pH, Calcium and Hydroxyl Ions Released from Nanoparticle Calcium Hydroxide as Intracanal Medicament

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Abstract

Ca(OH)2 has an antimicrobial effect through the dissociation of Ca2+, OH- ion and pH ideally used as an intracanal medicament. Nanoparticle Ca(OH)2 has been successfully obtained from natural limestone in Indonesia. Nanoparticle Ca(OH)2, aquades, propylene glycol (PG), methylcellulose (MS) and barium sulfate (BS) formulations can affect the mechanism of action.

This study aims to determine the effectiveness of the Ca(OH)2 nanoparticle formula as an intracanal medicament through the Ca2+, OH- ion release and pH at the evaluation on days 1, 7, and 14. The method of this study was an experimental study consisting of two groups, the first group is the nanoparticle Ca(OH)2 25%, aquades, PG 3%, MS 2% and BS 35% and Ca(OH)2 Calxipex II® (Nippon Sika-Yakuhin., Shimonoseki, Japan) as the control group. Samples were inserted into polyethylene tubes and immersed with 10 mL of distilled water, incubated at 37°C and 100% humidity. Evaluation of Ca2+, OH- ion release and pH has done with a spectrophotometer (Dirui DR-7000D, China) and a pH meter (Lutron/pH-207, Taiwan). The results showed the Ca2+ ion release in all groups increased to its peak on day 14 (11.29±0.196) mg/dL and (17.52±4,67) mg/dL.

The pH levels of the nanoparticle Ca(OH)2 formula have the same pattern and are increased until day 14. However, this is not the same as measuring OH ion release. The number of OH ions released was highest on day 7 and decreased on day 14. There were significant differences between the two study groups on the measurements of the Ca2+, OH- ion release and pH with different day variables. The commercial Ca(OH)2 group demonstrated significantly higher levels of Ca2+, OH- ion release and pH than the nanoparticle Ca(OH)2 group. This can occur due to several factors that are affected, such as the solvent, powder-liquid ratio, particle size, and contaminations.

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Introduction

Calcium hydroxide $Ca(OH)_2$ is a white powder with a molecular weight of 74.08. Chemically, Ca(OH)2 is classified as an alkaline because it is a strong base (pH 12.5 - 12.8) with a solubility level of 1.2 g/L at 25 ºC, when in contact with a solvent it will dissociate into Ca^{2+} ions and hydroxyl ions (OH^-). $Ca(OH)_2$ is an effective intracanal medicament because it has broad-spectrum antimicrobial properties, is biocompatible with tissues, reduces periradicular inflammation and can stimulate hard tissue [1,2]. As an intracanal medication, $Ca(OH)_2$ is also essential in cases of recurrent periapical tissue infections. The use of $Ca(OH)_2$ as an intracanal

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medicament is well-known due to its antimicrobial effect through the dissociation of Ca^{2+}, and OH^- ion. Furthermore, due to their capacity to generate high pH, which is imparted to the surrounding environment, the biological and antiseptic effects of calcium hydroxide depend on alkalinity and calcium ion release and promote tissue mineralization. Through an irreversible enzymatic process, the high pH of $Ca(OH)_2$ encourages the suppression of microbial growth [2]. Another crucial characteristic of $Ca(OH)_2$ is its capacity to encourage the inactivation of bacterial lipopolysaccharides found in gram-negative outer membrane bacteria [3]. The release of OH^- ions is primarily responsible for the rise in pH in the surrounding tissue when $Ca(OH)_2$ is used as a root canal medicament [4]. One of the most crucial elements that determine how effective they are is the quantity of OH^- ions that are present at the application site.

Due to their extremely small size, nanoparticles have a greater surface-to-volume ratio than bigger particles. As a result, a larger percentage of their atoms are concentrated on their surface, which increases their reactivity and responsiveness to their surroundings [5]. This larger surface area enables better interaction with the environment, which opens up a wide range of applications in disciplines including environmental research and medicine. The use of nanoparticles as antibacterial agents is an important application. Because of their small size and high surface-to-volume ratio, nanoparticles can interact with microbial cells more effectively, resulting in higher antibacterial action. The antibacterial activity of nanoparticles is also greatly influenced by their charge density [6].

