were filled with an intracanal medicament calcium hydroxide paste. Using a plastic plate and tip provided by the manufacturer, the sample's complete root canal was filled with Ca(OH)2 paste (Calcipex, Japan), with the coronal portion reaching up to 2 mm from the orifice. The coronal area was then temporarily sealed (to avoid leakage) using zinc oxide eugenol cement. For seven days, tooth samples were kept in an incubator set to 37 °C with 100% humidity by being wrapped in cotton that had been soaked in saline.3

Calcium hydroxide removal

After seven days, the specimens were removed from the incubator, and the Ca(OH)2 paste in the root canal was removed with K-file #30 to the working length. The procedure was followed a series of root canal irrigation with 5 mL of 2.5% NaOCl, rinsed with 5 mL of distilled water, and dried with paper points. The 30 tooth samples were randomly grouped into 3 groups and each group received a different final irrigation treatment:

a) Group 1: Teeth samples were irrigated with 1 mL of 2.5% NaOCl for 1 minute.
b) Group 2: Teeth samples were irrigated with 17% EDTA in 1 mL for 1 minute.
c) Group 3: Teeth samples were irrigated with 1 mL of CA for 1 minute.

Before obturation, the root canal is rinsed with up to 5 mL of distilled water and dried with a paper point.

Obturation of root canals

Thirty teeth samples were obturated with hydraulic compaction single cone technique. A bioceramic sealer (Ceraceal; Meta Biomed, Korea) was applied to the root canal wall using a lentulo instrument with clockwise movement. Gutta-percha was coated at the apical third with the bioceramic sealer and placed in the root canal to the working length. Gutta-percha was cut and condensed to 2 mm below the orifice and then covered with an ionomer cement barrier. Mesiodistal and bucco-lingual radiographs were taken to confirm obturation. Specimens were wrapped in moist cotton and stored in a 37oC incubator at 100% humidity for 72 hours.[20]

All samples were coated with clear fingernail polish twice, except for the apical 2 mm. All tooth samples were immersed in Indian ink for 72 hours in the same vertical position to ensure homogeneous hydrostatic pressure on all samples. After completion, the samples were washed under running water for 10 minutes and the nail polish layer was removed. Positive controls in this study were 2 teeth that had been filled with calcium hydroxide, but no removal was performed. The negative controls were 2 teeth that were obturated with bioceramic sealer without prior application of calcium hydroxide intracanal medicament and the entire apical portion was covered with clear fingernail polish.23

Clearing Technique

Tooth samples were cleared based on a technique described in other publications.24 All tooth samples were demineralized using 5% nitric acid in a sealed bottle for 72 hours. The acid was changed every 24 hours, and the bottle was agitated occasionally.

Demineralized teeth were rinsed using running water and then immersed in distilled water for 6 hours, with the liquid changed every hour. All of the specimens were dehydrated by successive immersion in 70% alcohol solution for 24 hours,

95% alcohol for 2 hours, and 100% alcohol for 2 hours. Demineralized tooth samples were air spray dried and then cleared with methyl salicylate.[24]

Measurement of apical leakage

Using a stereomicroscope with a 10x magnification and a scale of mm, apical leakage in the sample was calculated based on the penetration of black ink in the space between the sealer and the root canal wall. The data gathered is the mean of the measurement data made by two calibrated observers.

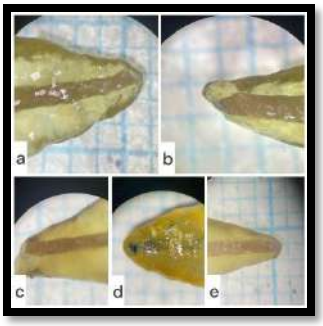

Figure 1. (a) Apical leakage from EDTA 17% group (b) Apical leakage from NaOCl 2,5% group (c) Apical leakage from CA 0,2% group (d) Positive control (e) Negative control

Statistical Analysis

Apical leakage data obtained from the results of this study were tested for normality using the Safiro-Wilk Test. Statistical calculations were performed with SPSS Statistics for Windows software version 25 (IBM, USA). The results showed that the data were not normally distributed ($p<0.05$). Therefore, a different test analysis was performed using the Krusskal Wallis Non-parametric test analysis to determine the difference in the apical seal of obturation using bioceramic sealer in all final irrigation treatment groups, which are: (1) 2.5% NaOCl final irrigation group, (2) 17% EDTA, and (3) 0.2% CA, with a significance level of $p < 0.05$.

Results

In this study, the effectiveness of the final irrigation solution was tested on the apical leakage of root canals that had previously beegiven calcium hydroxide intracanal medicaments and obturated with bioceramic sealer. The results of this study showed that the average value of leakage of NaOCl 2.5% was the same as EDTA 17% (0.03 + 0.04), and the smallest value of leakage was 0.2% CA solution (0.02 + 0.04). (Table 1)

The normality test used the Shapiro-Wilk test. The results showed that the leakage value data of the three test materials were not normally distributed ($p < 0.05$). Therefore, the hypothesis test continued with Kruskall-Wallis non-parametric test.

