ORIGINAL ARTICLE

Comparative Analysis of Porosity in *Anadara granosa* (Blood Cockle) Shells and Calcium Hydroxide for Pulp-Capping Applications

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ABSTRACT

Background: Maintaining tooth vitality is a pivotal aim of pulp capping treatments, which stimulate the formation of tertiary dentin as a reparative tissue response. Calcium hydroxide, commonly employed as the standard material in pulp capping, carries the risk of leakage due to tunnel defects in its porous structure. Blood cockle shells, comprising 98% calcium carbonate, offer an alternative material with bone-mimicking properties and structures. **Objective:** To compare and analyze the porosity discrepancies between calcium hydroxide and calcium carbonate sourced from blood cockle shells using scanning electron microscopy (SEM) image analysis, aiming to provide detailed insights into the material's structural characteristics. **Methods:** This study is a laboratory-based experimental investigation utilising a post-test control group design. Calcium hydroxide and calcium carbonate samples were freeze-dried and scrutinised using Scanning Electron Microscopy (SEM). Subsequent porosity analysis was conducted using ImageJ software. **Result:** The research results revealed an average porosity of 9.82% for $CaCO_3$, which was significantly lower than the porosity of Ca(OH)2 ($p < 0.05$), indicating superior mechanical properties. **Conclusion:** The porosity of calcium carbonate in blood cockle shells is lower than calcium hydroxide which is the gold standard pulp capping material so the calcium carbonate can be used to induce dentinogenesis.
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INTRODUCTION

Pulp disease represents a significant oral health concern, particularly impacting the Indonesian population. This observation is corroborated by data from Profil Data Kesehatan Indonesia, which indicates a high prevalence of dental pulp treatments.(1) Pulp capping serves as an essential therapeutic strategy for dental pulp diseases, aimed at preserving tooth vitality and stimulating reparative dentinogenesis. This regenerative process is mediated by both pulp cells and odontoblast cells.(2)

The treatment of dental pulp disorders necessitates the employment of materials that exhibit superior biocompatibility, inclusive of antibacterial, anti-inflammatory, and tissue-healing properties.(3) For decades, calcium hydroxide ($Ca(OH)_2$) has been considered the gold standard in pulp-capping materials due to its ability to inhibit bacterial proliferation and facilitate hard tissue mineralisation via the release of calcium and hydroxyl ions.(4) Nonetheless, the high solubility of calcium hydroxide poses an inherent drawback, creating tunnel defects in its porous structure that compromise the protective efficacy against pulp microleakage.(5) The role of porosity cannot be overstated; it influences a range of material properties including microleakage, adsorption, permeability, mechanical strength, and material density.(6) Specifically, optimal porosity—characterised by appropriate pore size, pore percentage, and inter-pore connectivity—can significantly enhance mechanical resilience during the formation of new hard tissues.(7)

Blood cockle shells are composed of 98% calcium carbonate ($CaCO_2$), making them highly biocompatible and a promising source of calcium for bone repair applications.(8) Given the compositional similarities between bones and teeth—both organically and inorganically—blood cockle shells emerge as viable alternative materials to induce dentinogenesis. Their high $CaCO_3$ content, anti-inflammatory properties, and excellent biocompatibility, combined with their structural integrity in wet dentine, make them suitable candidates for pulp-capping materials.(9,10) Moreover, $CaCO_3$ is not readily soluble in water and serves as an effective modulator of cellular growth.(11) Its angiogenic and vasculogenic properties contribute to the formation of tertiary dentine.(12)

The need to explore alternative materials for dentinogenesis arises from the limitations of currently employed materials, particularly their porous structures leading to microleakage. The focus of this research is to investigate the efficacy of blood cockle shells as natural sources of $CaCO_3$, by analysing the differences in porosity between $CaCO_3$ and calcium hydroxide ($Ca(OH)_2$).

MATERIALS AND METHODS

Samples

The research sample was bifurcated into two groups to assess the porosity differences between $CaCO_3$ derived from blood cockle shells and traditional $Ca(OH)_2$. According to Federer's sampling formula, each group consisted of 16 samples, totalling 32. The samples were cylindrical, with dimensions of 5 mm in diameter and 3 mm in height, and were utilised in post-test control groups only.

