Preparation of Hydroxyapatite Scaffold using Luffa Cylindrica Sponge as a Template

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Abstract

Hydroxyapatite (HA) scaffold has been widely applied in the biomedical field especially for bone implants. The purpose of this research is to determine the influence of dispersant addition and stirring time on HA scaffold which formed using gambas sponges as a template. HA slurry was made by mixing 10 grams of HA, 12 grams of aquadest, 0,2, 0,25, 0,3 grams of Darvan 821A and 1 gram of sago. The slurry was impregnated into Luffa cylindrica sponges. The sample then dried at 110°C for 3 hours. The green bodies were burned at 600°C for 1 hour and followed by sintering at 1250°C for 1 hour. The results showed that the addition of dispersant and increasing of stirring time lead to smaller porosity and shrinkage. In addition 0,2; 0,25; and 0,3 grams dispersant, the resulting porosity in the range of 63,85-70,78%; 58,74-70,35%; and 53,42-69,66%. Shrinkage 39,43%-53,71%; 27,75-50,68%; and 13,61-41,25%. Compressive strength 0,61-1,05 MPa; 2,88-3,81MPa; and 0,52-2,96 MPa. The macroporous and microporous average pore sized was 50 µm dan 5 µm.

Keywords: dispersant; gambas; hydroxyapatite; scaffold.

1. Introduction

Nowadays, it’s a lot of method to produced porous implants as prepared study to substitute the metal implants. The pores in a porous scaffold can be formed by adding pore-forming agents such as starch, using air; using protein and starch (protein foaming - consolidation method), or by using a template that impregnated by biomaterial suspension (replica method). Hydroxyapatite (HA) scaffold ideal is a biomaterial that has pores system which forms a secure bond with the tissues, increasing the mechanical fastening between an implant and bones (Swain et al., 2015). One way to achieve this pores system that uses the template as in the polymeric sponge method (Sopyan and Kaur, 2009). The main advantage of this technique is, that HA scaffold has very good interconnected pores (Dobos, 2012).

HA Scaffold should have an interconnected pore structure with high porosity. The pore diameter is about above 300 microns for penetrating the cell, tissue growth, and vascularization (Barbara et al., 2005). Luffa cylindrica is a kind of tropical plant and the vine of the family Cucurbitaceae (Tanobe et al., 2005). It has microcellular architecture fibers that form a net-like fibrous vascular system. The struts of Luffa cylindrical sponge which has 200-400 µm diameter form by microcellular architecture macro-pore with a diameter of 10–20 µm and yield a multimodal hierarchical pore system that can be used as a template in the manufacture of porous material (Zampieri et al., 2006). Sopyan and Kaur, 2009 have pointed out in their investigation based on the amount of solid loading HA. HA scaffold has been made by a polymeric-sponge method using commercial cellulose (Spontex Spa, Italy), dispersant 3% of solid loading HA, sintering temperature 1200oC for an hour. Solid loading HA amount is 38%w; 42%w; and 44%w. The result indicated the addition of 42%w solid loadings produced HA scaffold with density 2.08 g/cm3 and compressive strength 10.5 MPa.

Jamaludin et al, 2015 produced HA scaffold using commercial polyurethane sponge (20 pores/inch, CCT Automation Sdn Bhd). HA scaffold has been fabricated based on the influence of binder type (PVA, sago starch, and tapioca). The result shows that sago starch produced the highest density is 0.83 g/cm-3. Fu et al., 2008 have done their research to producing HA scaffold by the freeze-casting method based on the type of dispersant (Darvan C, Darvan 811, Darvan 812A, Targon 1128). The result shows darvan
821A usage producing 55% of good structure porous. This work focuses on the preparation of hydroxyapatite scaffold using Luffa cylindrica sponge, which amount of solid loading HA and darvan 821A were varied. This method is using a template to form macroporous ceramics by impregnation cell structure with ceramic suspense to produce similarity morphology as well as a template, where the ceramic suspense be made by varied solid loading and dispersant.