Based on the Kruskal Wallis test results, the mean rank value showed that NaOCl and EDTA have slightly higher leakage than CA. Considering that the significant value was higher than 0.05, it can be stated that there is no difference in the final irrigation effectiveness between EDTA and chitosan-acetate.(Table 2)

Discussion

The apical seal of the root canal is influenced by the nature of the obturation material used and the cleanliness of the root canal material before obturation.25 Calcium hydroxide has a high pH so it is mostly used for root canal intracanal medicaments. However, calcium hydroxide is difficult to remove completely from the root canal which may lead to microleakage in the long term. This study aims to determine the apical seal of obturation using bioceramic sealer after calcium hydroxide removal with three groups of irrigation materials, namely NaOCl 2.5%, EDTA 17%, and CA 0.2%. On observation under a stereomicroscope with 10 times magnification, there was no clear penetration of the dye in all samples, therefore the magnification was increased to 40 times. Measurements were taken by two observers with three repetitions each. Based on the results of the analysis, it was found that there was no difference in the effectiveness of final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of obturation of bioceramic sealer after calcium

Table.1 Mean and Standard Deviation of Apical Leakage

Group	N	Mean (mm) $\pm$ SD
NaOCl 2,5%	10	0,03 $\pm$ 0,04
EDTA 17%	10	0,03 $\pm$ 0,04
CA 0,2%	10	0,02 $\pm$ 0,04

Table.2 Comparison of the three irrigation groups, analyzed with Kruskal Wallis test (Sig>0,05).

Group	N	Mean	Sig.
Final irrigation using NaOCI 2.5%	10	16,00	
Final irrigation using EDTA 17%	10	16,00	0.848
Final irrigation using CA 0.2%	10	14,50	

hydroxide removal. The smallest mean value of apical leakage was the group with final irrigation with 0.2% CA. The apical leakage value of the 2.5%

NaOCl group was similar to that of 17% EDTA, but there was no statistically significant difference in the apical leakage value among the three groups.

In this study, the 2.5% NaOCl solution group was used to determine the leakage that occurred in the group that did not use chelation material during the final irrigation before obturation. Based on the study of Basrani, et al., NaOCl irrigation is recommended before using bioceramic materials. This research showed that the apical seal of the 2.5% NaOCl irrigation group was equivalent to the group using 17% EDTA chelation material. In root canal obturation using bioceramic sealer, the results of research by Yildirim, et al. and Torabinejad, et al. reported that the presence of a smear layer slightly increased the bond strength of bioceramic sealer to the root canal wall. The moisture contained in the smear layer has been shown to be the same as a hydrophilic bioceramic-based sealer adapted to the dentine and act as a bonding agent to the root canal dentine. 16

The group of teeth that were irrigated with 17% EDTA showed the same leakage value as in the group irrigated with 2.5% NaOCl. The results of this study are different from the results of study by Eman, et al., Dr. Prateek, et al., and Doaa, et al. who conducted research without preceding the application of calcium hydroxide. Their studies showed that obturation leakage after final irrigation with EDTA was smaller than with NaOCl. EDTA solution can reduce apical leakage because it is more effective in cleaning the smear layer in root canal dentine and allows the penetration of bioceramic silica in the dentineal tubules to improve the mechanical retention of the silica and the bonding strength of the silica to the root canal dentine.26–28 However, the results of research by Ariani, et al. showed that the apical leakage value after irrigation with EDTA was greater than that of NaOCl 2.5%. This might be because EDTA only dissolves inorganic particles, leaving the organic particles in the root canal. In addition, EDTA also dissolves dentine, causing erosion of peritubular and intratubular dentine, leading to irregularity of dentine structure and thus reducing the seal and adaptation between sealer and dentine. This theory was also proven in a previous study stating that EDTA causes dentine erosion through demineralization and excessive opening of dentinal tubules.29,30

Chitosan is a hydrophilic polymer that adsorbs into root dentine and allows it to penetrate deeper into the dentinal tubules. It consists of higher amounts of hydroxyl and amino groups, which make it cationic in nature, allowing ionic interaction with calcium ions from dentine. Following previous studies, a chitosan acetate solution at 0.2% concentration showed its efficiency in removing the smear layer at this low concentration with minimal dentine erosion.12,31

The results of this study showed that the mean leakage value of 0.2% CA solution was smaller than the NaOCl 2.5% and EDTA 17% groups. These results are supported by the study of Veeramachaneni, et al. which showed that the use of 0.2% chitosan as the final irrigation in bioceramic sealer obturation showed good apical seal. Chitosan is an effective chelating agent with less alteration to the root dentine and can be considered a less invasive substitute for 17% EDTA. Antunes, et al. reported that 0.2% chitosan on the dentine surface removed the smear layer and unblocked the dentine tubules with little erosion of the peritubular dentine.5 Based on the research of Medhat, et al. the apical seal of bioceramic sealer after the use of 0.2% CA as an irrigation solution is slightly better than the use of EDTA.32

