

Preparation of Porous Hydroxyapatite Bodies Using Bamboo and Rattan Templates

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Abstract—Hydroxyapatite (HA) has been widely used as bone graft in form of porous HA/scaffold. This research has a purpose to fabricate HA scaffold by using different biotemplates and determine which biotemplate will give well pore structure on produced HA scaffold. First, HA scaffold obtained used bamboo template. Templates were prepared by cutting bamboo into cylindrical shape and then extracted using ethanol and NaOH solution. Then dipped into mixed of sago starch, NaOH, and distilled water solution / grafting solution. Templates then soaked into CaCl_2 1 M solution and then impregnated in 0.6 M KH_2PO_4 solution. Templates then dried at 110°C for 2 h. Second, HA scaffold obtained used rattan template. Templates were prepared by cutting rattan into cylindrical shape. Slurry was prepared by mixing HA, sago, distilled water and Darvan 821 A. Templates then impregnated into slurry and stirred coincidentally. Templates then dried at 110°C for 1 h. Both templates obtained pore sizes at range 100-500 μm . According to physical properties, it is clear that rattan is more suitable for using as template in replica method. Sample of HA scaffold that produced at stirring time for 12 h has more similar physical properties with bone implant than other samples.

Keywords—hydroxyapatite, biotemplate, bamboo, rattan, scaffold

I. INTRODUCTION

Bone grafting is one of the most commonly used surgical methods to augment bone regeneration in orthopedic procedures [1]. There are several methods of implantation that are often commonly used, namely autografts, allografts and xenografts. However, those methods still have weaknesses such the limited source of implants and the risk of disease transmission [2], therefore alloplast methods that use synthetic biomaterials are

developed as bone implant material [3]. One of these synthetic biomaterials is hydroxyapatite [4].

Hydroxyapatite (HA) is an apatite compound which has the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. HA is the main inorganic component in bones and teeth [5]. HA has excellent biocompatibility, osteoconductivity and chemical and biological affinity for bone tissue [6]. These properties make the ideal HA used as a component of bone and dental engineering [7].

Nowadays, HA mostly used in terms of porous HA scaffold. HA scaffold is more resorbable and more osteoconductive compared to HA dense. In addition, porous HA scaffold has a large surface area and mostly used as implant for spongy (trabecular) bone. The presence of pores in the HA scaffold is needed for bone tissue formation for migration and proliferation of osteoblast and vascularization. In addition, the porous surface is able to increase the mechanical binding power between the implant and the bone so that giving greater mechanical stability [8]. However, it is well known that the mechanical strength of a material decreases as its porosity increases. This conflicting nature induces a challenge in developing porous scaffolds for bone tissue engineering. Numerous methods have been developed to fabricate the porous HA scaffolds including incorporation of volatile or combustible burnout of organic particles in the HA powder, gel casting of foams, and replication of a porous substrate [9].

Replica method is a method based on impregnation of cell structures with ceramic suspense to produce macroporous ceramics that have same morphology as the original porous substrates [10]. HA scaffold that produced by this method had an open, relatively uniform, and interconnected porous structure [11]. Replica method use template as pore former. The template can be either

synthetic or natural material. The mostly used of synthetic material on this method is polymeric sponge. Nowadays, the use of polymeric sponge has been improved by using natural template such luffa fibers [12]. This method use bamboo and rattan as template for pore former in different ways. Both templates are used because they have similar shape with bone in order to the pore structure. The use of both templates is done to see how the effect of using different biotemplates on the porous HA bodies that produced.

II. MATERIALS AND METHODS

A. Materials

Bamboo and rattan as biotemplate for pore former are bought from local market in Pekanbaru, Riau. Distilled water and ethanol were supplied by BratacoChemica Indonesia, sodium hydroxide (NaOH), calcium chloride (CaCl_2) and potassium dihydrogen phosphate (KH_2PO_4) as ingredients that used in the process of forming hydroxyapatite compounds on bamboo replicas were purchased from Merck (Germany). HA powder as solid loading was supplied by Lianyungang Kede Chemical Industry Co. Ltd, China. Sago starch as binder was supplied by Puri Pangan Sejahtera, Indonesia. Darvan 821 A as dispersant was supplied by Vanderbilt Company, USA.