Sample preparation

The preparation phase entailed two primary processes: (1) Fabrication of calcium carbonate ($CaCO_3$) specimens involved the precise weighing of 0.12 grams of $CaCO_3$powder utilising an analytical balance. Subsequently, a paste was prepared by blending this powder with 0.04 ml of distilled water at a ratio of 3:1. The mixture was then agitated with a dental cement spatula until a homogeneous paste was achieved. (2) Calcium hydroxide ($Ca(OH)_2$) samples were likewise prepared by weighing out 0.125 grams of $Ca(OH)_2$ powder with an analytical balance. A 1:1 ratio paste was then constituted by amalgamating 0.125 grams of $Ca(OH)_2$ powder with 0.125 ml of distilled water. The paste was thoroughly stirred using a dental cement spatula to ensure homogeneity. Following the preparation, the $CaCO_3$ and $Ca(OH)_2$ pastes were dispensed into cylindrical moulds for the subsequent stage.

The second phase entailed the lyophilisation of the samples employing the freeze-drying technique. Both sets of specimens were placed in a mould pre-cooled to -40°C and were subjected to a drying period lasting 48 hours. After lyophilisation, the samples were extracted from the moulds, and their dimensions were ascertained using a calibrated blade to adjust for any shrinkage that may have occurred during the freeze-drying process.

Analytical procedures

Sixteen samples for each group were prepared by applying a carbon tip for secure adhesion to pin holders before insertion into the scanning SEM instrument (Zeiss EVO LS-10, Carl Zeiss AG, Germany) at a magnification of 1000x. To enhance image clarity and color contrast, a sputter coater was used. The SEM images were then processed and analyzed using ImageJ software. To standardize the images, black edges were cropped out, ensuring accurate measurements by focusing solely on the actual material areas. The set scale function was employed to present the measurements in micrometers. Open pores were identified by analyzing pixel intensity from the image histogram, highlighting their distinct outlines. The Adjust/Threshold function was used to measure the area of these open pores. For accurate pore count and size determination, the Process/Binary/Watershed function was applied after the Adjust/Threshold function to separate closely packed pores that might otherwise be counted as one. The Analyze Particles function was subsequently used to configure the particle analyzer, while the Show/Outlines and Summarize functions provided automated numbering, total count, and average size of the pores. Porosity percentages were quantified based on these SEM images. Statistical analysis was conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), using SPSS version 25.0 (IBM Corp., Armonk, NY, USA),and the Mann-Whitney U Test was performed to evaluate any significant differences.

RESULTS

Figure 1 presents the SEM results for the samples of both calcium hydroxide and calcium carbonate. Further analysis of porosity percentages conducted using ImageJ software is shown in Figure 2.

The descriptive statistics for pore size and porosity of the two groups evaluated in this study are summarized in Table I. We assessed the characteristics of both calcium hydroxide ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$) derived from blood cockle shells as potential materials for pulp capping. Each group comprised 16 samples, enabling a robust analysis of the materials'

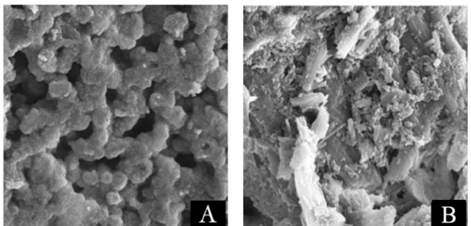

Fig. 1: SEM Observational Data: (A) Calcium hydroxide sample at 5,000x magnification, (B) Calcium carbonate sample at 5,000x magnification.

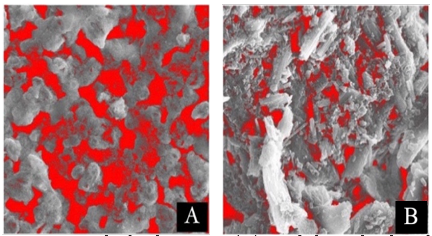

Fig. 2: ImageJ Analytical Data: (A) Calcium hydroxide sample at 5,000x magnification, (B) Calcium carbonate sample at 50,000x magnification.

Table I: Descriptive statistics of pore size and porosity for both groups

	Group	Sample Amount	Mean Pore Size (μm)	Mean Porosity (%)
	$Ca(OH)_2$	16	2.26 ± 1.55	23.25 ± 2.44
	$CaCO_3$	16	1.74 ± 1.30	9.82 ± 2.06
p* value			0.368	0.000

microstructural properties.

The mean pore size of the $Ca(OH)_2$ group was recorded at 2.26 μm with a standard deviation of 1.55 μm. In contrast, the $CaCO_3$ group demonstrated a smaller mean pore size of 1.74 μm with a standard deviation of 1.30 μm. Regarding porosity, $Ca(OH)_2$ exhibited a higher mean porosity percentage of 23.25% with a standard deviation of 2.44%, significantly greater than the $CaCO_3$ group, which had a mean porosity of 9.82% and a standard deviation of 2.06%.

