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Research Report

Differences in the solubility of $CaCO_3$ from blood clam shells and $Ca(OH)_2$ as a candidate pulp capping material

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ABSTRACT

Background: *Calcium hydroxide ($Ca(OH)_2$) is the gold standard in pulp capping treatment because the biocompatibility and capability to stimulate hard tissue formation and also antibacterial effect. However, this compound has the disadvantage of being easily soluble in saliva which will increase the risk of leakage in the cavity. Another alternative pulp capping material is calcium carbonate from blood clam shells. Blood clam shells contain 98% $CaCO_3$, $CaCO_3$ has low solubility and is difficult to dissolve in water due to the large ions on Ca^{2+} and CO_3^{2-} so that the attractive force between these ions is very strong and finally H_2O is not able to break down the $CaCO_3$ compound to be dissolved.* ***Purpose:*** *Explained the differences in the solubility of calcium carbonate from blood clam shells and calcium hydroxide as candidates pulp capping materials.* ***Methods:*** *This research is a laboratory experimental study with a pretest-posttest control group design method. The samples consisted of 12 pieces of $Ca(OH)_2$ and $CaCO_3$ then divided into 2 groups and given treatment. Group 1 $CaCO_3$ immersed in 1 day, group 2 $Ca(OH)_2$ immersed in 1 day, group 3 $CaCO_3$ immersed in 7 days, and group 4 $Ca(OH)_2$ immersed in 7 days. Solubility is calculated according to the solubility formula. Data testing with Anova-Welch.* ***Results:*** *There was a significantly difference in the solubility rate between the $CaCO_3$ dan $Ca(OH)_2$ groups immersed for 1 day and 7 days on the Anova-Welch results ($p<0.05$).* ***Conclusion:*** *The solubility of $CaCO_3$ in blood clam shells is lower than the solubility in $Ca(OH)_2$.*

Keywords: *Anadara granosa; blood clam shells; calcium carbonate; calcium hydroxide; solubility*

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INTRODUCTION

The prevalence of dental and oral diseases according to Indonesian basic health research data in 2018 reached 57.6% and those who received medical personnel services were 10.2%.[1] Based on the Indonesian Health Data Profile in 2011, it recorded pulp and periapex diseases in the 7th place of outpatient diseases in Indonesia.[2] The classification of pulp disease itself, consists of several of which are reversible pulpitis, irreversible pulpitis, hyperplastic pulpitis and pulp necrosis. Inflammation in reversible pulpitis will heal and the pulp returns to normal if the cause is eliminated. Untreated reversible pulpitis can develop into irreversible pulpitis and even pulp necrosis.[3]

One of the treatments performed on reversible pulpitis is pulp capping. Pulp capping aims to maintain the vitality of the pulp. The way pulp capping maintains the vitality of the pulp is by placing biocompatible materials in the open pulp to stimulate the formation of reparative dentin before a definite restoration.[4] One of the pulp capping materials that can be used is calcium hydroxide ($Ca(OH)_2$). This material is in demand by most dentists because it can accelerate the formation of tertiary dentin.[5]

Calcium hydroxide ($Ca(OH)_2$) has been used since the early 1920s and became a gold standard material in pulp capping. Ca(OH)2 has biocompatibility and ability to stimulate hard tissue formation as well as its antibacterial effects. Some of the known disadvantages regarding this compound are its high solubility, degradation of the material over time, the formation of a weak dentin barrier, poor adhesion properties to the tooth structure, and chronic uncontrolled inflammation of the pulp.[4] $Ca(OH)_2$ easily soluble in saliva will increase the risk of leakage in the cavity.[6] Materials pulp capping should not be toxic, biocompatible, have low solubility and have a great influence on the success rate of restorative procedures.[7] Solubility cause degradation chemical structure and decreased mass of the material.[8]

$CaCO_3$ is an important component in biological systems that can be obtained in the shells of marine biota, pearls, and eggshells.[9] Blood clam shells have a high calcium carbonate ($CaCO_3$) composition, which is as much as 98% which can

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be used as a source of calcium in the synthesis of compounds containing calcium metals such as hydroxyapatite.[10] $CaCO_3$ from the blood clam shell has the ability to stimulate TGF-β1 and VEGF-A which serves to modulate cell growth and is a good angiogenic and vasculogenic agent in the production of tertiary dentin.[11] Calcium carbonate ($CaCO_3$) is not easily soluble in water. The ionic bonds that make up this compound have a large charge on the Ca^{2+} and CO_3^{2-} ions. Therefore, the attractive force between these ions is so strong that H_2O is not able to break down the $CaCO_3$ compound to dissolve the Ca^{2+} ion.[12] Exploration of natural materials are needed to overcome water-soluble properties, this study is considered necessary to determine the difference in the solubility of $CaCO_3$ in blood clam shells and $Ca(OH)_2$ as candidates for pulp capping material.

MATERIALS AND METHODS

This study is a laboratory experimental study with pretest-posttest control group design. Samples are 24 cylindrical shaped with 15 mm in diameter and 1 mm tall based on ISO 4049 standardization. Samples are then divided into four groups with six samples for each group. The first group is $CaCO_3$ immersed in artificial saliva for 1 day (G1), group 2 $Ca(OH)_2$ immersed in artificial saliva for 1 day (G2), group 3 $CaCO_3$ immersed in artificial saliva for 7 days (G3), and group 4 $Ca(OH)_2$ immersed in artificial saliva for 7 days (G4).

