Flowability Of Nanoparticles Of Calcium Hydroxide Palimanan In Dentinal Tubule

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ABSTRACT

Background: *Intracanal medicaments $Ca(OH)_2$ must have the ability to contact directly with bacteria adhering to the dentinal tubules. However, the narrow and complex shape of the root canal makes it difficult for $Ca(OH)_2$ to penetrate the root canal apically. This study aimed to evaluate the flowability of $Ca(OH)_2$ nanoparticle synthesized from $CaCO_3$ source from natural limestone in Palimanan, Cirebon, West Java, Indonesia with an average particle size of 278 nm by quantifying the penetration of the coronal, middle, and apical thirds of the root canal.*
Methods: *This research comprised two distinct groups: one group included nanoparticles of $Ca(OH)_2$ Palimanan, while the other consisted of conventional $Ca(OH)_2$. The paste was then applied to standardized root canals (n=3 per group) with 5 times measurements for each sample. All samples were then incubated at 37°C, 100% humidity. The flowability of the root canals was measured using scanning electron microscopy (SEM) on day 14. The data was analyzed using ANOVA and a post-hoc t-test, with a significance level of $p<0.05$.*
Results: *The use of $Ca(OH)_2$ Palimanan has been found to be more effective in reaching deeper into the dentinal tubules of the root canal, particularly in the coronal, middle, and apical areas, compared to traditional $Ca(OH)_2$. This occurs because the particle size of $Ca(OH)_2$ Palimanan is smaller with a more rounded shape than conventional $Ca(OH)_2$ so that it to flow into narrow and complex areas, especially in the apical root canals.*
Conclusion: *Nanoparticle $Ca(OH)_2$ Palimanan flow deeper than conventional $Ca(OH)_2$*

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INTRODUCTION

Root canal treatment is an option for treating infections that develop in the inflamed periapical region. Root canal treatment success is determined by three major stages: biomechanical preparation, sterilization, and root canal filling.[1] Cleaning and shaping the root canal to remove all pathogens down to the periapical area is part of biomechanical preparation. The act of removing harmful microorganisms and debris from within the root canal by utilizing irrigation solutions and intracanal medications is what is referred to as disinfection.[2,3] However, since bacteria can enter the dentinal tubules and live for long periods in a starving environment, disinfection of the root canal is critical for treatment. *Enterococcus faecalis* has been recognized as the main contributor to the failure of root canal treatments.[4]

The average size of the dentinal tubules is 2-5 µm, while *E. faecalis* has a diameter of 0.5-1.00 µm allowing it to penetrate the dentinal tubules as deep as 50-100 µm. Therefore, a medicament must have sufficient flowability to penetrate the dentinal tubules. The size of the particles can have an impact on their flowability. In general, smaller particles are more spherical in shape, which enables them to penetrate more deeply into the dentinal tubules and enhance the antimicrobial effect.[5] Bacteria were discovered to have penetrated the dentinal tubules, with the highest concentration of bacteria being found in the coronal third of the root canal (81%), followed by the middle third (57%), and the apical third (47%). Notably, even after ultrasonic sterilization, bacteria were still present in the tubules, with the highest percentage being found in the coronal third of the root canal.[6,7]

Commonly used in endodontic treatment for root canal filling is calcium hydroxide ($Ca(OH)_2$). The use of intracanal medication aims to alleviate periradicular inflammation and discomfort, curb bacterial growth, thwart reinfection of the root canal, and function as a barrier against leakage in temporary restoration. To be effective, $Ca(OH)_2$ needs to penetrate the dentinal tubules and make direct contact with microorganisms.[8,9] In previous research Palimanan limestone ($CaCO_3$) has been successfully synthesized into $Ca(OH)_2$ with nanoparticle size (279,4±17,0 nm).[10,11] Therefore, this research aims to measure the penetration ability of nanoparticles $Ca(OH)_2$ Palimanan on dentinal tubules in the coronal, middle, and apical thirds.