In this study, a single cone obturation procedure with a bioceramic sealer was used. Previous studies have suggested using the single cone approach for applying bioceramic sealer. Due to the bioceramic sealer's slow hardening period, which gives it more time to expand, the sealer is pushed closer to the dentine wall in the tooth root canal.28,33 The results of this study proved that the minimum leakage in the three tested groups was due to the bioceramic sealer (Ceraseal) having a good chemical attachment to the dentine wall. The mechanism of the hardening reaction of bioceramic-based sealer starts by absorbing water from the dentineal tubules. The freshly mixed bioceramic sealer releases high amounts of Si^{4+}, Ca^{++}, and OH^- ions during hardening. This reaction produces the hydrogel calcium silicate and hydroxyapatite. The calcium silicate hydrogel chemically bonds with hydroxyapatite through hydroxyl groups. Then, the hydroxyapatite in the sealer undergoes a continuous crystal growth process, and both sealer compounds form a strong chemical bond with dentine. The sealer also flows within the dentinal tubules without shrinkage during hardening, resulting in less micro-leakage on the apical.34,35 Previous studies have shown that bioceramic sealers do not shrink during the setting process because they do not contain monomers, thus providing a good sealing capability between the sealer and the root canal's dentine walls.36

Most studies on the push-out bond strength after calcium hydroxide treatment are relevant to the quality of obturation. However, the clinical importance of push-out bond strength values' effects on the efficacy of endodontic treatment is not entirely understood.37 Some studies states that the presence of calcium hydroxide can reduce the push-out bond strength of sealer, but other research states that it has no effect. This may be due to differences in research methods.

Previous studies have shown that the amount of root canal microleakage with bioceramic sealer increases in the presence of Ca(OH)2.3 However, in this study, the apical leakage was very small. This study is in concordance with the results of push-out bond strength studies of bioceramic sealer after calcium hydroxide application conducted by Hegde, et al., Amin, et al., Akcay, et al., and Ustun, et al. Their result proved that calcium hydroxide had no significant effect on the push out bond strength of various root canal sealer even though those studies used different methods. Ustun et al. used 1% NaOCl irrigation with ultrasonic agitation, while Amin et al. used passive ultrasonic irrigation with 2.5% NaOCl and final irrigation with EDTA 17.38–41

These results are also supported by previous studies which showed that residual Ca(OH)2 can increase the bond strength of bioceramic sealer and improve the release resistance of calcium silicate-based sealer. Residual calcium hydroxide also improves the marginal adaptation of MTA.39,42 It is assumed that the Ca(OH)2 residue can chemically interact with the bioceramic sealer and positively affect the apical seal in this study. The results of this study are different from those by Araghi et al. and Ghabraei et al., which stated that intracanal medicament Ca(OH)2 had a negative effect on the apical seal of bioceramic sealer.3,37

In this study, bioceramic sealer obturation was performed after Ca(OH)2 removal. Prior to obturation, the final irrigation was conducted using NaOCl, EDTA, and CA irrigation materials which resulted in minimal leakage. Other factors may affect the apical seal, such as volume and flow rate during irrigation. The size of the needle diameter can influence the flow rate of irrigation. Therefore, using needles with a smaller diameter is preferred because it increases the flow rate and shear stress on the root canal walls.43,44

vIn this study, a manual irrigation technique was used with a 30G side-vented and close-ended irrigation needle, as suggested in previous studies. The needle was given a stopper 2 mm shorter than the apex and moved in and out at a speed of 5 mL/minute. This movement produces apical pressure with shear stress on the root canal wall to produce better cleanliness and does not cause injury to periapical tissues.45,46 The results of Ghabraei, et al. showed no significant difference in the effectiveness of Ca(OH)2 removal using manual and ultrasonic files. Based on observation by Scanning Electron Microscope, neither technique could remove all Ca(OH)2 from the root canal wall, but the Ca(OH)2 left after ultrasonic cleaning was less.37

The positive control group in this study was a dental root canal filled with calcium hydroxide intracanal medicament. The results showed that Indiana ink dye could penetrate root canals filled with calcium hydroxide 6 mm and 7 mm apically. This suggests that calcium hydroxide intracanal medicament is an unstable and soluble material, leaving gaps for the dye to penetrate. The results of this study showed that there is no effect of different types of final irrigation materials after calcium hydroxide cleaning towards the apical seal on obturation of bioceramic-based sealer.

The primary limitation of the study is that it is challenging to calculate the amount of leakage because all groups' leakage during bioceramic obturation is very small and almost unseen under a stereomicroscope with a 10 times magnification. However, according to various researchers, the dye penetration technique is still regarded as reliable to identify the micro-leakage rate; however, micro-computed tomography would provide a more precise result.3 The limitation of this study may be that the number of samples is still small so that the standard deviation of the measurement results is high. Further study on the effect of calcium hydroxide cleaning with various solutions and irrigation techniques needs to be carried out to obtain the apical seal of root canal obturation on various types of sealer so that the success of root canal treatment will increase.

Conclusion

The results of this study can be concluded that there is no difference in the effectiveness of the final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of bioceramic sealer obturation after cleaning the calcium hydroxide intracanal medicament. The smallest apical leakage value is the final irrigation group with CA 0.2%, so that it is recommended as a final irrigation material for root canals after calcium hydroxide intracanal medicament application in obturation using a bioceramic sealer.

