BOVINE BONE HIDROKSIAPATITE MATERIALS MECHANICS PROPERTIES AT 900°C AND 1200°C OF CALCINATION TEMPERATURE

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Abstract:

The aims of this study is to determine the mechanical characteristics of the calcinated scaffold material bovine hydroxyapatite (BHA) for bone filler applications. Scaffold BHA was obtained from femur section of bovine bones which cut into 10x10x10 mm. Scaffold BHA was calcinated by temperature variations of 900ºC and 1200°C for 2 hours with 10ºC/min as the amount of the increasing level. The study result of each scaffold BHA which had been calcinated by 900°C and 1200°C has a hardness value of 8.48 ± 0.1118 VHN and 12.37 ± 0.5803, meanwhile the compressive strength value of each scaffold BHA samples is 3.03 ± 0.6764 MPa and 1.96 ± 0.3450 MPa. The porous on scaffold BHA samples calcinated by 900°C and 1200°C which had been observed by SEM had porous size that is not much different, it was ± 200-400 μm, the difference can be seen from the smaller porous size of the scaffold BHA calcination 1200°C compared to the porous size of scaffold BHA calcination 900°C.

INTRODUCTION

Approximately 40% of body rigorous tissue damage due to brittle bones, bone cancer or accidents occurred in Indonesia, and the rest as congenital [1]. The bone filler needs for transplant and implantation were significantly increased on its restoration. An inexpensive bone filler material was necessary for Indonesian patients because there were fewer transplantation donors. Synthetic materials such as metal alloys or bioceramics were expensive because it is imported, whereas the cow bones waste as a bioceramics scaffolds raw material is abundant in Indonesia. Many studies were utilizing it to create hydroxyapatite material (HA) as one of the bioceramics products variants which used for the medical field that has economic value and environmental friendliness.

Hydroxyapatite (HA) is an excellent biomaterial for substituting bone because it has excellent biocompatibility. Hydroxylapatite or hydroxyapatite (HA) with $CA_5(PO_4)_3(OH)$ of molecular formula however, people often write as $CA_{10}(PO_4)_6(OH)_2$ is a calcium bioceramics apatite which can be found in human teeth and bones. It derived from natural sources which can form a strong bond to the bone tissue. Cow bone composition is consisting of 93% hydroxyapatite ($CA_{10}(PO_4)_6(OH)_2$) and 7% β-tricalcium phosphate ($Ca_3(PO_4)_2$, β-TCP) [2]. A porous HA became a fundamental requirement for the bone fractures or cracks reconstructions. The forming bones were served as a bone cells tissue structuring mediator that grows, which can increase bone regeneration properly [3].

HA itself is divided into two types: natural and synthetic. Natural hydroxyapatite is hydroxyapatite which is produced by using natural ingredients that contain a rich of calcium of carbonate $CaCO_3$. Calcium carbonate can be easily found in the environment around us, such as bones, shells, and malleable animal shields. Synthetic hydroxyapatite is hydroxyapatite which is made chemically. It is not only obtained by reacting synthetic compounds, but can also be achieved by reacting synthetic compounds with natural compounds. It is known as one of the important material because it has the bioactive, biocompatible, and osteoconductive properties which are similar to natural bone mineral, so it can be used as a human hard tissue substitution.

Bone filler is made from hydroxyapatite, hydroxyapatite can be made from bones, shells, and malleable animal shields. In health area, its orthopedic surgeon needs is a direct scaffold which utilized to fill the bone damage. Therefore, HA powder has to be further processed into a scaffold. This process requires high temperatures of about 1400ºC, so it is costly. The metal plate applications can not be done because it could not be used as a bone filler. Due to, it will be taken back if the bones has been constructed.