RESEARCH METHOD

This study used a controlled experimental design to evaluate the flowability of $Ca(OH)_2$ nanoparticles synthesized from CaCO3 from Palimanan, West Java. Ethical clearance was obtained from the Institutional Review Board (IRB) Komite Etik Penelitian Universitas Padjadjaran number 956/UN6.KEP/EC/2021. A total of six mandibular first premolars with one root canal, without fracture and caries, and completely formed apices were obtained from extraction due to periodontal abnormalities and orthodontic purposes. The samples were divided into two groups: Nanoparticle $Ca(OH)_2$ Palimanan synthesized like previous method[10] (n=3) and conventional $Ca(OH)_2$ (Merck, Germany) (n=3). All the specimens were stored at 100% humidity during the experimental period. The samples were cut at their crowns using carborundum discs to establish a standardized root canal length of 14 millimeters. The root canals were prepared using a rotary instrument with a working length of 13 millimeters in the crown-down technique, starting with files XS-F3 (VDW Silver Reciproc, Germany), Rotary instruments (Protaper Gold Sterile Files Assortment, Dentsply, Germany) with irrigation NaOCl 2.5%, NaCl and EDTA 17% as lubrication every file. Final irrigation to remove the smear layer was performed using 5 ml of 2.5% NaOCl for 3 min, and finally 5 ml of saline solution (0.9% NaCl) was dried using paper points. The proportion of intracanal medicament was considered ideal, with the consistency of the paste being homogeneous 0.8 gr/ml. The paste was injected into the standardized root canal using a disposable syringe (3 ml until the orifices. The orifices were then covered with a flowable resin composite (3M ESPE Filtek Z350 XT, USA) and placed in a tube for incubation (JEIO TECH BW-10E Water Bath, South Korea) at 37°C and 100% humidity for 14 d.

The surface of the sample was notched vertically with a diamond disc bur, and the root was separated into two halves using a needle holder. Afterward, the tooth was divided into three equal segments: the coronal third, the middle third, and the apical third. The extent to which the $Ca(OH)_2$ found in Palimanan and conventional $Ca(OH)_2$ penetrated the dentinal tubules was assessed using a Scanning Electron Microscope (SEM) (JEOL JSM IT300). All data were then tabulated in the form of mean and standard deviation, and a one-way ANOVA test was performed to compare three sections of the root canal (coronal 1/3 crown, middle 1/3, and apical 1/3) in each group. Post hoc test to compare the depth of penetration of $Ca(OH)_2$ Palimanan and conventional $Ca(OH)_2$ nanoparticles in the same section using an independent t-test.

RESULTS

The particle sizes of the Ca (OH) 2 Palimanan and conventional Ca (OH) 2 nanoparticles were determined using SEM. As illustrated in **Figure 1**, the results show that the particle sizes varied.

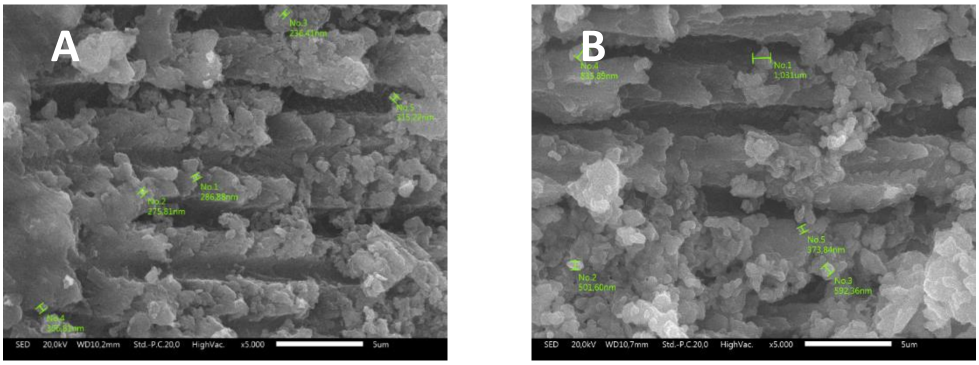

Figure 1. Size of nanoparticles $Ca(OH)_2$ Palimanan (A) and conventional $Ca(OH)_2$ in dentinal tubules (5000x magnification)