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Conflict of Interest

The authors declare that there are no conflicts of interest

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The Capability of Three Final Irrigation Materials on apical leakage of Bioceramic Sealer Obturation after Calcium Hydroxide Cleaning

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Original Articles

The Capability of Three Final Irrigation Materials on apical leakage of Bioceramic Sealer Obturation after Calcium Hydroxide Cleaning

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ABSTRACT

Background: Obturation is an important step in root canal treatment. Obturation has to result in a tight root canal closure and eliminate all apical and coronal connection pathways. Calcium hydroxide intramedicament residue left behind may interfere with the endodontic sealer penetration, and cause an apical leakage. Chitosan acetate 0.2% has been shown to have antibacterial effects and can remove the smear layer. **Objective**: To determine the effectiveness of different types of final irrigation on the apical seal of bioceramic sealer obturation after calcium hydroxide intramedicament cleaning. Method: The study is performed on a sample of lower first premolar teeth that have been prepared and filled with calcium hydroxide intramedicament paste. One week later, calcium hydroxide removal is performed with NaOCl. The samples were divided into 3 groups based on the final irrigation material before obturation: group 1 was irrigated with 2.5% NaOCl, group 2 with 17% EDTA, and group 3 with 0.2% chitosan acetate. All samples have been obturated using the single cone technique and bioceramic sealer, then immersed in Indiana ink. Apical leakage was measured based on the deepest penetration of the dye into the root canal. Results: The smallest apical leakage value was the final irrigation group with 0.2% Chitosan Acetate, and the apical leakage value of the 2.5% NaOCl group was the same as 17% EDTA, but there was no statistically significant difference between the three study groups. Conclusion: There is no difference in the effectiveness of final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of bioceramic sealer obturation after calcium hydroxide removal. Chitosan-acetate 0.2% can be used as an alternative as a final irrigation material for root canals after calcium hydroxide intramedicament, in obturation using a bioceramic sealer.

Keywords: Chitosan acetate, EDTA, Bioceramic sealer, $Ca(OH)_2$, Apical Leakage

Background

The primary goal of endodontic treatment is to remove as much bacteria from the root canal system as possible before establishing a setting in which the remaining organism are unable to survive.[1] Obturation is an important phase in the procedure of root canal therapy. Obturation should remove all apical and coronal connection paths and create a tight root canal seal.[2] Poorly hermetic obturation of the root canal is the most common cause of root canal therapy failure. To prevent harmful bacteria from percolating into and out of the root canal, the apical of the root canal is required to be sealed tightly.[2] The periapical disease is mainly brought on by microorganisms that remain in the root canal. Even though mechanical and chemical root canal cleaning methods have improved over time, no method has yet been able to eliminate all bacteria from the root canal system. As a result, when pulp necrosis or root canal re-treatment occurs, intracanal medications are applied to inactivate the bacteria that remain in the root canal system.[1,3]

Calcium hydroxide ($Ca(OH)_2$) is a commonly used material as an intracanal medicament in endodontic treatment due to its biological ability, antimicrobial activity, and ability to inactivate bacterial endotoxins.[1,3] However, numerous research on the removal of intracanal medications that contained $Ca(OH)_2$ using chemical and mechanical methods have shown that there is no effective method of completely removing calcium hydroxide from the root canal system. A root canal treatment's long-term prognosis could be affected by incomplete $Ca(OH)_2$ removal, which could also significantly impact bonding and sealing.[3]

Obturation of the root canal aims to stop root canal reinfection by forming an adequate seal against bacteria reinfection and their toxin. Solid core material and sealer were the two types of materials employed for canal obturation. There are a lot of various kinds of sealer base materials that exist at present, such as sealers based on silicone, methacrylate, calcium hydroxide, glass ionomer cement, epoxy resin, and zinc oxide eugenol (ZnOE). Manufacturers claim that these sealers are insoluble, radiopaque, aluminum-free, and water-hardenable. The hydrophilic nature of this particular sealer gives it the ability to slightly expand after hardening, enhancing sealing against the root canal wall.[3] However, previous research has shown that the number of microleakage in canals filled with bioceramic materials and resins increases with the presence of $Ca(OH)_2$ intramedicament left behind.[1,3]

The most common technique used to remove calcium hydroxide is using a primary apical file combined with irrigation using 2.5-5% sodium hypochlorite (NaOCl) solution followed by 17% ethylenediaminetetraacetic acid (EDTA).[4,5] NaOCl has the advantage of being antibacterial, proteolytic and can dissolve organic tissue. However, NaOCl is unable to remove inorganic tissue from the smear layer on the root canal wall. In addition, NaOCl is toxic to tissues, corrosive, and has a strong odor.[6] EDTA is frequently used in the clinic and as the gold standard for final irrigation solutions due to its ability to bind to dentine calcium ions, and dissolve inorganic components of the smear layer. However, prolonged application time of EDTA on the dentine wall may cause erosion and reduce dentine microhardness. In addition, EDTA does not have antibacterial properties.[2,7,8]