Statistical analysis employing the Mann-Whitney U test revealed that the differences in mean pore size between the two groups were not statistically significant ($p = 0.368$). However, the differences in mean porosity percentages were statistically significant ($p < 0.001$), indicating a substantially lower porosity in the $CaCO_3$ group compared to the $Ca(OH)_2$ group.Morphological characterization from the SEM images provides further insight into these findings. The SEM image of the calcium hydroxide sample at 5,000x magnification (Figure 1A) shows a rough and granular surface. The particles are irregularly shaped with a high degree of agglomeration, creating a porous structure. The $Ca(OH)_2$ sample exhibits numerous open pores, which are relatively larger and more frequent compared to the $CaCO_3$ sample. This is reflected in the mean pore size of 2.26 ± 1.55 μm and a mean porosity of 23.25 ± 2.44%. The texture is uneven, indicating a high level of porosity, which contributes to its lower mechanical properties. The distribution of pores and particles is somewhat uneven, leading to an inconsistent internal structure.

In contrast, the SEM image of the calcium carbonate sample from *Anadara granosa* at 5,000x magnification (Figure 1B) reveals a more compact and crystalline structure. The particles are well-defined and closely packed, reducing the overall porosity. The $CaCO_3$ sample shows fewer and smaller open pores, with a mean pore size of 1.74 ± 1.30 μm and a mean porosity of 9.82 ± 2.06%. The texture is smoother and denser, indicating better mechanical properties due to the reduced porosity. The distribution of particles and pores is more uniform, contributing to a consistent internal structure.

These results suggest that while the two materials exhibit comparable pore sizes, the porosity of $CaCO_3$ is significantly lower, which could be advantageous in terms of material stability and durability when used as a pulp-capping material. The substantially lower porosity observed in the $CaCO_3$ group may contribute to its enhanced mechanical properties and reduced susceptibility to microleakage, potentially making it a superior alternative to traditional $Ca(OH)_2$ in dental applications.

DISCUSSION

This study revealed that the average pore size for $Ca(OH)_2$ is 2.26 μm with a corresponding porosity of 23.25%, while $CaCO_3$ exhibits a smaller average pore size of 1.74 μm and a lower mean porosity of 9.82%. These findings validate the hypothesis of $CaCO_3$'s lower porosity. The synthesis of $Ca(OH)_2$ resulted in granular, stone-shaped nanoparticles, whereas rod-like aragonite crystals were identified in the cockle shell powder.

The elevated porosity in calcium hydroxide can be attributed to its propensity to readily dissolve in water. As the water content increases, so does the material's porosity, consequently diminishing its mechanical strength. This relationship between solubility and porosity influences both the stability and durability of the material. Additionally, research on cement-stabilized soils has shown that the porosity-water/binder ratio critically impacts strength, stiffness, and durability. Increased water content results in higher porosity, which weakens the material and affects its mechanical properties.(13) Specifically, $Ca(OH)_2$ contains hydroxyl ions—free radicals that are readily released—making the material more susceptible to water absorption, which results in increased porosity.(12)

The utilisation of natural materials such as blood cockle shells promotes dentinogenesis due to their high concentrations of $CaCO_3$, which possess biocompatible, osteoconductive, and biodegradable properties.(4) The ionic bonds in $CaCO_3$ contribute to its limited solubility, attributed to the magnetic forces between ions, thereby leading to a denser material with reduced porosity.(12) Lower levels of porosity, in turn, enhance the mechanical properties of the material.

This research utilises the freeze-drying technique, corroborating the findings by Yu(14), who asserted that cooling yields porous calcium carbonate products with pore sizes ranging from 100 nm to 1 μm and 20-90 μm. Our study demonstrated an average pore size of 1.74 μm for $CaCO_3$. Blood cockle shell extract exhibits low porosity, non-toxicity, and favourable viability. It accelerates vascularisation, promotes fibroblast growth and osteoblast proliferation, and stimulates bone growth. Moreover, $CaCO_3$ serves as an effective medium for growth factors and facilitates cellular attachment, growth, dispersal, and differentiation. As a result, $CaCO_3$ has proven its potential as a material for inducing dentinogenesis and initiating tissue deposition with the formation of tertiary dentine, characterised by its smaller pore sizes and lower porosity.(11) As a result, $CaCO_3$ has proven its potential as a material for inducing dentinogenesis, characterised by smaller pore sizes and lower porosity.