The discovery of nanoparticle $Ca(OH)_2$ from natural limestone in Indonesia presents a groundbreaking opportunity for revolutionizing endodontic treatments [7,8]. Despite its promising potential, questions remain regarding the effectiveness of this new formulation when combined with other substances such as aquades, propylene glycol, methylcellulose, and barium sulfate. To fully unlock the potential of this innovative discovery, it is crucial to conduct thorough experimental studies. Therefore, this study aims to evaluate the Ca^{2+}, OH^- ion release and pH of a nanoparticle $Ca(OH)_2$ formula and compare it to a commercial $Ca(OH)_2$ product over 14 days. The results of this study will provide invaluable insights into the potential use of nanoparticle $Ca(OH)_2$ in endodontic treatments and could ultimately pave the way for more effective and efficient treatments for patients.

Materials and methods

This is a true experimental study using $Ca(OH)_2$ as an intracanal medication to evaluate ions Ca^{2+}, OH^-, and pH specifically on days 1, 7, and 14. There were two research groups (Table 1). Commercial $Ca(OH)_2$ paste (Calcipex II®, Nippon Sika-Yakuhin, Shimonoseki, Japan) containing $Ca(OH)_2$ 24% added with $BaSO_4$ 24%; the other group with nanoparticle $Ca(OH)_2$ Palimanan 25% paste added with $BaSO_4$ (Pudak Scientific, Indonesia) 25%; PG (DOW PuraGuard™, USA) 3%; and MS 2%, 10 samples in each measurement group. The nanoparticle $Ca(OH)_2$ powder was manipulated to form a paste and then put into a polyethylene tube with a diameter of 2x1 cm and immersed in a plastic vial filled with 10 mL of distilled water. The container was kept at a temperature of 37°C and a humidity level of 100%. Each sample was measured for the release of Ca^{2+} ions on day 1, day 7, and day 14 using a spectrophotometer (DIRUI DR-7000D, China). The pH measurement uses a pH meter that has previously been calibrated using a buffer solution with a pH of 7. The pH (Lutron / pH-207, Taiwan), measurement process is carried out by inserting the electrode into each solution from the sample group. After obtaining the pH value, the rate of OH ions release was calculated. The numerical data normality test was tested using the Shapiro-Wilks test with a follow-up test using the independent t-test.

Component	Nanoparticle $Ca(OH)_2$ (%wt)	Commercial $Ca(OH)_2$ paste (%wt)
$Ca(OH)_2$	25%	24%
$BaSO_4$	25%	24%
Aquades	45%	52%
Propylene glycol (PG)	3%	-
Methylcellulose (MS)	2%	-

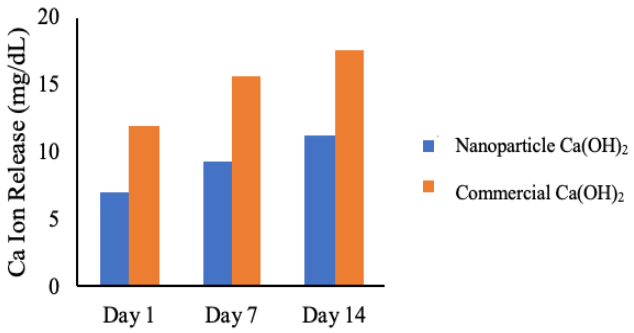
Table 1. Intracanal Medicament Composition.

Results

Ca Ion Release

Graph 1 Illustrates the release of Ca^{2+} ions in the commercial $Ca(OH)_2$ and nanoparticle $Ca(OH)_2$ groups on day 1, day 7, and day 14. The nanoparticle $Ca(OH)_2$ group showed a Ca^{2+}

ion release of (6.97 ± 0.741) mg/dL on day 1, (9.20 ± 0.573) mg/dL on day 7, and (11.29±0.196) mg/dL on day 14. In comparison, the commercial $Ca(OH)_2$ group had a Ca^{2+} ion release of (12.00 ± 1.990) mg/dL on day 1, (15.63 ± 1.257) mg/dL on day 7, and (17.52 ± 4.669) mg/dL on day 14. Both groups gradually released Ca^{2+} ions throughout the 14 days, but the commercial $Ca(OH)_2$ group had a higher and more stable ability to release Ca^{2+} ions over time.



Graph 1. Release of Ca ion Release in commercial $Ca(OH)_2$ and $Ca(OH)_2$ nanoparticle groups.