Calcination on beef bones to turn it into hydroxyapatite is necessary. It was used to eliminate bacteria or agents which cause disease [4]. The higher calcination temperature was also very influential in cow bone hydroxyapatite crystallinity level [5]. Its intensity was increased significantly with the increasing of temperature and becomes more prominent as the temperature rises above 600°C. Hydroxyapatite calcination temperature has an optimum temperature to produce the best hydroxyapatite from various temperature calcination levels. Its variations affect the cow bones mechanical properties. Therefore, it needs a temperature that can produce hydroxyapatite which had the best results. On several studies suggest that temperatures of 900°-1200°C had the best results. The best performance of the scaffold was 900ºC. Its treatment was important to create a resilient scaffold HA [6]. The optimum temperature was 900°C for having formed crystals with a high degree of crystallinity or a perfect crystal [7]. Sintering temperature of 1200 ° C and 1300 ° C had higher results containing HA crystals, which have a high density and good mechanical properties [8]. HA was sintered at 1000°C, 1200°C, and 1300°C. Its hardness value increased as the sintering temperature, and maximum compressive strength values obtained on a sample of sintering temperature 1200°C [9]. HA which was generated in this study will be calcined by varying the calcination temperature, ie 900ºC and 1200ºC.

This study was conducted a preliminary study of scaffold bovine hydroxyapatite manufacture with cow bone waste raw material with calcination methods. Cow's femur was then cut to obtain the size of 10x10x10 mm. Cow bone calcination process was performed by 900ºC and 1200ºC of variations in temperature. HA calcination process was using 10ºC of temperature rise every 1 minute until it reaches 900ºC and 1200ºC of temperature respectively. It was accomplished to determine the value and material properties of HA from bovine bones. This study results were expected to be purposed as a new biomaterials and bone filler for fractured patients.

RESEARCH METHODOLOGY

Initial assembling

The initial step in research was to set up the cow bone, thigh one (femur) was obtained from a slaughterhouse cattle/ slaughter house (RPH) in Jagalan street No. 26, Jagalan Village, District Jebres, Surakarta, Central Java Province with cow age range of 2-3 years. The bones were cleaned of meat and fat, then was boiling for 2 hours about 2-3 times until the existing oil at bone and fishy smell disappeared. Afterthat, it was carried out the drying bones to get bone dry, and it was cut using a bone saw machine to get shape/ size of 10x10x10 mm/ pieces. The following process can clearly be seen on Figure 1.

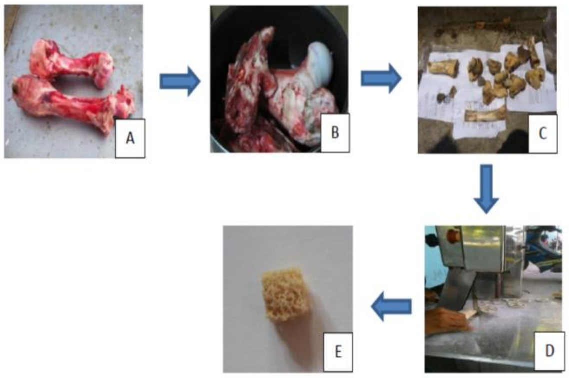


Figure 1. Execution Process

Annotation:
(A) Raw bones
(B) Boiling process
(C) Drying process
(D) Cutting process, 10x10x10 mm of size
(E) Scaffold bovine 10x10x10 mm

Calcination process

The calcination process was performed to obtain a pure HA, calcination was executed by varying 900ºC and 1200ºC of temperature. HA calcination process was using 10ºC of temperature rise every 1 minute until the temperature reaches 900ºC then held for 2 hours and then cooled again to room temperature (27°C) and the same was operated at a temperature of 1200ºC. The following Figure 2 shows a furnace.

Figure 2. Furnace

Testing

This study was implemented three tests, namely hardness, compressive strength test, and HA material characteristics. Hardness testing was using a Vickers Hardness Number (HVN) testing machine with 100g of load and pushing the indenter 10 seconds.