CONCLUSION

The porosity of calcium carbonate derived from blood cockle shells is demonstrably lower than that of calcium hydroxide, which is considered the gold standard for pulp capping materials, thereby presenting itself as an effective substitute for dentinogenesis induction. The significantly lower porosity of $CaCO_3$ may contribute to enhanced mechanical properties, reduced susceptibility to microleakage, and greater material stability and durability. This suggests that blood cockle shell-derived calcium carbonate can be an effective alternative material for inducing dentinogenesis and providing better protective barriers in pulp-capping applications. Further studies on other properties such as biocompatibility, bioactivity, and long-term clinical outcomes are recommended to fully establish the potential of blood cockle shell-derived calcium carbonate in dental treatments.

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REFERENCES

1. Ismiyatin K, Wahluyo S, Soetojo A, Rahayu, RP, Utomo H, Anindya C. The expression of pulpal substance P after dentinal application Escherecia coli lipopolyaccharide. Saudi Endodontic Journal. 2019; 9(3): 169-173. doi: 10.4103/sej.sej_92_18.
2. Hanafi, MGS, Izham A, Bahtiar WE. 2018. Biokompabilitas bahan kaping pulpa. Cakradonya Dental Journal. 2018; 13(1): 14–21. doi: 10.24815/cdj.v13i1.20912.
3. Kumala YR, Rachmawati D, Hersanto K. Stimulasi dentin reparatif direct pulp capping menggunakan ekstrak ikan teri (Stolephorus sp). E-Prodenta Journal of Dentistry. 2017; 1(2): 45–53. doi:10.21776/ub.eprodenta.2017.001.02.1.
4. Gandolfi MG, Siboni F, Botero T, Bossш M, Riccitiello F, Prati C. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. Journal Applied Biomaterials & Functional Materials. 2014; 13(1): 43-60. doi: 10.5301/jabfm.5000201.
5. Paula BA, Laranjo M, Marto CM, Paulo S, Abrantes MA, Fernandes MB, Casalta-Lopes J, Marques-Ferreira M, Botelho FM, Carrilho. Evaluation of dentinogenesis inducer biomaterials: an in vivo study. J Appl Oral Sci. 2019; 28:e20190023. doi: 10.1590/1678-7757-2019-0023.
6. Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus biodentine: review of literature with a comparative analysis. J Clin Diagn Res. 2017;11(8):ZG01-ZG05. doi: 10.7860/JCDR/2017/25840.10374.
7. Abbasi M, Madani M, Kazemi A. Fluid flow in fractured reservoirs: Exact analytical solution for transient dual porosity model with variable rock matrix block size. Journal of Petroleum Science and Engineering. 2018; 164: 571-583. doi:10.1016/j.petrol.2018.01.010.
8. Hirayama S, Komine C, Takahashi C, Matsui S, Matsushima K. Effects of calcium carbonate on odontoblas differentiation and calcification ability of human dental pulp cells. Journal Of Oral Tissue Engine. 2013; 11(2): 123–134. doi: 10.11223/jarde.11.123.
9. Saraswati W, Dhaniar N, Wahjuningrum DA, Nuraini N, Bhardwaj A.The effect of exposure calcium carbonat from blood cockle (*Anadara granosa*) shells to the expression of the NF-κβ on dentin pulp complex. Journal of International Dental and Medical Research. 2021; 14(2): 549-553. Available from: http://www.jidmr.com/journal/contents-of-jidmr-2021-vol-14-no-2/.
10. Praja HA, Dhaniar N, Santoso RM, Kusumaputri DP, Putri ASA, Soetojo A, Saraswati W. Calcium carbonate of blood cockle (*Anadara granosa*) shells induced VEGF-A expression in dentin pulp complex: an in vivo study. Malaysian Journal of Medicine and Health Sciences. 2022; 18 (SUPP6): 24-30. doi: 10.47836/mjmhs.18.s6.5.
11. Overby J, Chang R. Chemistry. 14th ed. New York: McGraw Hill; 2022. p. 131
12. Saraswati W, Soetojo A, Dhaniar N, Praja HA, Santoso RM, Nosla NS, Rahardia N. $CaCO_3$ from *Anadara granosa* shell as reparative dentin inducer in odontoblast pulp cells: in-vivo study. J Oral Biol Craniofac Res. 2023; 13(2): 164-168 doi:10.1016/j.jobcr.2023.01.003. 2023.
13. Baldovino JA, de la Rosa YEN, Calabokis PO. Analysis of novel porosity–water-to-binder index for prediction of strength, stiffness and durability for cemented soils. Materials. 2023, 16(19): 6354. doi:10.3390/ma16196354.
14. Yu HD, Tee SY, Han MY. Preparation of porosity-

controlled calcium carbonate by thermal decomposition of volume content-variable calcium carboxylate derivatives. Chemical Communications. 2013; 49(39): 4229-4231. doi: doi.org/10.1039/C2CC35339C.