The average size of $Ca(OH)_2$ Palimanan nanoparticles ranged from 237 µm to 357 µm with an average of 294.31 µm. Meanwhile, for conventional $Ca(OH)_2$ measurements ranging from 374 µm to 1,031 µm with an average of 666.93 µm. While the results of the $Ca(OH)_2$ Palimanan nanoparticle sizes varied in size as shown in **Table 1**. Data using the T-Independent test showed that the size of the $Ca(OH)_2$ Palimanan and conventional $Ca(OH)_2$ nanoparticles had a significant difference p = 0.033.

Table 1. Size of $Ca(OH)_2$ in dentinal tubules.

	Nanoparticle $Ca(OH)_2$ Palimanan (µm)	**Conventional $Ca(OH)_2$ (µm)**	**P-value**
Mean (SD)	294,31 (44,85)	666,94 (264,52)	0,033*
Min-Max	237 - 357	374-1031	

(*) significance

According to the results of morphological analysis of $Ca(OH)_2$ using SEM, nanoparticles $Ca(OH)_2$ Palimanan have a more rounded particle shape than conventional $Ca(OH)_2$, as shown in **Figure 2**. The particle shapes of the two groups are illustrated below.

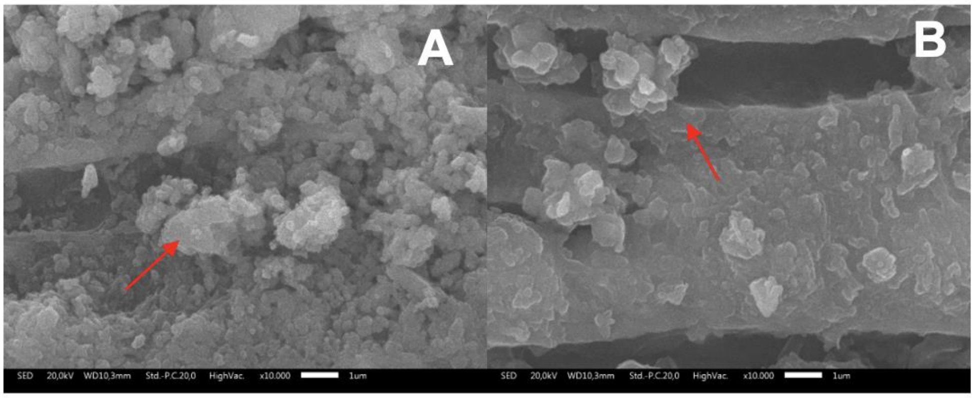

Figure 2. SEM characterization results of $Ca(OH)_2$ in dentinal tubules conventional $Ca(OH)_2$ (A) and nanoparticles $Ca(OH)_2$ Palimanan (B)

The maximum penetration depth of nanoparticles $Ca(OH)_2$ Palimanan and conventional $Ca(OH)_2$ was measured in µm using SEM by measuring the maximum depth of $Ca(OH)_2$ particles in the dentinal tubules calculated from the edge of the inner dentinal tubules. The measurement results are tabulated in **Table 2.** The mean and standard deviation of the two groups were measured in the coronal, middle and apical third parts.

Table 2. Comparison of nanoparticle $Ca(OH)_2$ Palimanan with conventional $Ca(OH)_2$

Groups	Nanoparticle $Ca(OH)_2$ Palimanan	Conventional $Ca(OH)_2$	P-Value
Coronal	356,08	296,9	0,007*
Middle	362,4	209	0,006*
Apical	388,1	190,2	0,000*

*: significance

The data in Table 2. show comparison of nanoparticle with conventional $Ca(OH)_2$ using the T-Independent test showed that between groups of $Ca(OH)_2$ Palimanan nanoparticles and conventional $Ca(OH)_2$ there was a significant difference p value <0.05. In the coronal part of $Ca(OH)_2$ Palimanan nanoparticles with conventional $Ca(OH)_2$ there is a significant difference in the yield (0.007 < 0.05), the middle part with the value (0.006 < 0.05) and the apical part (0.000 < 0.05). The following is a comparative **Figure 3**, of the penetration ability of $Ca(OH)_2$ Palimanan and $Ca(OH)_2$ nanoparticles in the dentinal tubules seen in the coronal, middle and apical third.