B. Methodology

This method used bamboo and rattan as template. Both templates have macropores that are similar with spongy bone. It is possible for slurry to fulfill the pores of both templates and produce HA scaffold.

1) *Synthesis of HA scaffold by using bamboo as template:* Templates were prepared by cutting bamboo into cylindrical shape with a diameter of 1 cm, with a length of 1 cm. Bamboo which has cylindrical shape might prevent from having outer and inner layers of bamboo. It was extracted by using ethanol for 7 days then dried at 40°C for 12 h, then reprocessed with NaOH solution for 20 min and dried at 40°C for 1 h. To form a binder solution, 12 g of sago starch, 4 g of NaOH and 88 g of distilled water are mixed and left for 30 min. Templates are then dipped into grafting solution for 3 h and then rinsed with distilled water. Templates were immersed in a CaCl_2 1 M solution with different temperatures (100°C and 150°C) and immersion times (12 h, 14 h and 16 h) and then dried at 80°C . The results then immersed in 0.6 M KH_2PO_4 solution at 110°C for 18 h. Furthermore, templates that have been impregnated by phosphate solution were dried using an oven at a temperature of 110°C for 2 h.

2) *Synthesis of HA scaffold by using rattan as template:* Rattan is cut by using a saw with a length of ± 1 cm. The surface of the rattan then cleaned so that the pores were open perfectly. Slurry is made by mixing HA, sago, distilled water and Darvan 821 A with a magnetic stirrer. First, the ingredients are weighed according to the research variable. Then the beaker is prepared on a magnetic stirrer. Darvan 821 A and distilled water are poured into a beaker. Next, HA and sago are then poured slowly into a beaker while stirring slowly with a stirring rod. After all parts of HA and sago are mixed evenly, then the magnetic stirrer is set at a

speed of 400 rpm. The duration of stirring varied for 8, 12, and 16 h. After the slurry has been mixed, rattan is impregnated into the slurry and stirred coincidentally so that the slurry enters the rattan pores and covers all parts of rattan. Furthermore, the rattan which has been impregnated by slurry is dried using an oven at a temperature of 110°C for 1 h to produce HA scaffold.

3) *Characterization:* Characterization of crystalline structure was carried out by using XRD analysis, characterization of morphology and pore size was carried out by using SEM analysis. The mechanical strength of the porous HA bodies was achieved by using universal testing machine. The average pore size was calculated by using ImageJ v.151 software. The result of analysis was pore surface area. The pore size is calculated as the diameter of the pore by using circle area formula on the pore surface area resulted.

III. RESULT AND DISCUSSION

A. XRD Analysis

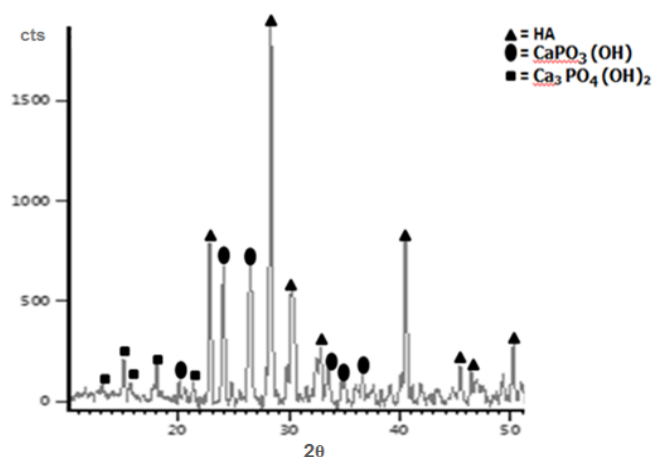
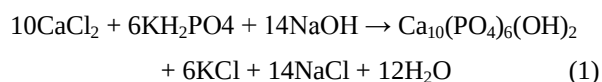


Fig. 1. Diffractogram results of synthesis of porous HA at immersion time of 14 h and temperature of 150°C

XRD analysis was carried out to determine the chemical composition of the product. This analysis was carried for sample with bamboo template at immersion time for 14 h and temperature of 150°C .