2. Materials and Methods

2.1. Materials

The material used in this study were pure HA powder as solid loading was supplied by Lianyungang Kede Chemical Industry Co. Ltd, China. Luffa cylindrica sponge as a template was supplied by Trendy, Indonesia. Sago starch as binder supplied by Puri Pangan Sejahtera, Indonesia. Darvan 821A as dispersant supplied by Vanderbilt Company, USA. And distilled water as solvent was supplied by Brataco Chemica, Indonesia.

2.2. Preparation of HA scaffold

Porous template prepared by soaking Luffa cylindrica sponge for 12 hours in order to make it expands. Sponges were cut into circular samples of ± 10 mm diameter then dried in the open air for an hour. The slurries were prepared by dissolving 10, 11 and 12 g hydroxyapatite powder with 10 g sago starch, 2%; 2.5%; and 3% Darvan 821A and 12 g distilled water. The slurries were stirred using magnetic stirrer with a rate of 350 rpm for 20 hours. The sponges then impregnated with the slurry and dried using an air oven at 110°C for 3 hours.

The organic matrix is eliminated through the burning process using a muffle furnace at 600°C for 1 hour and followed by sintering at 1250°C for 1 hour.

2.3. Measurement of shrinkage, density, and porosity

The shrinkage was determined by measuring the volume of the sample before and after the sintering process. The shrinkage was then calculated by using Equation (1).

\[ \text{\% Shrinkage} = \frac{V_b - V_a}{V_b} \]  

(1)

Vb and Va were sample volumes before and after sintering. The apparent density of sintered porous HA obtained by using Archimedes principles in an Electronic densimeter (Alfa Mirage, MD300S model). The theoretical density of HA 3.16 g/cm3 was used as a reference to calculate the total volume of the fraction of porosity.

2.4. Evaluation of Compressive Strength

A compressive strength test is performed to determine the durability of the sample when given loads. Compressive strength is obtained from stress-strain curves by burdening the sample at a certain rate until the sample is a failure. The compressive strength was evaluated using Universal Testing Machine.

2.5. SEM and XRD analysis

Phase analysis was carried out on the porous HA samples after sintering by using X-ray diffraction (XRD) analysis using Panalytical XRD XPERT POWDER operating from 10 to 90° 2θ at a step size of 0.026 2θ with CuKα radiation (Kα = 0.15406 nm) at 30 mA and 40 kV. Scanning Electron Microscopy (SEM: Hitachi-SU 3500) was performed to characterize the morphology and microstructure of the sintered porous samples. The average pore size was calculated by using ImageJ v.151 software.

3. Results and Discussion

3.1. Body Properties of HA scaffold

This work is using Luffa cylindrica as a template to form interconnected macrostructure pores. Fig 1 shows the green bodies before sintering and after sintering processes. It is shown that the size of samples after burning and sintering processes are smaller than the samples before treatment. It caused by the decreasing of volume samples during both of processes. During the burning process, organic materials such as template and starch were removed as a pore-forming agent. Then the particles which were initially weakly bonded close together and contacted each other after the sintering process. The release of organic materials formed pores on the ceramic body and caused volume shrinkage (Sopyan et al., 2012). The volume was decreasing because of densification when sintering processes (Kang, 2005), the densification caused the matrix of bodies dense along with the loss of organic compound (Li et al., 2013).
3.2. Shrinkage

The addition of dispersant and stirring time in this study affected the physical properties of the HA scaffold. Physical properties measured include a percentage of shrinkage, density and porosity.

The addition of dispersant and stirring time in the process synthesis of slurry influences the percentage of shrinkage of the HA scaffold formed. Figure 2 shows the relationship between the addition of dispersant mass and stirring time against shrinkage of HA Scaffold. Addition of dispersant 0.2; 0.25; and 0.3 gram on each stirring time of 16, 20, and 24 hours decreasing percentage of shrinkage.

