Evaluation of calcium ion release in calcium hydroxide prototype as intracanal medicament

Atia N. Sidiqa,1* Myrna N. Zakaria,2 Ira Artilia,1 Zwista Y. Dewi,1 Arief Cahyanto1

Abstract

Objective: Calcium Hydroxide (Ca(OH)2) has been widely used in many dental treatments such as pulp capping, dentin hypersensitivity and as an endodontic intracanal medicament. Ca(OH)2 is highly alkaline, has antimicrobial, and remineralization action by releasing Ca2+ and OH-. Ca2+ release plays an important role in cell proliferation and remineralization of hard tissue healing. Indonesian limestone can be synthesized to produce Ca(OH)2 that can be an alternative to commercial Ca(OH)2 available. Therefore this study aimed to synthesized a Ca(OH)2 prototype and evaluate the Ca2+ released by the Ca(OH)2 prototype.

Material and Methods: Ca(OH)2 prototype was synthesized from limestone by calcination process and characterized by XRD and FTIR. The Ca(OH)2 prototype was then manipulated to a pasta form by mixing the powder with distilled water in 0.8 w/p ratio then inserted to a polyethylene tubed (2x10mm) using and immersed in 10 ml distilled water. Samples were divided into three groups for different periods of Ca2+ evaluation (1, 7 and 14 days). The Ca2+ concentration released was measured by a Spectrophotometer (DIRUI DR-7000D). Data obtained were analyzed by Anova.

Results: The observations on one day immersion was 3.589 mg/dL, the observation for seven days was 3.736 mg/dL, and for 14 days was 3.850 mg/dL. Statistically p<0.05.

Conclusion: Calcium hydroxide prototype released a sustainable amount of Ca2+ up to 14 days with the highest concentration achieved on day 14th.

Keywords: Ca2+ release, Calcium hydroxide, Limestone

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Introduction

Root canal infections often occur due to progressive caries. Microorganisms in the root canal system can penetrate to the apical foramen and enter the periapical region resulting in tissue damage and bone resorption. Root canal therapy is a treatment to prevent and control periapical infections. The outcome of this treatment is strongly influenced by the elimination of intracanal microorganisms.1-2 Ca(OH)2, has advocated as the most commonly used intracanal medicament disinfection agent. Ca(OH)2, mechanism action is through dissociation of Ca2+ which play an important role in the process of tissue mineralization and OH- ions that elevate the surrounding pH thus inhibits bacterial growth. Ca2+ ions activate the Transforming Growth Factor type β (TGF-β) which biomineralization and Adenosine Triphosphate (ATP) which accelerates the process of bone and dentin mineralization. When applied to the root canal as an intracanal medicament, Ca(OH)2 breaks down to Ca2+ and OH- then diffuse through the dentinal tubules and kill the bacteria inside.3,4 In general, the diameter of the dentinal tubules is about 1 to 2.5 μm which can be penetrated by bacteria such as enterococcus faecalis and other anaerobic bacteria. Therefore, material that can penetrate into dentinal tubules is needed to reach to small and complex sites. Ca(OH)2 with smaller particle size than dentinal tubules can penetrate into dentinal tubules and work more effectively.5-7 Limestone (CaCO3) is one of the natural resources in Indonesia. Limestone molecules contain atoms of calcium and oxygen. CaCO3 can transform to calcium oxide (CaO) through calcination process at 900°C. Ca(OH)2 paste was produced by dissolving CaO powder with distilled water for 24 hours. The Ca(OH)2 paste was then dried at 80°C to get the final result in the form of Ca(OH)2 powder. In this way, limestone can be utilized in the medical field, such as material research for clinical applications and its development in the manufacture of materials to increase the economic value of limestone itself.8-9 The Palimanan limestone has been successfully synthesized and characterized by our previous study, however improvement and in vitro evaluations regarding its physical/mechanical properties is yet to be carried out. Ca(OH)2 can be applied as inter appointment dressing, as well as pulp capping agent, therefore, additional material such as retarders, accelerators, radiopacifier or modification on its particle size must be adjusted according to its clinical application needs.10-12 Atomic absorption spectrophotometry can be utilized for qualitative and quantitative analysis.
Qualitative analysis can analyze organic compounds, while quantitative analysis ensures the quantity of molecules absorbed. The basic mechanism of a spectrophotometer is the light absorption as a Beer-Lambert law which states that when a beam of light is passed through a transparent cell containing a solution will be absorbed.9 A spectrophotometer runs by using a solution and measure the absorbance at the right wavelength. The selected wavelength is usually the maximum absorption wavelength (λmax), if there is a small error in setting the wavelength scale then there is an effect on the absorbance measurement. Ideally, the concentration must be adjusted to absorb it so that the optimal measurement accuracy and accuracy are obtained.12-14

Since the release of inorganic ions namely, Ca2+ plays an important task in the effectiveness of dental materials, many studies have been carried out to measure the Ca2+ ions released by different kinds of dental material.15-18 Considering the newly developed material has not comprehensively evaluated particularly in its bioactivity by releasing Ca2+ ions, our study aimed to evaluate the Ca2+ release rate of the Ca(OH)2 prototype from Indonesian limestone at different interval of time using an atomic spectrophotometer.