From Fig. 1, HA peaks were found at 2θ i.e 22.8585° , 28.3445° and 40.4914° . The reaction of formation HA that occurred as follow below:



Besides of HA, sample contains another chemical compounds such as $\text{CaPO}_3(\text{OH})$ and $\text{Ca}_3\text{PO}_4(\text{OH})_2$. It showed that the reaction produced side products which are undesired for bone implant.

B. SEM Analysis

Soaking time ratio affects the produced coating. Figure 2 shows SEM results of coating surface of sample with immersion time on CaCl_2 for 12 h and 16 h. The longer of immersion time made formed pores being greater. Formed

open pores have sizes between 200 - 500 μm . These results have met the criteria for porous bone implant prerequisites ranging from 100-400 μm [13]. It is important to keep the pore size of produced HA scaffold. Greater pore size will affect the porosity and next affect the mechanical strength of HA scaffold. HA scaffold with greater pore size than the range has lower density, it caused by the space because of the greater pores. Low density makes HA scaffold has greater porosity, so that HA scaffold will be brittle, then the mechanical strength will be decreased according to the increasing of porosity [14].

Figure 3 shows the morphology of HA scaffold at 12 h and 16 h of stirring time. The formed open pore has sizes between 100-400 μm . Both of templates given well pores size for bone implant. Rattan gives more stable pore size than bamboo. The pore size of HA scaffold by using rattan template achieved the range for qualified bone implant than bamboo template that has some greater pore size than the range. It is because bamboo has greater pore structure than rattan.

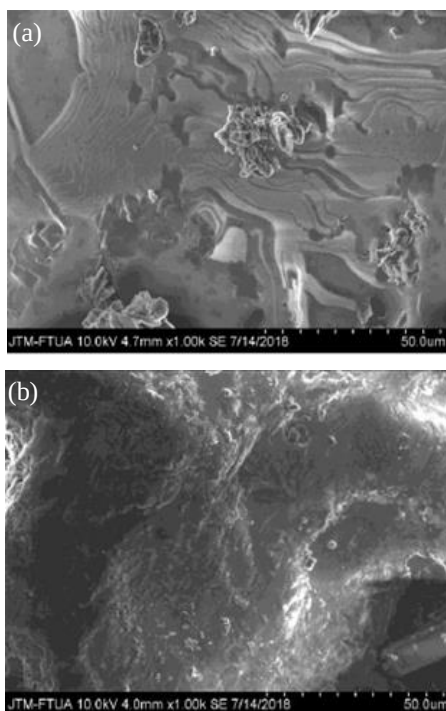


Fig. 2. SEM result of coating surface at 1,000x magnification at immersion time of (a) 12 h (b) 16 h

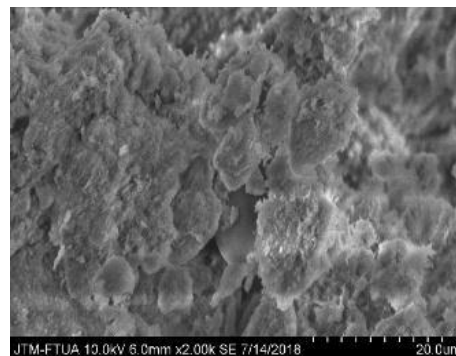
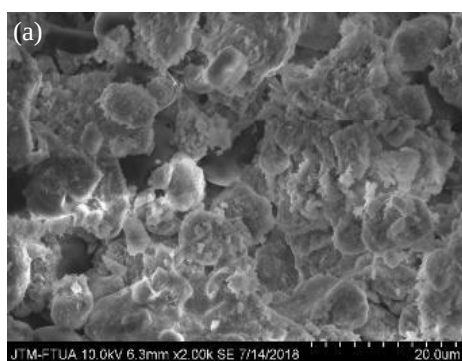


Fig. 3. Morphology of HA scaffold at 2,000x magnification for stirring time (a) 12 h (b) 16 h

C. Density, Porosity, and Compressive Strength

Figure 4 shows the obtained density for both templates. HA scaffolds that were produced by using rattan template have higher density (0.84-0.90 g/cm^3) than bamboo (0.166-0.1986 g/cm^3). A Commercial bone implant has density at range 0.402-3.941 g/cm^3 [15]. From Figure 4 it is clear that HA scaffold that used rattan template is able for bone implant in terms of density. HA scaffold that used bamboo is too dense, so that it doesn't suitable for bone implant.