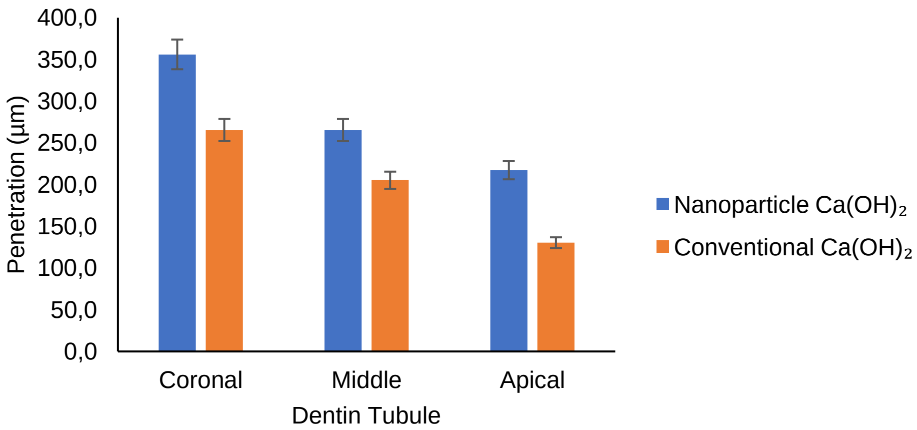

Figure 3. Depth comparison of conventional $Ca(OH)_2$ and nanoparticles $Ca(OH)_2$ Palimanan penetration on dentinal tubules

DISCUSSION

This study compared the penetration ability of nanoparticles $Ca(OH)_2$ Palimanan with conventional $Ca(OH)_2$ in the coronal, middle and apical third of the dentinal tubule. Following ionic dissociation of Ca^{2+} and OH^+ in water, $Ca(OH)_2$ can both induce the formation of hard tissue and have an antimicrobial impact.[12] For a longer antibacterial action in preventing reinfection, the slow dissolution rate of $Ca(OH)_2$ (approximately 1.2 g/L at 25°C) must be maintained.[13]

Particle size is one of the factors that can influence intracanal medicament penetration depth. The smaller the particle size, the easier it is to interact with water, as such small particles produce high flowability. The larger the particle size, the more difficult it is to interact with water, resulting in low flowability.[14] In addition, the particle size will affect the shape of $Ca(OH)_2$, the smaller the particle size, the more rounded the particle shape so that it can penetrate better into the dentinal tubules. Whereas when the particle size increases, the particle shape will become more rectangular which causes less $Ca(OH)_2$ to penetrate the dentinal tubules.[15] Flowability is the ability of medicaments to enter the dentinal tubules, this is related to viscosity. Viscosity is a measure of the consistency of a liquid and its resistance to flow. The lower the viscosity, the higher the ability of $Ca(OH)_2$ to flow, making it easier to penetrate the dentinal tubules.[16] Besides viscosity, several other factors affect flowability, including particle size, particle shape, solvent and W/P ratio.[17] The use of $Ca(OH)_2$ nanoparticle paste mixed with distilled water, propylene glycol, and polyethylene glycol leads to improved flowability compared to conventional $Ca(OH)_2$ paste. This is due to the fact that smaller particle sizes are more easily movable and spreadable than larger particle sizes in conventional $Ca(OH)_2$ paste. Additionally, smaller particle sizes can enhance particle dissolution and minimize unreacted particle residue, which allows $Ca(OH)_2$ to penetrate more deeply into dentinal tubules and increase its antimicrobial activity.[17] The shape of the particles is affected by particle size. As a result, as particle size decreases, particle shape becomes more rounded and can penetrate deeper into the dentinal tubules.[18]

