Characterization of porous hydroxyapatite-alumina composite scaffold produced via powder compaction method

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Abstract. Recently research on hydroxyapatite bioceramic material has been carried out rapidly to support the needs in the medical field. The study aims to develop porous bioceramic from hydroxyapatite Al₂O₃ composites. Porous hydroxyapatite Al₂O₃ composites were prepared with variation of the weight fraction of Al₂O₃ reinforcement and green bean starch space holder used as porous maker. The manufacturing process begins by mixing hydroxyapatite powder (200 μm), Al₂O₃ powder (55 μm) and green bean powder (200 μm) using a Ballmill with a rotating speed of 225 rpm for 1 hour. The mixture is then put into a mold and compressed in a unidirectional compression device at a pressure of 2000 psi. Green body specimens are then sintered at a temperature of 1200°C and holding time for 3 hours. The apparent density test was carried out using the Archimedes’ method and the highest density was 1.95 g/cm³ with 41.915% porosity in the specimen with 25% Al₂O₃ weight fraction. The results of the compressive test showed that specimens with a weight fraction of 25% Al₂O₃ had the highest compressive strength of 1.01 MPa with a porosity of 41.915%. The morphology observation using the Scanning Electron Microscopy (SEM) showed interconnecting porous had been formed.

1. Introduction
Hydroxyapatite (HA) is one of the ceramic based biomaterials that have been widely used as implant material. Generally HA is used to repair, graft, fill or replace the human hard tissue, especially bones and teeth. This synthetic material HA has similarities with bones and teeth in humans in terms of chemical composition and its crystal structure. This material belongs to the family of calcium phosphate compound with molecular formula Ca₁₀(PO₄)₆(OH)₂ [1]. In addition HA is also able to meet the absolute requirements of biomaterials such as excellent biocompatibility with hard tissue, bioactivity properties that capable of reconstructing damaged tissue either in hard or soft tissue and bioresorable properties that can facilitate the dynamic process of both bone formation and reabsorption inside bones [2].

Currently researchers have developed several methods of hydroxyapatite synthesis including so-gel [3], mechanochemical [4], hydrothermal [5] and precipitation method [6]. These methods are normally performed using synthetic materials and complex equipment. Currently, HA has developed the HA synthesis method using natural materials with a simpler process. However some natural resources can be used as raw materials for HA synthesis such as egg shells [7], coral [8] and bovine bone [9]. In general, each both synthesis method and row material used will affect the composition, size and morphology of hydroxyapatite.

In Indonesia, based on BPS data in 2016 the number of beef cattle increased by 3.06 percent compared to 2015 and continued to increase significantly in 2017 with a total of 16,599,247 [10]. These numbers indicate potential utilization of beef bones as raw material in HA synthesis. Beef cattle is definitely producing waste, one of which is bovine bone. Utilization of these waste provide not only
added value to bovine bone waste but also one of the environmentally friendly HA raw materials. The process of HA synthesis from bone waste generally can be done via a simple method that begins from the washing and boiling to remove fat attached, then the cutting process becomes a small size and ends with calcination.

Bone is a composite material consisting of calogen matrix reinforced by crystalline HA. The bones are composed of two main structures: cortical bone and porous bone (cancellous bone) that naturally have their respective functions. Porous bones generally function to dampen shock waves that arise because of various human activities such as jumping, running, and walking. Cancellous bone also has the function to spread vitamins and nutrients to the bones and other parts of the body through the tissues and caliper blood vessels and it has also function for the metabolism of calcium and the production of red blood cell [11].

The method of development of porous hydroxyapatite has been carried out by researchers such as method of slip casting [12], gel-casting [13], sequential freeze casting [14], polymeric sponge replication [15], protein foaming-consolidation method [16] and powder metallurgy methods with applying a material as space holder [17]. The Porosity formed via space holder method depend the size of the short-term constituent part mixed to the matrix. This constituent will burn in the sintering process and leave marks as pores. Various type of space holder have been applied in producing porous structures by some researchers [18-23].

Hydroxyapatite intrinsically owing to low mechanical properties such as compressive strength and bending strength, therefore it is necessary to improve its mechanical properties by reinforcing with Al₂O₃. The main objective of this work is to characterize fabricated porous hydroxyapatite-Al₂O₃ composites with the application of space holder materials (i.e. green bean starch) through the powder compaction method.