Ion Release Ca^{2+} (mg/dL)	Groups		P value
	Nanoparticle $Ca(OH)_2$	Commercial $Ca(OH)_2$	
Day 1	6.97±0.741	12.00±1.990	0.001**
Day 7	9.20±0.573	15.63±1.257	0.0001**
Day 14	11.29±0.196	17.52±4.669	0.018**

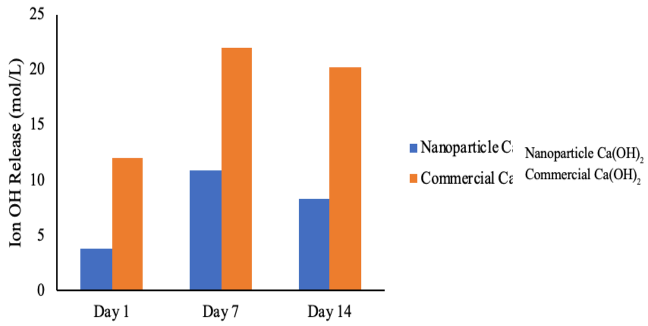
Table 2. Comparison between Ca^{2+} ion release on day 1, day 7, and day 14 in commercial $Ca(OH)_2$ and nanoparticle $Ca(OH)_2$ groups.
*P value <0.05 which means statistically significant.

The average Ca^{2+} ion release was then analyzed using the Shapiro Wilks test as the results for the nanoparticle $Ca(OH)_2$ group obtained $p>0.05$, which means that the data were normally distributed on day 1 and day 14; while on day 7, $p<0.05$ means the data is not normally distributed. The normality test results in the commercial $Ca(OH)_2$ group had P values on day 1, day 7, and day 14 ($p>0.05$), which means that the data were normally distributed as shown in Table 2.

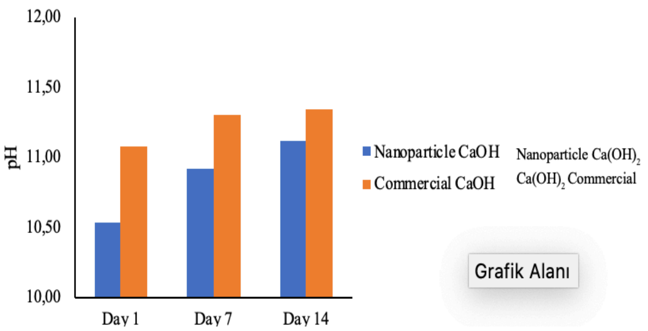
The results of the study indicate that commercial $Ca(OH)_2$ released significantly higher amounts of Ca, hydroxide OH ions, and pH compared to nanoparticle $Ca(OH)_2$ over a period measured on days 1, 3, and 7, across all measurement categories.

Hydroxyl Ion Release

The commercial $Ca(OH)_2$ group had the highest levels of OH^- ion release on day 7 according to Graph 2: Overall, the commercial Ca(OH)2 group had a higher rate of OH ion release than the nanoparticle $Ca(OH)_2$. The release rate of OH ions increased for the commercial $Ca(OH)_2$ and nanoparticle $Ca(OH)_2$ formulations from day 1 to day 7, then it dropped on day 14. The measurements of OH ions in all groups revealed a release rate of OH ions on alkaline pH that was greater than 4.002x10-4 mol/L.



Graph 2. Hydroxyl Ion Release in Nanoparticle and Commercial $Ca(OH)_2$.



Graph 3. pH levels of two different types of $Ca(OH)_2$.

pH Evaluation

Graph 3: shows the pH levels of two different types of $Ca(OH)_2$ over 14 days. The comparison pH levels of three different periods (day 1, day 7, and day 14) between two groups: the nanoparticle $Ca(OH)_2$ group and the commercial $Ca(OH)_2$ group. The nanoparticle $Ca(OH)_2$ group average pH on day 1 was (10.52 ± 0.214), on day 7 it was (10.91 ± 0.316), and on day 14, it was (11.11 ± 0.083). The commercial $Ca(OH)_2$ group average pH on day 1 was (11.07 ± 0.058), on day 7 was (11.29 ± 0.097), and on day 14 was (11.34 ± 0.040).

Discussion

In this study, there were two distinct groups of intracanal medicaments, each possessing unique compositions and formulations. The first group denoted Group 1, composed of nanoparticles $Ca(OH)_2$ obtained from Indonesian limestone, supplemented with propylene glycol (PG) and methylcellulose (MS) as thickening agents to ensure appropriate consistency. Meanwhile, the second group denoted Group 2, consists of Ca(OH) with distilled water as a vehicle. Both groups were further characterized by the incorporation of $BaSO_4$, which imparts a radiopacity, facilitating visualization in clinical settings. The careful characterization of these two groups using advanced techniques and meticulous measurements provides valuable insights into the role of these intracanal medicaments in endodontic practice. The findings of this study may serve as a basis for further research aimed at optimizing the formulation and delivery of intracanal medicaments to achieve enhanced clinical outcomes.