Shrinkage is also related to the densification process that occurs when sintering. Shrinkage will cause a decrease in sample volume, on the other hand, samples that have undergone a sintering process will become denser. With this shrinkage, the pore density will increase and automatically the mechanical properties of the material will also increase, especially the strength of the sample after sintering. The higher sintering temperature causes greater shrinkage. The increase in temperature will increase the rate of densification of the sample so that the ceramic particles become more dense and unite strongly.

3.3. Density and Porosity

The addition of dispersant and stirring time increases the sample density in Figure 3, in addition to the dispersant 0.2; 0.25; and 0.3 gram and the stirring time of 16 hours the sample density obtained is 0.92; 1.11; and 1.14 gr/cm$^3$. When stirring 20 hours, the density of the sample obtained is 0.; 1.25; and 1.31 gr/cm$^3$, while at stirring 24 hours the sample density obtained was 0.96; 1.31; and 1.47 gr/cm$^3$. The increase in density caused by the addition of dispersants causes the slurry to become thick and maintain the colloidal properties of the slurry. The addition of dispersant is done so that the quality of the formed slurry is maintained and avoids precipitation. Ramay and Zhang, 2003 stated that dispersants can maintain slurry properties and stabilize slurry from the formation of solids. The stirring time slurry also affects the density of the sample produced.
Stirring time of slurry also affects the density of the resulting sample. Abdurrahim and Sopyan, 2008 stated that the longer of mixing time the density and homogeneity would increase. In addition, when there is an increase in temperature during the sintering process, the density of the sample will increase, this increase is caused by particles that are increasingly compact and compact (densification) at high temperatures. In the process of sintering, the structure of particle material will grow (coarsening) and unite to form a unity of mass (densification). Fu et al., 2008 also mentioned that sintering at temperatures between 1250°C-1350°C will increase the density of HA scaffold without changing its microstructure.

Figure 4 shows the relationship between the addition of dispersants and the stirring time to the porosity formed. At the addition of the mass of dispersant 0.2; 0.25; and 0.3 gram and stirring 16 hours of HA scaffold the porosity are 70.63%; 70.35%; and 69.66%; on stirring 20 hours the porosity formed were 69.60%; 59.50%; and 58.74%; in the 24 hour stirring the porosity formed were 63.85%; 58.74%; and 53.42%.

The addition of dispersants decreases the porosity formed in the sample, Fu et al., 2008 stated that the increase in the amount of dispersant mass reduces flocculation formation in the suspension. This causes the suspension formed to have good colloidal stability with a high degree of homogeneity so that the sample porosity decreases.

3.4. Compressive Strength

Compressive strength obtained ranged from 0.61 to 3.81 MPa. Overall the addition of dispersant affect to the increased compressive strength. The increased of compressive strength investigated affected by the reduction of porosity. Rahman and Guan, 2007 mentioned that the lower compressive strength affected by size and degree of porosity.

It is known that the compressive strength change with time of stirring (Figure 5). In Figure 5 (a) and (c) the compressive strength of the sample rises as the duration of stirring takes place. in Figure 5 (b) the compressive
strength of the sample has fluctuations for each variation of stirring time. This is caused by several factors that influence the compressive strength of the material including the shape, size, and structure of the sample being tested, the surface area of the test sample, application of the test load, water content in the sample, and the type of load used.

**Figure 5.** Effects addition of dispersant; 0.2 Gram (a), 0.25 gram (b), and 0.3 gram (c) and Stirring Time 16, 20 and 24 Hours against Compressive Strength and Porosity
Figures 5 (a), (b) and (c) also show the effect of adding dispersants to the compressive strength of the resulting HA scaffold, when the stirring time is 20 hours with variations in the addition of dispersant mass of 0.2; 0.25; and 0.3 gram; HA scaffold compressive strength increased by 2.03 MPa; 2.10 MPa; and 2.42 MPa. It shows that the addition of mass dispersants will increase the compressive strength of the material. This is related to the decrease in porosity that occurs due to the addition of dispersants.