2. Materials and Method
Waste bovine bone from local market is used as a raw material to produce HA. Firstly, bone is cleaned from tissue and substances that are on the bone surface by washing then proceed with the process of boiling the beef bones for 5 hours to clean the marrow and tendons easily. After that the drying process is carried out until the color of the cow bone becomes yellowish white. Then the bone is cut into small pieces to reduce dimensions. Subsequently bone is calcined using an electric furnace up to temperature of 600°C and heating rate of 3°C/min then hold for 30 minutes before cooled inside furnace. During calcination process the organic substances decomposed and water evaporated. Calcined bone beef is then crushed using mortar to produce powder. Raw material for space holder is green bean (Vigna radiate). Following washing and drying, green bean is then blending and sieving until achieve preferred particle size.

| No. | Sample       | HA (%wt) | Al₂O₃ (%wt) | Space Holder (%wt) |
|-----|--------------|----------|-------------|--------------------|
| 1   | HA-5Al₂O₃    | 95       | 5           | 20                 |
| 2   | HA-15Al₂O₃   | 85       | 15          | 20                 |
| 3   | HA-25Al₂O₃   | 75       | 25          | 20                 |

Powder of hydroxyapatite (200 μm), Al₂O₃ (55 μm) and green beans (200 μm) were mixed using a Groschopp Viersen FRG ballmill for 1 hour with a speed of 225 rpm. Composition of each samples are listed in table 1. with percentages of space holder refer to total weight of composite. Cylindrical samples with length 12 mm and diameter 10 mm are formed via a unidirectional compaction. The compaction process has been done with applying pressure of 2000 Psi for ten minutes. Subsequently conventional electrical furnace Nabertherm GmbH was used in sintering process with 5°C/min heating rate. Two step holding process was applied during sintering where firstly hold for 60 minutes at temperature of 600°C to facilitate complete burn of green bean and secondly 3 hours holding time at final temperature of 1200°C. Heating schema in sintering process can be seen in figure 1.
The Archimedes’ principle was applied to calculated apparent density and relative density was calculated using eq. 1.

$$\rho_{\text{relative}} = \frac{\rho_{\text{apparent}}}{\rho_{\text{theoretical}}} \times 100\% \quad (1)$$

where:

- $\rho_{\text{apparent}}$: apparent density (g/cm$^3$)
- $\rho_{\text{relative}}$: theoretical density (g/cm$^3$)

Theoretical density was calculated using the Role of Mixture and porosity was calculated using eq. 2

$$\rho_{\text{relative}} = 1 - \Phi \quad (2)$$

where:

- $\Phi$: Porosity (%)
- $\rho_{\text{relative}}$: Relatives density (%)

Various experimental method have been done to characterize powder green bean, HA and Al$_2$O$_3$ as well as porous HA- Al$_2$O$_3$ composite including Scanning Electron Microscopy (FEI Inspect S50), and Thermo Gravimetric Analyzer (TA Instruments TGA Q500), X-Ray Diffraction (Rigaku MiniFlex 600) and Hydraulic Universal Material Tester (Bongshin).

3. Results and Discussion

Thermo gravimetric analyzer (TGA) was carried out to determine the rate of change in weight to the temperature function of green bean powder from room temperature to a temperature of 600°C. From figure 2 can be seen, the recording data test starts at a temperature of 50°C and unto the temperature of 180°C the green bean starch has experienced a severe reduction due to the loss of water content. Continuously increasing heat from temperature of 230°C to 400°C resulted the green bean starch burn out its organic constituent and the weight decreases significantly from 84.76% to the remaining of 3.989%. It can also be seen at a temperature of 580°C, green bean powder still has 3.082% by weight, visual investigation showing that the sample remain in the form of black color ash and has not been burned out.
As can be seen from figure 3 the XRD results of calcined bovine bone at a temperature of 600°C in the red graph while hydroxyapatite standard according to ICDD 09-432 in the blue graph. It can be seen that the XRD graph of the sample close to the ICDD standard 09-432 at 2θ of 26.27, 29.61, 32.43, 33.31, 35.41, 40.19, 51.52, and 64.65°. It is also confirm that calcined powder is pure hydroxyapatite since there is no α-TCP, β-TCP or TTCP peaks detected.
Figure 4. XRD result of Aluminum Oxide (Al$_2$O$_3$) Powder

The XRD result of Al$_2$O$_3$ powder shown in figure 4. It can be seen that the XRD results obtained peaks of Al$_2$O$_3$ samples close to the standard ICDD 46-1212 for aluminum oxide (Al$_2$O$_3$) material. Peak-peak shown confirm the pure Al$_2$O$_3$ since similarities found at 20 of 8.120, 15.929, 25.882, 35.451, 38.071, 41.94, 43.638, 52.835, 57.792, 61.591, 66.785, 68.467, 77.137, 77.477°.

Before the sintering process the specimen has a black color while after undergoing the sintering process at a temperature of 1200°C for 3 hours the specimen changes color to white. The color change from black to white shows the decomposition of organic matter. White color appearance due to the complete decomposition of organic matter.