Recently, chitosan has been widely researched and utilized in the field of dentistry due to its biocompatibility, biodegradability, bioadhesion, and non-toxic properties. Chitosan is a polysaccharide from chitin, which is obtained from the shells of crustaceans and shrimp, through a process called deacetylation.[2,9] Chitosan has been used in various fields such as food, cosmetics, biomedicine, and pharmaceuticals. In acidic conditions, chitosan research as an irrigator in the field of dental research is particularly interesting because of the substance's ability to bind (chelate) various metal ions, such as the treatment of dentine tubule infections, direct pulp capping and in tissue regeneration in pulp injuries.[10-13] On previous studies have shown that chitosan has high antibacterial properties against Enterococcus faecalis and Candida albicans. Chitosan has a chelation ability so that it can dissolve the smear layer.[2,14,15]

Root canal irrigation with chitosan acetate on the dentine surface can remove the smear layer and open the dentine tubules with little erosion of the peritubular dentine.[16] Chitosan is an effective chelation material with little change to the root dentine and can be considered a less invasive substitute than 17% EDTA.[17] On previous research showed that the apical seal and bond strength of epoxy sealer after final irrigation using 0.5% chitosan nanoparticle solution was created by dissolving 0.5 grams of chitosan powder in 1% acetic acid was equivalent to final irrigation with 17% EDTA. An application time of 3 min resulted in greater apical sealing and push-out bond strength than an application time of 1 min.[17]

Previous studies have shown that microleakage in canals obturated with epoxy and bioceramic resins increases in root canals previously treated with $Ca(OH)_2$ intramedicament. With the increasing use of large tapering file systems and single cone guta perca obturation techniques using bioceramic sealer, it is necessary to study the effectiveness of final irrigation with chitosan-acetate on the apical sealing of bioceramic sealer obturation after calcium hydroxide intramedicament removal.

Material and Methods

This research is a laboratory experimental with post-treatment with control carried out in the Microbiology Center of Research and Education (MiCore) laboratory at the Teaching Dental and Oral Hospital (RSGM-P), Faculty of Dentistry, Trisakti University,
West Jakarta, Indonesia from November to December 2022. This study used a sample of mandibular premolar teeth after extraction of orthodontic treatment with a total of thirty-four teeth taken by random sampling from forty teeth that have been collected. The thirty teeth became the experimental sample group for the final irrigation with each group having ten samples, These samples have been calculated by Lwanga and Lemeshow's formula concerning the bioceramic sealer apical seal research using the clearing tooth technique that had been carried out previously.[19] The four teeth become the control group in this research that divide into two groups control, which are positive control and negative control.

The sample inclusion criteria in this study were teeth that were perfectly formed, had no caries, no calcification, had never had root canal treatment before, had straight roots, the roots of the teeth had grown completely, the apical size was no more than #20 and sample exclusion criteria were teeth that had more than one root canal and apical foramen, teeth that had been treated with root canals, open apex immature teeth, internal/external resorption, caries/cracks/fractures on the root surface, and/or root canal curvature of more than 10°.[20]

Preparation of 0,2% chitosan acetate

Chitosan acetate (CA) prepared from shrimp shell chitosan (CHIMULTIGUNA Indonesia, *medical grade* DDA 96%). Preparation of acetic acid solution with 99 ml of distilled water and 1 ml of 100% acetic acid to obtain 1% acetic acid solution. A total of 0.2 grams of chitosan was then added to 100 mL of 1% acetic acid solution and stirred using a magnetic stirrer for 2 hours to obtain 0.2% CA.[21]

Preparation of specimens

The collected teeth according to the inclusion criteria were randomly selected 34 teeth to be used in this study. The selected teeth were crowned (decorated) using a diamond disk or a round-end taper to obtain the root length. K-file #10 was inserted into the root canal until it was visible at the main apical foramen, and the working length was determined by subtracting 1 mm from this size.

Root canals were prepared using ProTaper Universal (Dentsply, Maillefer) to F3 (size 30/.06). At each file change, the root canal was apically patency with K-file #10 and the root canal was irrigated with 2 mL of 2.5% NaOCl. The final rinse used 1 mL of 5% NaOCl for 60 seconds and 1 mL of 17% EDTA for 60 seconds. Irrigation with a working length of 12 mm, which is 2 mm from the apical foramen with in-out movements, ended with 5 mL of distilled water flushing. The specimen was dried using a paper point and given a calcium hydroxide intramedicament.[20,22]

Calcium hydroxide application

In this research, 32 tooth samples were filled with an intramedicament calcium hydroxide paste. Using a plastic plate and tip provided by the manufacturer, the sample's complete root canal was filled with $Ca(OH)_2$ paste (Calcipex, Japan), with the coronal portion of the paste reaching up to 2 mm from the orifice. The coronal area was then temporarily sealed (to avoid leakage) using zinc oxide eugenol cement. For seven days, tooth samples were kept in an incubator set to 37 °C with 100% humidity by being wrapped in cotton that had been soaked in saline.[3]

Calcium hydroxide removal

After seven days, the specimens were removed from the incubator, and the $Ca(OH)_2$ paste in the root canal was removed with K-file #30 to the working length, then the root canal was irrigated with 5 mL of 2.5% NaOCl, rinsed with 5 mL of distilled water and dried with paper points. The 30 tooth samples were randomly grouped into 3 groups and each group received a different final irrigation treatment:

a) Group 1: Teeth samples were finally irrigated with 1 mL of 2.5% NaOCl for 1 minute.
b) Group 2: Teeth samples were finally irrigated with 17% EDTA in 1 mL for 1 minute.
c) Group 3: Teeth samples were finally irrigated with 1 mL of CA for 1 minute.