Material and Methods
Limestone (Ca(CO)3) was obtained from Palimanan, Cirebon and used as an alternative raw material for synthesizing Ca(OH)2 as an intra-canal medication material. The synthesis process to produce Ca(OH)2 includes combustion, grinding, filtering and drying. Firstly Ca(CO)3 was cleaned with distilled water, then calcined for 1 hour with 900°C, crushed to obtain the powder, and sieved using a 400 mesh sieve to obtain a fine powder. After CaO powder was obtained, stirring with a magnetic stirrer was carried out at a temperature of 50°C for 1 hour then left 24 hours to get the sediment and dried at 80°C to produce a Ca(OH)2 prototype.9 FTIR analysis was done to characterize the synthesized cement. Moreover, to evaluate the Ca2+ release, the Ca(OH)2 prototype powder was mixed with distilled water to 0.8 w/p ratio to form a paste consistency as study samples. The Ca(OH)2 paste was inserted into a polyethylene tube (2x10mm) using a plastic syringe and immersed in 10 ml distilled water with a neutral pH (7.0), and stored at 37°C water bath (Schutzart DIN 40050-IP 20). Samples were divided into 3 groups (n=9) for different periods of Ca2+ calculation (1,7 and 14 days). A spectrophotometer (DIRUI DR-7000D) was used to measure the amount of Ca2+ released.

Results
FTIR functions to identify functional groups in the sample. The functional group to be identified is O, H in the range 4000 to 600 cm-1 as shown in figure 1.

This figure shows the diffractogram of the calcium hydroxide prototype FTIR characterization. OH group was detected at wavenumber 3641.6 cm-1 which showed the presence of covalent bonds with the vibration of the O-H functional group. The C=O bond was also detected at a wavelength of 1845.88 cm-1 and a C-O bond was detected at a wavelength of 1176.58; 1082.07 cm-1 which shows there is a group CO3

The Ca2+ release rate for all tested groups are shown in table 1. The observations on day 1 immersion was 3.589 mg/dL, day 7 was 3.736 mg/dL, and the observation for day 14 days was 3.850 mg/dL. The results of statistical analysis using ANOVA test at 95% confidence level showed significant differences in the Ca2+ ion release rate on observations of immersion for 1 day, 7 days and 14 days with a value of p = 0.033 (value p≤0.05). The results of further tests using Poshoct test at 95% confidence level showed that there was a significant difference in the Ca2+ ion release rate of 14 days of immersion compared to 1 day of immersion with a value of p = 0.02 (p≤0.05), whereas there was no significant difference on 7 days immersion observation compared to 1 day immersion with p = 0.27 figure 2.

Discussion
Ca(OH)2 is an intracanal medicament that has an antimicrobial effect. Ca(OH)2 works by the dissolution Ca2+ and OH- through a solvent vehicle. This dissolution elevates its alkalinity and acts as

| Table 1 | Mean Ca2+ released of calcium hydroxide prototype |
|---------|-----------------------------------------------|
| Group   | Ca2+ Ion Release Prototype (mg/dL) | P-value |
| Day 1   | 3.589 ± 0.14                          | 0.03    |
| Day 7   | 3.736 ± 0.22                          | 0.27    |
| Day 14  | ± 0.18                                | 0.02*   |

p value ≤ 0.05
Ca²⁺ donor to support mineralization by apatite formation. Solvents play a role in determining the speed of dissociation or the release of Ca²⁺. Distilled water has neutral pH and low viscosity, it is commonly used for Ca(OH)₂ paste. As this study used only distilled water, when Ca(OH)₂ powder is dissolved with distilled water, it will rapidly decompose and becomes easily soluble, causing rapid release of Ca²⁺ and increases when the immersion time is longer. The viscosity of the solvent vehicle affects the release of Ca²⁺, distilled water has low viscosity therefore they are easier to dissolve an ingredient, so the Ca²⁺ release rapidly.¹⁶,¹⁷,²² This is consistent with the previous research by Grover, that Ca(OH)₂ dissolved by distilled water showed higher ionic liberation compared to other solvent used (propylene glycol, chitosan and gutta percha points impregnated with Ca(OH)₂ on day 1, while the highest yield using distilled water occurred on day 14 and lowest on day 1, they gradually decreased over 15 days to 30 days of observation.²⁶

In line with the results of our study that the Ca²⁺ rate increased over time from day 1, 7 to the last day of observation on day 14. Another study by Misra also observed an increase of Ca²⁺ rate of Ca(OH)₂ in double distilled water from day 1 to day 2 and day 7, however it then declined in day 15 and 30 period of observation, they concluded that the use of aqueous vehicle for Ca(OH)₂ resulted in a rapid initial ionic dissociation which will decreased consequently.¹⁷ Several factors can affect the release of Ca²⁺ namely, solvent, viscosity, particle size, the ratio of powder to liquid and contamination.¹³,¹⁶,¹⁸ Viscosity of a material affects the release of Ca²⁺, a low viscosity vehicle will dissociate the Ca²⁺ and OH⁻ ions faster because they will have less inner particle interaction, but not as stable as a viscous solvent.¹⁷ Short period of observation is one of the limitation of our present study, hence the trend of Ca²⁺ ions after 14 days was not recorded. However, the vast release of ions usually will eventually be tapered down in longer period observations, leaving undissolved particles. Therefore addition of other substances or the use of viscous solvents to Ca(OH)₂ paste will enhance its bio-availability to release Ca²⁺ as well of OH⁻, because it reduces the dispersion of the Ca(OH)₂ due to its molecular weight.¹⁷,¹⁹,²³ This might improve its clinical efficiency since it can stay longer in the root canal with stable antibacterial action therefore need less appointments time during treatment.²¹ Smaller particle size might also enhanced the particle dissolution and reduce the number of residual unreacted particle, it will also allow the Ca(OH)₂ to penetrate deeper to the dentinal tubules and improve its antimicrobial activity.

Conclusion
The Ca²⁺ release rate of the Ca(OH)₂ prototype gradually increased from day 1 to 14 days of immersion.

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Conflict of Interest
The authors report no conflict of interest.
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