Figure 5. XRD result of Porous HA-25Al$_2$O$_3$ Composite Sintering Temperature 1200°C

The results of XRD on Porous HA-25Al$_2$O$_3$ sintered at temperature of 1200°C shown in Figure 10. It can be seen that the porous HA-25Al$_2$O$_3$ composite which was sintered at 1200°C produced a more crystalline hydroxyapatite phase. This can be seen by comparing the intensity of the hydroxyapatite powder from the calcined 600°C with the sintering results at temperature of 1200°C. In addition, the β-TCP phase (ICDD 09-169) also detected at 20 of 25.789° and 39.850°. The presence of β-TCP phase is
also confirmed the partial dehydration that leading decomposition of bovine bone derived hydroxyapatite sintered at 1200°C as reported earlier by Ooi. The reaction process of hydroxyapatite in the sintering temperature producing CaO and water vapor that modify the location of the phase boundaries and promote hydroxyapatite decomposition into TCP [24].

The sintered samples are weighing and measuring the dimensions to determine changes in weight and shrinkage volume. It can be seen from figure 6 the highest shrinkage is found in sample of HA-5Al₂O₃ with an average of 22.88%. The lowest shrinkage specimens are found in specimens with a weight fraction of HA-25Al₂O₃ with an average of 7.99%.

The apparent density results that have been carried out shown in figure 7. The highest apparent density is 1.95 g/cm³ for porous HA-25Al₂O₃ and the lowest apparent density is 1.68 g/cm³ for hydroxyapatite composites Porous-Al₂O₃ is the composite with a weight fraction of 5% alumina.
Similarly, the theoretical density depicts the increasing trend with alumina addition where the highest density of 3.324 g/cm³ in the specimen with a weight fraction of 25% Al₂O₃ and the smallest density of 3.188 g/cm³ in the specimen with a 5% Al₂O₃ weight fraction respectively. Figure 7 also depicts porosity trend of the sample and it is found in the specimen with a weight fraction of 5% Al₂O₃ with porosity 47.305% and the lowest porosity is in the specimen with a weight fraction of 25% Al₂O₃ with porosity 41.34%. This result is the same as the research that has been done before, increasing the weight fraction of Al₂O₃ will reduce the porosity formed in the composite [16]. The porosity trend also can be confirmed with XRD results. As explained earlier at a sintering temperature of 1200°C, hydroxyapatite decomposed to the β-TCP phase, more hydroxyapatite weight more the β-TCP phase produced and vice versa. An increasing third constituent i.e. the β-TCP phase in the sample suppressed diffusion rate among particle that decreasing densification.

![Compressive Strength Results of Composite at Various Al₂O₃ Weight Fraction](image1)

**Figure 8. Compressive Strength Results of Composite at Various Al₂O₃ Weight Fraction**

![SEM Results of HA-15Al₂O₃ Composite at Two Different Point](image2)

**Figure 9. SEM Results of HA-15Al₂O₃ Composite at Two Different Point**

The results of compressive strength to weight fraction of Al₂O₃ in the samples can be seen in figure 8. It can be seen that the highest compressive strength was found in samples with 25% weight fraction of Al₂O₃ and the lowest strength specimens found in specimens with 5% weight fraction of Al₂O₃. The
decreasing compressive strength at high content of hydroxyapatite due to high porosity of the samples as depicted at figure 7. The increasing $\beta$-TCP phase at high content of hydroxyapatite affected to compressive strength as well. The $\beta$-TCP is a kind of biodegradable calcium phosphate material owing to low mechanical property.

Microstructure observation using a Scanning Electron Microscopy (SEM) was carried out in the sample with a 15% alumina weight fraction. Two different observation point from figure 9 shows clearly the interconnecting porosity. Two distinct size of porosity can be detected noticeably in the samples. The wider porosity size suggested the effect of space holder while smaller one associated with diffusion of particles in sintering process.

4. Conclusion

1. Porous structure of hydroxyapatite-alumina composite has successfully produced using green bean as space holder.
2. Density characteristics of the sample show that the composite with 25% $\text{Al}_2\text{O}_3$ weight fraction had the highest apparent density with an average of 1.95 g/cm$^3$ and composites with 5% $\text{Al}_2\text{O}_3$ weight fraction had the lowest apparent density with an average of 1.68 g/cm$^3$.
3. Compressive strength results show that the addition of $\text{Al}_2\text{O}_3$ weight fraction can increase the compressive strength of porous hydroxyapatite-$\text{Al}_2\text{O}_3$ composites. Specimens with 25% $\text{Al}_2\text{O}_3$ weight fraction have the highest compressive strength, with an average of 1.01 MPa with an average porosity of 41.915% and specimens with 5% $\text{Al}_2\text{O}_3$ weight fraction have the lowest compressive strength, with an average of 0.2 MPa and average porosity of 47.44%.
4. SEM observations of microstructure confirm that an interconnected porous composite successfully developed.

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