In this study, the average penetration ability of $Ca(OH)_2$ showed that the penetration ability of conventional $Ca(OH)_2$ Palimanan and $Ca(OH)_2$ nanoparticles decreased from 1/3 coronal to 1/3 apical. The SEM characterization results from this study indicated that the smaller particle geometry of the nanoparticle group enables calcium hydroxide particles to penetrate the open dentin tubules. These findings align with previous research, indicating anatomical variations in the number and size of dentinal tubules from the coronal to the apical sections of the root canal. The morphological variability of the dentinal tubules shows a progressive decrease towards the apical region, with tubular lumen areas reducing from 15.47 ± 7.06 μm² in the coronal zone to 12.77 ± 10.23 μm² in the middle zone, and reaching a minimum of 3.033 ± 2.43 μm² in the apical zone.[22]

Another explanation for the penetration results of $Ca(OH)_2$ in the root canal is the complex anatomy of the root canal and the progressive narrowing of the dentinal tubules from the coronal region to the apical part.[19] Dentin tubules are generally considered to have a diameter of 2 to 5 μm.[5] Other influencing factors are the difficulty of intracanal medicaments to penetrate the apical part which has less number of dentinal tubules accompanied by sclerotic dentine, and irregular direction of dentinal tubules and accessory root canals. Endodontic instruments that are difficult to access the apical 1/3 cause the effectiveness of smear layer elimination to decrease so that intracanal medicament penetration is lower in the apical part.[20,21] This is following Giudice, et.al showed that the diameter of the dentinal tubules in the coronal to the apical section has decreased, the coronal section has an average size of 4.324 µm, the middle part is 3.749 µm and 1.731 µm in the apical part. Whereas the area of the dentinal tubules progressively decreased from 15.47 μm^2 in the coronal section, 12.77 μm^2 in the middle and 3.033 μm^2 in the apical section. [22] The number of dentinal tubules increased from 15,000 to 20,000/mm^2 in DEJ and in pulp from 45,000/mm^2 to 65,000/mm^2. In coronal dentin, the average tubule diameter at DEJ is 0.5-0.9 µm but increases by 2-3 µm near the pulp. The diameter of the dentinal tubules in the coronal section has an average size of 4.324 µm, in the middle it is 3.749 µm and 1.731 µm in the apical part.[23]

Furthermore, these results are in line with Zand, that $Ca(OH)_2$ in the form of nanoparticles is superior and significantly penetrates deeper into the dentinal tubules than conventional $Ca(OH)_2$ and shows that conventional $Ca(OH)_2$ particles appear trapped in the orifices. dentinal tubules, due to their rectangular shape. Although the penetration of $Ca(OH)_2$ decreases in the apical portion, this will not be a problem because the penetration of $Ca(OH)_2$ is expected to penetrate deep into the dentinal tubules in the coronal and middle third, based on Harrison showed that the highest percentage of bacteria was in the coronal (81%), followed by the middle (57%) and apical (47%).[24] Even though sterilization has been carried out, bacteria are still present in the dentinal tubules. Therefore $Ca(OH)_2$ must be able to penetrate into the dentinal tubules to come into direct contact with bacteria to produce an antimicrobial effect.[25] More research is needed to assess the ability of intracanal medications to penetrate using other methods. The advantage of measuring penetration using SEM is that it produces very detailed images of the dentinal tubules and allows observation of material within the dentinal tubules in areas remote from the root canal walls. However, the main disadvantage of SEM is that it is difficult to analyze at low magnifications.[9]

CONCLUSION

The nanoparticles $Ca(OH)_2$ Palimanan could penetrate deeper into the dentinal tubules than conventional $Ca(OH)_2$ in all areas of the coronal, middle, and apical third.

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