Figure 5 and Figure 6 shows the porosity and compressive strength of produced HA scaffold respectively. From both figures, it's seen that the porosity was inversely proportional on the compressive strength which was the increasing of porosity caused the compressive strength decreased. HA scaffolds that were produced by using bamboo template have higher porosity (80.95-83.93%) than rattan template (71.34-73.25%) but its compressive strength are lower (29-35.2 MPa) than rattan template (37-45 MPa). For implantation, HA scaffolds should have porosity value at range 30-90% [16] and compressive strength at range 2-230 MPa [17]. According to their porosity and compressive strength, both of templates give well qualification in terms of porosity and compressive strength.

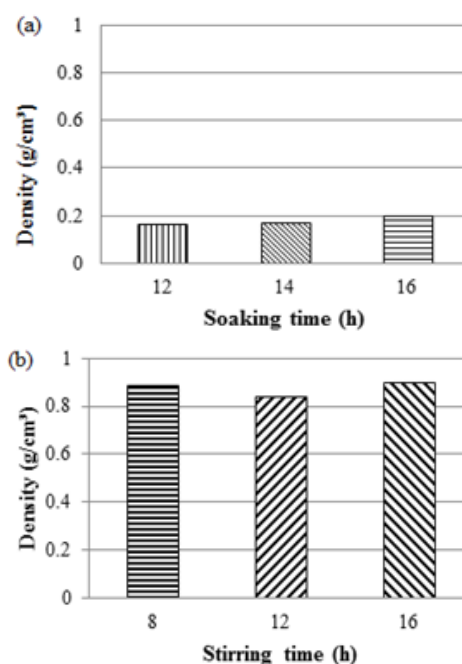


Fig. 4. The density of HA scaffold (a) bamboo and (b) rattan

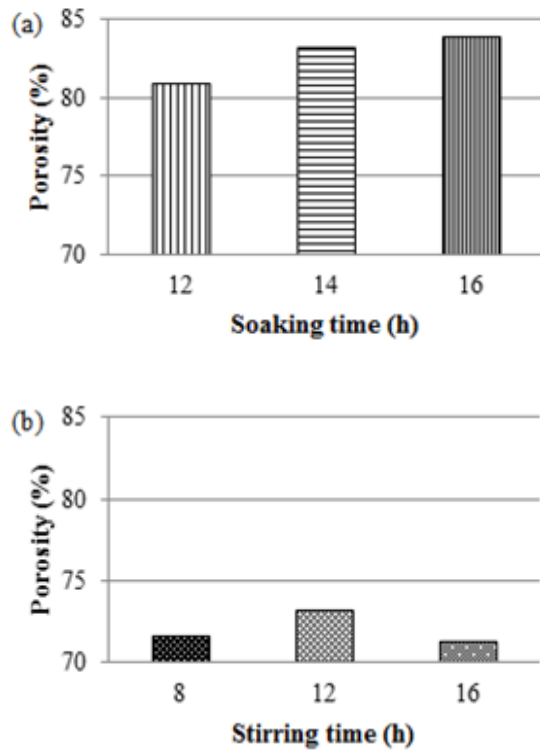


Fig. 5. The porosity of HA scaffold (a) bamboo and (b) rattan

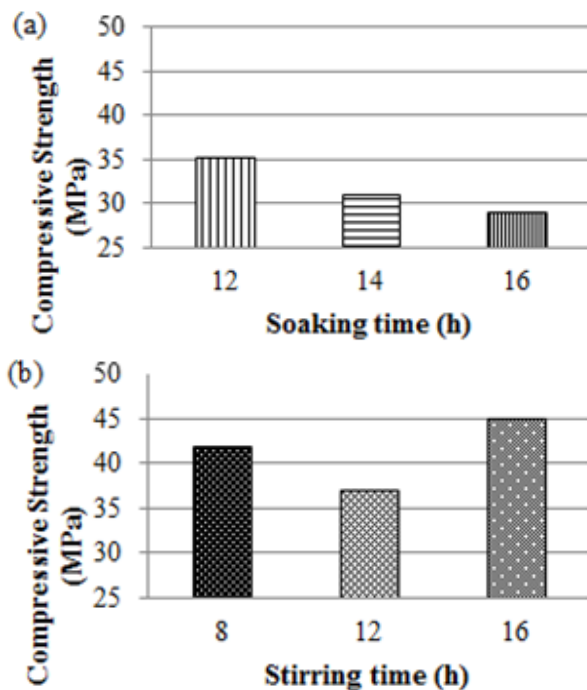


Fig. 6. The compressive strength of HA scaffold (a) bamboo and (b) rattan

D. Result on Visual Analysis

The visual analysis that were carried out on HA scaffolds by using bamboo template show that almost all samples had layer that are formed evenly. Only sample that was soaked at 100°C for 12 h didn't have evenly formed layer. This is due to by a lack of immersion time that caused the layer didn't settle perfectly on the bamboo.