Before obturation, the root canal is rinsed with as much as 5 mL of distilled water and dried with a paper point.

Obturation of root canals

Thirty teeth samples were obturated with hydraulic compaction single cone technique. A bioceramic sealer (Ceraceal; Meta Biomed, Korea) was applied to the root canal wall using a lentulo with clockwise movement. Guttapercha was coated at the apical third with the bioceramic sealer and placed in the root canal to the working length. Gutta-percha was cut and condensed to 2 mm below the orifice and then covered with an ionomer cement barrier. Mesiodistal and bucco-lingual radiographs were taken to confirm obturation. Specimens were wrapped in moist cotton and stored in a 37°C incubator at 100% humidity for 72 hours.[20]

All samples were coated with clear fingernail polish for two times, except for the apical 2 mm. Each coat waited for the fingernail polish to dry. All tooth samples were immersed in Indian ink for 72 hours in the same vertical position to ensure homogeneous hydrostatic pressure on all samples. After completion, the samples were washed under running water for 10 minutes and the nail polish layer was removed. As positive controls in this study were 2 teeth that had been filled with calcium hydroxide but no removal was performed. The negative controls were 2 teeth that were obturated with bioceramic sealer without prior application of calcium hydroxide intramedicament and the entire apical portion was covered with clear fingernail polish.[23]

Clearing Technique

Tooth samples were cleared based on a clearing technique similar to that used by previous researcher Hosoya.[24] All tooth samples were demineralized using 5% nitric acid in a sealed bottle for 72 hours. The acid was changed every 24 hours and the bottle was agitated occasionally. Demineralized teeth were rinsed using running water, then immersed in distilled water for 6 hours, the liquid was changed every hour.

All the specimens were dehydrated by successive immersion in 70% alcohol solution for 24 hours, 95% alcohol for 2 hours, and 100% alcohol for 2 hours. demineralized tooth samples were air spray dried and then cleared with methyl salicylate.[24]

Measurement of apical leakage

Using a stereomicroscope with a 10x magnification and a scale of mm, apical leakage in the sample was calculated based on the penetration of black ink in the space between the sealer and the root canal wall. The data gathered is the mean of the data made by two calibrated observers.

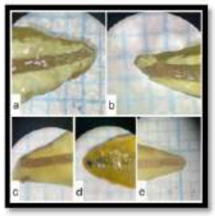

Figure 1. (a) Apical leakage from EDTA 17% group (b) Apical leakage from NaOCl 2,5% group (c) Apical leakage from CA 0,2% group (d) Positive control (e) Negative control

Statistical Analysis

Apical leakage data obtained from the results of this study were tested for normality using the Safiro-Wilk Test. Statistical calculations were performed with SPSS Statistics for Windows software version 25 (IBM, USA). The results showed that the data were not normally distributed ($p<0.05$). Therefore, a different test analysis was performed using the Krusskal Wallis Non-parametric test analysis to determine the difference in the apical seal of obturation using bioceramic sealer in all final irrigation treatment groups, which are: (1) 2.5% NaOCl final irrigation group, (2) 17% EDTA, and (3) 0.2% CA, with a significance level of $p < 0.05$.

Results

In this research, the effectiveness of the final irrigation solution was tested on the apical leakage of root canals that had previously beegiven calcium hydroxide intramedicaments and obturated with bioceramic sealer. The results of this study showed that the average value of leakage of NaOCl 2.5% was the same as EDTA 17% (0.03 ± 0.04), and the smallest value of leakage was 0.2% CA solution (0.02 ± 0.04). (Table 1)

The normality test used the Shapiro-Wilk test. The results showed that the leakage value data of the three test materials were not normally distributed ($p < 0.05$). Therefore, the hypothesis test continued with Kruskall-Wallis non-parametric test.