Compressive strength testing was operating by Universal Testing Machine (UTM) in accordance with ASTM standard F-451-95 using a Universal Testing Machine (JTM Technology Machine, 0.5T Capacity) with a load of 50 kg at a speed of suppression 5 mm / min.

SEM (Scanning Electron Microschopy) observations was using SEM (VEGA3 TESCAN) with 20.0 kV HV SEM, Low Vac 17 Pa, 31 Pa, and

15 Pa. It was conducted to determine the bones porous wall surface detail.

RESULT AND DISCUSSION

Calcination Temperature Effect to Bovine Bone Powder

Bovine bone was processed by 2 different calcination temperatures were 900°C and 1200°C. A calcined bovine bone from room temperature (27°C) up to temperatures of 900°C with an increase 10°C/ min and then hold for 2 hours and cooled again to room temperature (27°C), the same method was performed on samples of cow bones calcined at a temperature of 1200°C. During the calcination process, the bone color has been changed between before and after calcined. After calcination, it has a white color and been become bovine hydroxyapatite (BHA). Bovine bone scaffold color, before calcination process at room temperature was yellowish white. The following figures portray bovine bone before and after the calcination process is shown in Figure 3.

(a) (b) (c)

Figure 3. Bovine bone color: (a) Before calcined, (b) After calcined at 900°C, (c) After calcined at 1200°C.

Figure 3 displays the color change from white yellowish to white after calcination, it caused by the organic material decomposition. The white one was generated as a result of the organic material decomposition completely and obtained only the inorganic material.

This was reinforced by studies which claim that the calcination results at 400°C, 500°C, and 600°C of temperature, the bone samples color were turn black, dark gray, and light gray respectively. The calcined bone at ≥700°C of temperature, it became white which was indicating the organic matter loss on the bone. This change was believed that linked to the organic matrix burning process (protein and collagen) in a bovine bone. The dark color of bovine bone samples under a temperature of 700 ° C were showed incomplete decomposition of organic material. The results were obtained from FTIR analysis showed that bovine bones gradually release OH- ions at a temperature above 1100°C (Ooi et al, 2006).

Same as Ooi et al, (2006), Herliansyah et al, (2009) stated that the bovine bone which was containing large pores and small pores but generally a raw bovine bone microstructure was very dense because of their organic substance impregnated with inorganic minerals associated with it. After it has been heated at 900°C for 2 hours, microstructure was changed due to the water content evaporated (about 9 wt %) and organic substances such as collagen (approximately 20 wt.%), proteins, polysaccharides, and lipids which were also contained in a small number, and only a hard inorganic part with high porosity was remaining. The XRD results showed that BHA at a temperature above 1000°C which indicates its decomposition, it decomposed and turned into tri-calcium phosphate (TCP).

[1] declared that the increasing of a bovine bone scaffold calcination temperature showed discoloration, and dark color indicates the incomplete decomposition of organic matter composition. At room temperature, a white bovine bone scaffold slightly yellowish, black at a temperature of 300ºC, gray at a temperature of 600ºC, 900ºC, and white color temperature and 1200°C.

The same was done by [10] that the increasing temperature of bovine bones calcination showed the same color product, HA at room temperature (before calcined) was white yellowish color, at temperature of 700ºC, 900ºC, and 1200ºC were white. Dark gray color was occurred at 400°C of temperature and light gray color appeared at a temperature of 600 ° C, this happens due to the organic substances presence inside the bone. HA was decomposed to another calcium phosphates phase like tri-calcium phosphate (TCP) when calcined at temperatures over 1000°C, so that the calcination temperature played an important role in controlling the HA phase stability and mechanical properties.

From [11], [12], [1], and [10] explanation, it can be concluded that the color change from yellowish white to white after calcination due to the organic substances decomposition (protein and collagen).