The materials used in this study were $CaCO_3$ powder (blood clam shells) from PT. Pertiwi Parahita Technology, $Ca(OH)_2$ powder (hidroxido calsio P.A) from Biodinamica, aquadest, celluloid strip and artificial saliva. $CaCO_3$ is mixed with sterile aquadest in a ratio of 3:1 (0.9 grams of $CaCO_3$ powder and 0.3 ml of aquadest). Calcium hydroxide is mixed with sterile aquadest in a ratio of 1:1 (according to factory rules) (0.9 grams of Calcium hydroxide and 0.9 ml of aquadest). Then the paste $CaCO_3$ and $Ca(OH)_2$ is placed on a mold/disc with a diameter of 15 mm and a height of 1 mm. At the bottom of the sample mold is given a celluloid strip and placed on a glass slab.

Each sample was put into an incubator at 37°C for overnight and then weighed with a precision scale of 0.1 mg (Mettler Toledo AL204 *Analytical Balance)* to get a constant initial weight (M1). Petri dish are filled with 40 ml artificial saliva then place in incubator at 37°C for 24 hours. After 24 hours, the petri dish was removed from the incubator and then each group was immersed in artificial saliva with interval examination period 1 and 7 days. After that artificial saliva is removed, use specific paper to absorb the liquid of samples, then weighed with a precision scale of 0.1 mg for each group then flattened to get a constant initial weight (M2). Each sample is put in an incubator with a temperature of 37 °C for 24 hours. Then the samples are weighed until get the constant weight. The samples are weighed three times for mean weight value (M3).

$$Solubility = \frac{M1 - M3}{V}$$

M1 = initial constant weight
M3 = final constant weight
V = volume

The data were analyzed by Shapiro Wilk normality test, Levene's homogeneity test, and Anova-Welch to examine the significantly difference of the four groups. Then used one-way Anova (post hoc test) with the Games-Howell multiple comparison method to determine significant differences between each group.

RESULTS

The data results examination of solubility for each group is shown in Table 1. The data are then run through a statistic Shapiro Wilk normality test and the results are the significance value for G1 is 0.248, G2 is 0.540, G3 is 0.324, G4 is 0.429. All four groups had data significance values higher than 0.05 ($p>0.05$) meaning data from each group is normal distributed. The results of Levene's homogeneity test is significance value lower than alpha which is 0.001 ($p < 0.05$), therefore the value indicates that the variation in the data is not homogeneous (heterogeneous). The Anova-Welch test is an alternative test of one-way Anova for normally distributed but not homogeneous data. The significant value is lower than 0.05 shows that there is a difference in the average solubility value in four groups which indicates a significant difference for each group (H0 rejected and Ha accepted).

Then the data examined using one-way Anova (post hoc test) with the Games-Howell multiple comparison method to determine significant differences between each group. The results showed significant difference was obtained for a P value lower than 0.05. Then for the $CaCO_3$ group immersed 1 day and $CaCO_3$ immersed 7 days obtained a p value greater than 0.05 indicating the data did not different significantly.

DISCUSSION

Based on this study, the data is analyzed and the mean value of G1 group $CaCO_3$ immersed in 1 day is 0.248, while G2 group $Ca(OH)_2$ immersed in 1 day is 0.540. From this

Table 1. Mean and standard deviation solubility of $Ca(OH)_2$ and $CaCO_3$ in 1 day and 7 days immersion

Group	N	Mean	SD
$CaCO_3$ immersed 1 day (G1)	6	0.099	10.26
$Ca(OH)_2$ immersed 1 day (G2)	6	0.025	2.78
$CaCO_3$ immersed 7 days (G3)	6	0.370	20.91
$Ca(OH)_2$ immersed 7 days (G4)	6	0.025	4.02

result it is shown that the mean value of G1 is lower than the mean value of G2. In statistically, there is significant difference between the two groups. This result are consistent with the hypothesis that solubility of $CaCO_3$ is lower than $Ca(OH)_2$.

This study is appropriate with the theory by Otto[13], $Ca(OH)_2$ compound dissociates completely when dissolved in water and $Ca(OH)_2$ also has hydrogen bonds. Hydrogen bonds are the weakest bonds. Compounds that have hydrogen bonds will dissolve easily.[14] Weakened chemical bonds cause release of Ca^{2+} ions from calcium hydroxide and binding OH^- ions with H_2O resulting solubility of the material and reduced sample mass. Meanwhile, the $CaCO_3$ has ionic bonds caused by the large ion of each compound.[13] Ionic bonds are the strongest bonds than the other chemical bonds. The ions in this bond strongly attract electrons from nearby molecules that have opposite electrons. Positive ions with a large electron such as the Ca ions in $CaCO_3$ which are 2+ have a small radius cause the ions in this bond have a high density so that ionic bonds in a compound will not be easily solve.[14]

In this study, the mean value of G2 group $Ca(OH)_2$ immersed in 1 day was significantly different with G4 group $Ca(OH)_2$ immersed 7 days. The longer immersing time, the more the bonds is broken because the H^+ ions that diffuse into the calcium hydroxide.[15] This opinion is also in accordance with the theory from Chemistry[16] that immersing for the first 3 days to a certain time cause the solution to become saturated and water content in the material increases resulting a solubility process and the precipitate formation.

The solubility of a material affected by several factors including the type of solute, the type of solvent and temperature. In this study, the type of solvent used for both materials were the same is artificial saliva which was dominated by H_2O and for the incubator temperature when immersing the two materials were also same (37°C). Types of solutes an important role in this study, calcium hydroxide has hydroxyl ions (OH^-) which easily bind to H_2O cause this compound is easily soluble in water. Meanwhile, calcium carbonate has strong ionic bonds that are difficult to dissolve. This causes the solubility of $CaCO_3$ lower than the solubility of $Ca(OH)_2$.

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