Calcium and hydroxide ions should be able to be released gradually and slowly in the optimum carrier [9]. The proposed vehicles are classified as aqueous and oily [10]. The conductivity of saturated calcium hydroxide solutions in distilled water and propylene glycol may reduce the effectiveness of calcium hydroxide as a root canal medicament [11]. Additionally, the consistency of the PG enhances its handling properties and induces the most favourable release of Ca and hydroxyl ions compared to other vehicles. The sudden release of hydroxyl ions by the commercial $Ca(OH)_2$ group after 7 days can be attributed to the aqueous nature of DW, which promotes rapid ion release. This characteristic makes DW beneficial in clinical situations requiring short-term interappointment disinfection and in cases such as dental replantation and extreme exudative cases [12]. The application of $Ca(OH)_2$ distilled water paste resulted in the faster and more significant release of Ca and OH ions. This difference may be caused by the different methodologies used as well as the addition of barium sulphate in these pastes [13]. The study revealed that the liberation of hydroxyl ions from propylene glycol as a vehicle exhibited its maximum concentration on day 7, with a subsequent steady increase until day 14. This phenomenon can be attributed to the inherently high viscosity and limited solubility of propylene glycol, which lead to a gradual and protracted discharge of the hydroxyl ions [14]. Moreover, the incorporation of calcium hydroxide $(CaOH)_2$ and propylene glycol in medicament intracanal pastes demonstrated an enhancement in the calcium ion release kinetics [15].

The low solubility and poor ability of $BaSO_4$ to diffuse make it difficult for oil paste containing calcium hydroxide compounds to reach maximum pH levels in a short period [16]. The results showed that intracanal medicament should be used for a minimum of 7 days to achieve maximum therapeutic effectiveness [17]. Barium sulphate ($BaSO_4$) in these pastes is added to increase radioopacity. Water-based radiopaque $Ca(OH)_2$ pastes with barium sulphate in ready to use paste form which could be easily cleaned and removed from the canal whenever required. Barium sulphate has better chemical stability and nondrying nature due to the ability of antibacterial and bacteriostatic [18,19].

Nanoparticles $Ca(OH)_2$ in aqueous suspensions structurally and morphologically with XRD and TEM, respectively and found that they were crystalline, regularly shaped, and hexagonally plated with dimensions 30 nm to 300 nm or less [20]. Due to their extremely small size, nanoparticles have a greater surface-to-volume ratio than bigger particles. As a result, a larger percentage of their atoms are concentrated on their surface, which increases their reactivity and responsiveness to their surroundings [6]. This larger surface area enables better interaction with the environment, which opens up a wide range of applications in disciplines including environmental research and medicine. The use of nanoparticles as antibacterial agents is an important application [21]. Because of their small size and high surface-to-volume ratio, nanoparticles can interact with microbial cells more effectively, resulting in higher antibacterial action. The antibacterial activity of nanoparticles is also greatly influenced by their charge density. Furthermore, nanoparticles can also influence the pH of their environment, further enhancing their antimicrobial activity. By increasing the pH, nanoparticles can disrupt the membrane potential of microbial cells, causing further damage and leading to their destruction. Overall, the higher surface-to-volume ratio and charge density of nanoparticles make them an excellent candidate for use as antimicrobial agents. These findings suggest that the use of commercial $Ca(OH)_2$ may result in greater therapeutic efficacy due to its ability to release higher concentrations of these ions over an extended period. Through the use of cutting-edge techniques and rigorous statistical analysis, we have established a reliable dataset that can serve as a valuable resource for researchers and clinicians alike. We believe that this study contributes to the advancement of knowledge in the field of intracanal medicaments and has the potential to inform future directions of research in this area.

Conclusions

The results of the present study indicate that the release kinetics of Ca and OH ions, as well as pH, from $Ca(OH)_2$ nanoparticles are inferior to those of commercial $Ca(OH)_2$. Nevertheless, the observed biological activity of $Ca(OH)_2$ nanoparticles highlights their potential as an antimicrobial agent, attributed to their ability to elevate pH levels via the release of Ca, OH, and pH ions. Consequently, $Ca(OH)_2$ nanoparticles hold promise as an alternative to traditional intracanal medicaments in dentistry. The temporal profile of Ca, OH, and pH release revealed a periodic increase from day 1 to day 7, followed by a decline on day 14, with the peak levels of OH ion release observed on day 7.

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Declaration of Interest

The authors report no conflict of interest.

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