Vickers Hardness Testing Analysis

From the data in Figure 4, hardness test results show that the hardness of scaffold BHA samples after calcination was increased. At calcination temperature of 1200°C was higher than 900°C of temperature. It portrays that the high temperature of calcination was affecting an increasing of bovine bone scaffold hardness. This could be happen due to the organic material disappearance on the bones surrounds, so bones became harder. Then, the highest hardness sample was chosen as the best sample which was BHA with calcination temperature of 1200°C with ± 0.5803 12:37 HVN.

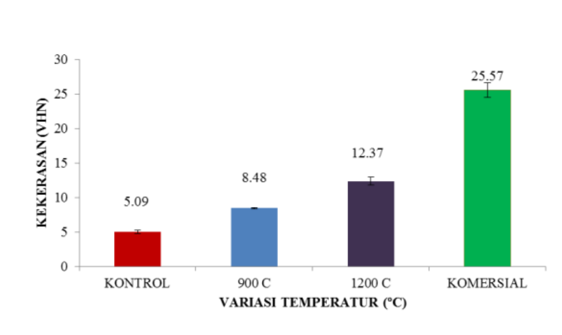


Figure 4. Microvickers scaffold BHA hardness.

It was stated by studies that claim that the HA hardness increases with the increasing of calcination temperature. HA hardness was increased from 152.2 MPa (15:52 VHN) at a temperature of 600°C, 172 MPa (17:54 VHN) at a temperature of 750°C, and then to 192 MPa (19:58 VHN) at 1000°C. [13]. From [13] explanation concluded that the effect of calcination temperature increasing generated the bovine bone material hardness. However, scaffold BHA hardness was exhibited still far from 25.57 ± 1.0692 VHN of commercial HA hardness.

Axial Compressive Strength Analysis

From Figure 5 can be clearly seen that compressive strength of the scaffold BHA specimens is decreased. At 900°C of calcination temperature, it produced 3:03 ± 0.6764 MPa of a compressive strength. At a temperature of 1200°C the compressive strength was decreased, compared to scaffolds BHA calcination temperature of 900°C which was 1.96 ± 0.3450 MPa. Scaffold BHA compressive strength test results portrays that bovine bone before calcined was produced higher compressive strength compared to BHA after calcined. The decreasing of compressive strength was provoked of the calcined bovine bones organic material disappearance, only produced inorganic materials that make BHA calcination becomes more porous. Hardness was inversely proportional to the compressive strength due to the samples hardness. Then, it was also more brittle than calcined sample with a lower hardness [9].

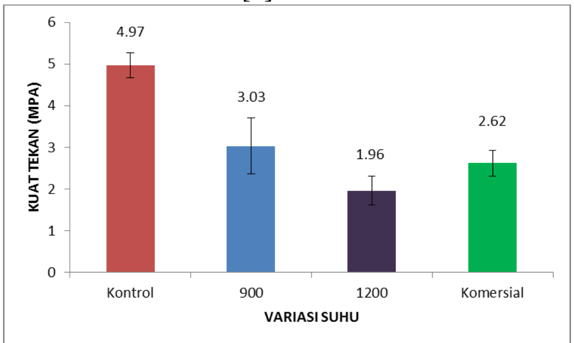


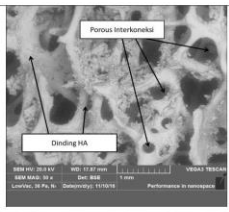
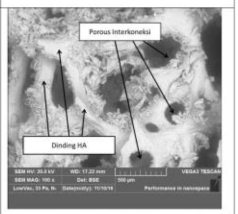
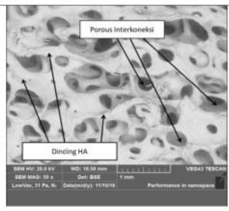
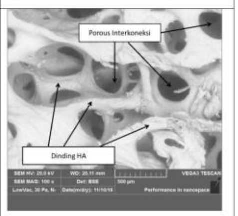
Figure 5. *Axial Compressive Strength scaffold* BHA testing result