Figure 6 shows the display of HA scaffolds with different surface layers. Figure 1 (a) sample with an evenly formed layer and (b) sample with an uneven formed layer. In Figure 1a bamboo template was covered perfectly by slurry on its surface, meanwhile in Figure 1b bamboo template wasn't covered perfectly on its surface.

Figure 7 shows samples after dried at 110°C for 1 h for rattan template. Visually it can be seen that there was HA formed a body like rattan. It was expected that in each HA sample it can fill every space or pore that appears on the rattan. Based on the three samples with variations in stirring time, sample with the longest stirring time (16 h) has a better structure compared to other samples. It is because the longer rattan was impregnated into slurry more slurry will fulfill the pores and cover the rattan.

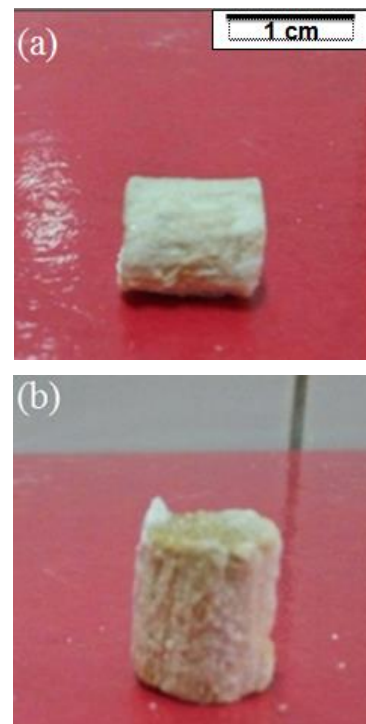


Fig. 7. Optical image of sample at 100°C for (a) 14 h and (b) 12 h of soaking time

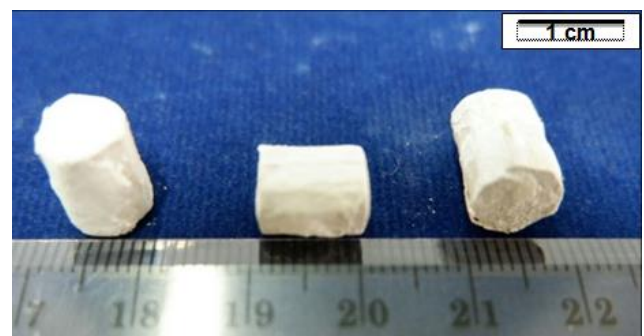


Fig. 8. Porous HA bodies by using rattan as template.

IV. CONCLUSION

HA scaffold have been fabricated by using replica method with bamboo and rattan as template for pore former with pore sizes at range 100-500 μm . According to physical

properties, it is clear that rattan is more suitable for using as template in replica method. On this method, sample of HA scaffold that produced at stirring time for 12 h has more similar physical properties with bone implant than other samples.

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