Table.1 Mean and Standard Deviation of Apical Leakage

Group	N	Mean (mm) ± SD
NaOCl 2,5%	10	0,03 ± 0,04
EDTA 17%	10	0,03 ± 0,04
CA 0,2%	10	0,02 ± 0,04

Based on the Krusskall Wallis test results, the mean value obtained by final irrigation using NaOCl 2.5% (16.00), EDTA (16.00) and CA 0.2% (14.50) which means that NaOCl and EDTA have slightly higher leakage than CA, Krusskall Wallis test results obtained a sig. value of 0.848, that the value p> 0.05. Based on the Sig. value, it can be stated that after cleaning calcium hydroxide intramedicament, there is no difference in the final irrigation effectiveness between EDTA and chitosan-acetate.(Table 2)

Table 2. Kruskall Wallis Test

Group	N	Mean	Sig.
Final irrigation using NaOCl 2.5%	10	16,00	
Final irrigation using EDTA 17%	10	16,00	0.848
Final irrigation using CA 0.2%	10	14,50	

Sig>0,05

Discussion

The apical seal of the canal is influenced by the nature of the obturation material used and the cleanliness of the root canal material before obturation.[25] Calcium hydroxide has a high pH so it is mostly used for root canal intramedicaments. However, calcium hydroxide is difficult to remove completely from the root canal, it's hicnder the penetration of the sealer into the dentine tubules of the root canal and reducing the sealer to the canal wall. This may lead to microleakage in the long term.

This research aims to determine the apical seal of obturation using bioceramic sealer after calcium hydroxide removal with three groups of irrigation materials namely NaOCl 2.5, EDTA 17%, and CA 0.2%. On observation under a stereomicroscope with 10 times magnification, there was no clear penetration of the dye in all samples, therefore the magnification was increased to 40 times. Measurements were taken by two observers with three repetitions each. Based on the results of the analysis, it was found that there was no difference in the effectiveness of final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of obturation of bioceramic sealer after calcium hydroxide removal. The smallest mean value of apical leakage was the group with final irrigation with 0.2% CA. The apical leakage value of the 2.5% NaOCl group was similar to that of 17% EDTA, but there was no statistically significant difference in the apical leakage value among the three groups.

In this research, the 2.5% NaOCl solution group was used to determine the leakage that occurred in the group that did not use chelation material during the final irrigation before obturation. Based on the research of Basrani, et al. NaOCl irrigation is recommended to be used before bioceramic materials. The results of this research showed that the apical seal of the 2.5% NaOCl irrigation group was equivalent to the group using 17% EDTA chelation material. In root canal obturation using bioceramic sealer, the results of research by Yildirim, et al. and Torabinejad, et al. reported that the presence of a smear layer slightly increased the bond strength of bioceramic sealer to the root canal wall. The moisture contained in the smear layer has been shown to be the same as a hydrophilic bioceramic-based sealer to adapt to dentine and act as a bonding agent between root canal dentine. [16]

The group of teeth that were final irrigated with 17% EDTA, in this study showed the same leakage value as the leakage in the group that was final irrigated with 2.5% NaOCl. The results of this study are different from the results of research by Eman, et al., Dr. Prateek, et al., and Doaa, et al. who conducted research without preceding the application of calcium hydroxide, showing that obturation leakage after final irrigation with EDTA was smaller than with NaOCl. EDTA solution can reduce apical leakage because EDTA is more effective in cleaning the smear layer in root canal dentine and allows the penetration of bioceramic silica in the dentineal tubules to improve the mechanical retention of the silica and the bonding strength of the silica to the root canal dentine.[26–28] However, the results of research by Ariani, et al. showed that the apical leakage value after final irrigation with EDTA was greater than that of NaOCl 2.5%. This can be caused because EDTA only dissolves inorganic particles so that organic particles are still left in the root canal. In addition, EDTA also dissolves dentine, causing erosion of peritubular and intratubular dentine, leading to irregularity of dentine structure and thus reducing the seal and adaptation between sealer and dentine. This theory was also proven in a previous study which said that EDTA causes erosion of dentine through the process of demineralization and excessive opening of dentineal tubules.[29,30]

Chitosan is a hydrophilic polymer that adsorbs into root dentine and allows it to penetrate deeper into the dentineal tubules. Chitosan consists of higher amounts of hydroxyl and amino groups which make it cationic in nature, allowing ionic interaction with calcium ions from dentine. Chitosan acetate solution at 0.2% concentration following previous studies showed its efficiency in removing the smear layer at this low concentration with minimal dentine erosion.[12,31]

The results of this study showed that the mean leakage value of 0.2% CA solution was smaller than the NaOCl 2.5% and EDTA 17% groups. These results are supported by the research of Veeramachaneni, et al. which showed that the use of 0.2% chitosan as the final irrigation in bioceramic sealer obturationshowed good apical seal. Chitosan is an effective chelating agent with less alteration to the root dentine and can be considered a less invasive substitute for 17% EDTA. Antunes, et al. reported that 0.2% chitosan on the dentine surface removed the smear layer and unblocked the dentine tubules with little erosion of the peritubular dentine.[5] Based on the research of Medhat, et al. the apical seal of bioceramic sealer after the use of 0.2% CA as an irrigation solution is slightly better than the use of EDTA.[32]

In this research, a single cone obturation procedure with a bioceramic sealer was the method used. The single cone approach has been suggested by previous research to be used for applying bioceramic sealer. Due to the bioceramic sealer's slow hardening period, which gives it more time to expand, the sealer is pushed closer to the dentine wall in the tooth root canal.[28,33]