It was reinforced by researches which declare that bovine bone before calcined showed highest compression and higher it has been calcined. The higher the calcination temperature provides lower compressive strength. The declining of compress strength due to organic binder was reduced and porousnya bovine bone increased. The BHA strength with PVA coatings was higher than without coating. The PVA percentage expansion will enhance the BHA compressive strength [1]. From [1] explanation was achieved that its high temperature calcination can decrease the compress strength rather than it has not been calcined. The commercial HA has higher compress strength compared to the calcined BHA Scaffold at 900°C was 5.80 ± 0.9702 MPa, however the calcined BHA scaffold at 1200°C has a lower compress strength.

***Scanning Electron Microschopy* (SEM) *Scaffold Bovine Hydroxyapatite* (BHA) Observation**

Table 1 at 900 ° C illustrates the BHA porous scaffold interconnection. It can be recognized the interconnected porous in ± 200-400 μm sized picture. This calcination process removed an organic material and the remaining inorganic material, it was hydroxyapatite. Table 1 at 1200°C of temperature demonstrated the BHA porous scaffold interconnection with porous size that was not much different from 900°C was ± 200-400. Its temperature of 1200°C has a size smaller its wall, which means the size of the porous getting bigger than its porous wall temperature of 900°C. Its growing porous size caused it became more brittle, thus decreasing the compress strength.

Table 1. BHA scaffold micro structure after calcination of 900°C and 1200°C

Suhu Kalsinasi	Perbesaran 50x	Perbesaran 100x
900°C		
1200°C		

This was reinforced by studies which confirmed that the BHA scaffold calcination temperature of 900°C. It was demonstrated porous interconnections exhibit ± 200-300 μm of porous. Bovine bone material calcination can eliminate organic and inorganic residual materials were hydroxyapatites. Calcination process also made it becomes more porous [1]. Similar to [1], [14] announced that 600°C of the calcination temperature, HA grain, and the tissue porous structure was not clearly visible, with the increasing calcination temperature of 750°C and 900°C, porous interconnected structure became quite noticeable. The constructed porous was very nice as bonding tissue when it purposed in ortopedi devices. [10] reported that it still looks collagen and proteins in bovine bone SEM results at 400-500°C of temperature. BHA SEM results at 600-1000°C of temperature to manufacture a special porous interconnection formations and the organic matter disappearance. From [1] and [14], [10] explanations can be concluded that the SEM result at 900°C and 1200°C of calcination temperature were seen an interconnection and BHA walls diminution porous occurred since temperature calcination was climbed up.

In scaffold bovine hydroxyapatite (BHA) research which has been given 900ºC and 1200°C of calcination treatment temperature for 2 hours with a 10ºC/ min of rising temperature. It has 12:37 ± 0.5803 HVN of hardness, 8:48 ± 0.1118 HVN of minimum requirement. The maximum compress strength was 3:03 ± 0.6764 MPa, minimum compressive strength was 1.96 ± 0.3450 MPa. SEM observations indicated that 200-400 lm of the porous interconnect size. Calcination temperature of 900°C was identified to be the best calcination temperature than the calcination temperature of 1200°C with8:48 ± 0.1118 HVN of hardness and has 3:03 ± 0.6764 MPa of maximum compress strength.

CONCLUSION

In this study were obtained several conclusions as follows:

1. BHA scaffold hardness was increasing due to the calcination temperature. It produced 12:37 ± 0.5803 HVN at 1200°C which was higher than 8:48 ± 0.1118 VHN at 900°C of temperature.
2. Bovine bone before calcination had compress strength was higher than bovine bone that has been calcined ie 5419 ± 1.3758 MPa. The BHA scaffold compress strength at 900°C of a temperature 3.03 ± 0.6764 MPa which created higher than 1200°C BHA scaffold was 1.96 ± 0.3450 MPa.
3. SEM observations at 900°C and 1200°C of temperature presented an interconnection porous and produced ±200-400 μm of sized.

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