Based on the compressive strength obtained, the longer stirring time will increase the compressive strength of the material to the amount of dispersant addition of 0.2 and 0.3 grams. This is consistent with research conducted by Barnes and Cooper, 2015 which states that stirring carried out on a material mixture will increase the compressive strength of the material.

3.5. Morphology of HA Scaffold

The size and arrangement of pores that are getting bigger and bigger will cause thinning of the struts which result in a decrease in compressive strength (Ramay and Zhang, 2003). In this study, fluctuations and decreases in compressive strength in the sample were caused by the uniformity of surface and pore morphology formed on a scaffold it can be proven by SEM analysis in Figure 6. The addition of dispersants affects the structure and pore size formed on green bodies HA it shows in Figure 7.

![Figure 6](image1.png)

**Figure 6.** Morphology of HA Scaffold on 0.2 Gram Darvan Addition with Stirring Time Variations (a) 16 Hours (b) 20 Hours and (c) 24 Hours

![Figure 7](image2.png)

**Figure 7.** Morphology of HA scaffold on 20 h stirring time with variations of gram Darvan addition (a) 0,2 Gram (b) 0,25 gram and (c) 0,3 gram
The size and arrangement of pores that are getting bigger and bigger will cause thinning of the struts which result in a decrease in compressive strength (Ramay and Zhang, 2003).

In this study, fluctuations and decreases in compressive strength in the sample were caused by the uniformity of surface and pore morphology formed on a scaffold it can be proven by SEM analysis in Figure 6. The addition of dispersants affects the structure and pore size formed on green bodies HA it shows in Figure 7. In Figure 7 (a) the pores formed are larger and more numerous than in Figures (b) and (c). In Figure (a) the pores formed are large and scattered throughout the surface of the sample, and have more pores of large size. Figure (b) has many open pores of relatively large size but looks denser than in Figure (a), and Figure (c) the entire surface of the sample has pores but with a smaller size than the sample (a) and ( b). The addition of a number of dispersants decreases the porosity formed in the sample, Fu et al. [8] state that the addition of a mass amount of dispersant reduces the formation of flocculation in the suspension. This causes the suspension formed to have good colloidal stability with a high degree of homogeneity so that the resulting slurry is able to maintain its structure in the template before it is sintered and produces good pore after sintering.

3.6. XRD (X-Ray Diffraction) Analysis

The results of the hydroxyapatite diffractogram below are obtained from samples with stirring times of 16 and 24 hours and the addition of 0.25-gram dispersant mass. Figure 8 is a diffractogram of the resulting green bodies, where the hydroxyapatite peaks in the image are similar to the standard hydroxyapatite characterization patterns of JCPDS (Joint Committee on Powder Diffraction Standards) No. 09-0432 data, namely (002), (112) and (300) angle 2θ which is 25,879°; 32,196° and 32,902°.

![Hydroxyapatite diffractogram analysis](image)
4. Conclusions

HA scaffold has been successfully made using Luffa cylindrica as a pore-forming template. The addition of dispersant and stirring time causes shrinkage and decreases porosity and increases sample density. Compressive fluctuations that occur in the addition of 0.25 grams of dispersant and stirring time variations of 16 hours; 20 hours; and 24 hours, caused by differences in the surface area of the test sample which is 1.36; 1.14; and 1.44 cm with the resulting compressive strength is 2.88; 2.10; and 3.81 MPa. The optimum results were obtained by variations in the addition of 0.3 grams of dispersant and stirring time of 24 hours with a density of 1.47 gr/cm³; porosity of 53.42%; and compressive strength of 0.61 MPa.

The resulting HA scaffold has a density in the range 0.92-1.47g/cm³; Porosity of 53.42-70.78%; and compressive strength of 0.61-3.81 MPa. The optimum results were obtained by variations in the addition of 0.3 grams of dispersant and stirring time of 24 hours with a density of 1.47 gr / cm³; porosity of 53.42%; and compressive strength of 2.59 MPa.

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