The results of this study proved that the minimum leakage in the three tested groups was due to the bioceramic sealer (Ceraseal) having a good chemical attachment to the dentine wall.
The mechanism of the hardening reaction of bioceramic-based sealer starts by absorbing water from the dentineal tubules. The freshly mixed bioceramic sealer releases high amounts of Si4+, Ca++, and OH- ions during hardening. This reaction produces the hydrogel calcium silicate and hydroxyapatite. The calcium silicate hydrogel chemically bonds with hydroxyapatite through hydroxyl groups, then the hydroxyapatite in the sealer undergoes a continuous crystal growth process and both sealer compounds form a strong chemical bond with dentine. The sealer also flows within the dentineal tubules without shrinkage during hardening, resulting in less micro-leakage on the apical.[34,35] Previous studies have shown that bioceramic sealers do not shrink during the setting process because they do not contain monomers, thus providing a good sealing capability between the sealer and the dentinee walls of the root canal.[36]

Most research studies on the push-out bond strength after calcium hydroxide treatment are relevant to the quality of obturation. The clinical importance of push-out bond strength values' effects on the efficacy of endodontic treatment, however, is not entirely understood.[37] Some research states that the presence of calcium hydroxide can reduce the push-out bond strength of sealer, but other research states that it has no effect. This may be due to differences in research methods.

Previous research has shown that the amount of root canal microleakage with bioceramic sealer increases in the presence of $Ca(OH)_2$.[3] However, in this research, the apical leakage was very small. This research reinforces the results of push-out bond strength studies of bioceramic sealer after calcium hydroxide application conducted by Hegde, et al., Amin, et al., Akcay, et al., and Ustun, et al. which proved that calcium hydroxide had no significant effect on the push out bond strength of various root canal sealer even though the studies used different methods. Ustun et al. used 1% NaOCl irrigation with ultrasonic agitation, Amin et al. used passive ultrasonic irrigation with 2.5% NaOCl and final irrigation with EDTA 17.[38–41]

These results are also supported by previous studies showing that residual $Ca(OH)_2$ can increase the bond strength of bioceramic sealer and improve the release resistance of calcium silicate-based sealer. Residual calcium hydroxide also improves the marginal adaptation of MTA.[39,42] It is assumed that the residue of $Ca(OH)_2$ can chemically interact with the bioceramic sealer and have a positive effect on the apical seal in this study. The results of this research are different from the results of research by Araghi, et al. and Ghabraei, et al. who stated that intramedicament $Ca(OH)_2$ had a negative effect on the apical seal of bioceramic sealer.[3,37]

In this research, obturation using bioceramic sealer was performed after $Ca(OH)_2$ removal, before obturation the final irrigation was performed using NaOCl, EDTA, and CA irrigation materials which resulted in minimal leakage. There may be other factors that affect the apical seal, such as volume and flow rate during irrigation. The flow rate of irrigation can be influenced by the size of the needle diameter. The use of needles with a smaller size diameter is preferred because it increases the flow rate and shear stress on the root canal walls.[43,44]

In this study, a manual irrigation technique was used with a 30G side-vented and close-ended irrigation needle as suggested in previous research. The needle was given a stopper 2 mm shorter than the apex and moved in and out at a speed of 5 mL/minute. This movement produces apical pressure with shear stress on the root canal wall so that it can produce better root canal wall cleanliness and does not cause injury to periapical tissues.[45,46] The results of Ghabraei, et al. showed that there was no significant difference in the effectiveness of $Ca(OH)_2$ removal using manual and ultrasonic files. Based on observation by Scanning Electron Microscope, both techniques could not remove all $Ca(OH)_2$ from the root canal wall, but the $Ca(OH)_2$ left after ultrasonic cleaning was less.[37]

The positive control group in this study was a dental root canal filled with calcium hydroxide intramedicament. The results showed that Indiana ink dye could penetrate root canals filled with calcium hydroxide 6 mm and 7 mm apically. This suggests that calcium hydroxide intramedicament is an unstable and soluble material, leaving gaps for the dye to penetrate.

The primary weakness of the research is that it is challenging to calculate the amount of leakage because all groups' leakage during bioceramic obturation is very small and almost unseen under a stereomicroscope with a 10 times magnification. According to various researchers, the dye penetration technique is still regarded as helping identify the micro-leakage rate, however, micro-computed tomography would provide a more precise result.[3]

The results of this research show that there is no effect of different types of final irrigation materials after calcium hydroxide cleaning towards the apical seal on obturation of bioceramic-based sealer Further research on the effect of calcium hydroxide cleaning with various solutions and irrigation techniques needs to be carried out to obtain the apical seal of root canal obturation on various types of sealer so that the success of root canal treatment will increase.

Conclusion

The results of this research can be concluded that there is no difference in the effectiveness of the final irrigation between EDTA 17% and chitosan-acetate 0.2% on the apical seal of bioceramic sealer obturation after cleaning calcium hydroxide intramedicament. The smallest apical leakage value is the final irrigation group with CA 0.2% so that it can be used as an alternative as a final irrigation material for root canals after calcium hydroxide intramedicament application in obturation using a bioceramic sealer.

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Conflict of Interest

The authors declare that there are no conflicts of interest

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