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Mechanical Properties and Microstructure of Artificial Bone Prototype Made of Bovine Bone Powder by Mixing Method

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ABSTRACT

The purpose of this study is to obtain bovine bone powder with high content calcium-phosphorus coumpound through combination of ball milling and heating processes. These processes was carried out in four stages at a temperature of 800°C for heating process. The obtained powder was then mixed with zirconia and resin, and then molded into a metal mold to obtain artificial bone prototypes in the form of dog-bone tensile test specimen. The tensile test was then conducted to the specimens to obtain the strength and strain capacity of the artificial bones. Fractured specimen surface morphology and sample microstructure were then examined using a scanning electron microscope (SEM) equipped with Energy Dispersive X-ray (EDX). The result of this study shows that the highest levels of calcium and phosphorus contents is 35.07% and 19.04%, respectively, with powder fineness of 63 µm. The strength of artificial bone varies according to the powder volume fraction, where the fraction of 90% powder and 10% resin gives the strength 5,57MPa. While the fractions of 80-20, 70-30 and 60-40 give the strength of 11.84 MPa, 13.66 MPa, and 9,56 MPa, respectively. The microstructure of the specimens shows micro cracks, voids, detachement particles, and agglomeration with different extent depend on the fraction of the powders.

KEY WORDS: *Calcium Compound; Tensile Strength; Mechanical Properties; Microstructure.*

1.0 INTRODUCTION

The high number of human bone fractures due to traffic accidents and osteoporosis in Indonesia has led to increase of bone implants needs [1]. Nowdays, most of implants is imported from abroad with high cost. Therefore, it is necessary to find an alternative material that has low cost and widely available in the field. Bovine bone is potentially be used as a base material for artificial bone and other implants, because it has structure and properties similar to those of human bone. Availability of bovine bone waste from food processing is a huge potential for the raw material of artificial bone. However, the bovine bones cannot be used directly. Organic elements such as protein, fat/lipid, bacteria etc that is existing in the bone must be removed because it can lead to decompose by time during application. Only inorganic substances such as hydroxyapatite (HA), that is contain calcium-phosphorous compound, can be used as raw material for artificial bones. Bovine bone waste in Indonesia is abundant because of the needs of the national beef meat each year reachs 593 tons /year [2]. Since the bovine is approximetly 1/3 of the bone weight, there will be around 200 tons/year of bovine bone waste in Indonesia. Theoretically, bone contains around 1/3 part of HA. Thus, a simple counting, a potential of bovine bones is around 60-70 tons/year. When 10% of the bone waste can be processed to be HA, it will be obtained as much as 6-7 tons/year, a fantastic amount. On the other hand, metal implants have many inherent limitations include physical and mechanical properties that are different from the bones. Moreover, the use of such kind magnetic and electrical material makes patients less comfortable.

Artificial bone made of such bioceramic material can overcome unmerits of the metal implants. HA is biocompatible or acceptable by the immune system of human body. In addition, their use effect may be not so severe physiological damage as compared to metal, because HA is non-toxic and also resorbable [3]. The mechanical properties are also a factor that limits the use of HA as an implant in the high load. As for ceramic materials, HA has a low tensile strength and brittle. Therefore, the mechanical properties of HA implants should be improved in order more potential to be used widely for implants in future. The factor that affects mechanical properties of bioceramic HA is the form of powder, pores, grain size and fabrication methods [4]. The step in HA preparation process will determine the quality and color of bones. Prior to calcination process the color bovine bone powder at room temperature is yellowish white. After calcination temperature of 800°C for 3 hours and followed by cooling slowly to room temperature (27°C), the color changed to be white. Bone weight decreases continuously as the burning of organic substances in the bone. A yellowish-white bovine bone at room temperature can be also change to black at a temperature of 400°C, and then to be gray at a temperature of 600°C, and finally to be white at a temperature above 700°C [5]. Other studies have also found that the process of color change may also occur at a different heating temperature. Some bone powders changes to be gray color at a temperature of 500°C and will turn into white at a temperature of 800°C [6]. The powder may also change into pure white color at a range temperature of 900-1100°C [7]. Meanwhile, other researcher states that the white powder is started to form at temperatures of 800°C and it is completed at temperature of 1100°C [8]. However, researchers agree that the change of color from yellowish white to pure white because decomposition of organic compounds [6-8]. The discoloration is caused by the decomposition of organic material in powder bone where it changes into a darker color shows the burning of carbon and organic material in bone powder and then turned into a clean white of HA [6]. This indicates that there are no clear process steps that are really exact to produce a high-quality HA as indicated by an optimum mineral content (especially calcium and phosphorus). The mechanical properties are also a factor that inhibits the use of hydroxyapatite (HA) as implants especially in part of to bear high loads. HA implant with great mechanical properties is necessary to be expanded further for new biomaterial in the future. Generally, mechanical properties of artificial bone depend significantly on the form of powder, pores, grain size and fabrication methods [9]. Method of fabricating includes how to prepare the artificial bone. Powder/HA can be mixed with resin binder material and other particulates to obtain high strength biocomposite as described below.

2.0 MATERIALS AND METHODOLOGY

Material used in this study is a waste bovine bone obtained from households and self-curring acryilic resin of ortho binder resin that is common for dental application. The waste bone was taken as much as 2,000 gr. The powder preparation were carried out in 4 stages: stage 1 is pulverizing the bones of bovine bone waste. Stage 2 is, namely the manufacture of HA, combines grinding and calcination processes. Stage 3 that is microstructure observation and monitoring of the levels of HA, and stage 4, namely molding and mechanical testing. HA is molded using the mentioned resin with volume fraction of 10% resin and 90% HA. The content of resin is increased to be 20%, 30% and 40% resin with keeping a balance HA. The mechanical testing of bone samples that have been made in a mold, in this study is only tensile testing.

The process of making high calcium hydroxyapatite was also done in several step of the process. The first process began with washing and cleaning processes bone waste, then waste the bones were dried using a special soap and then do the process of size reduction by cutting the bone using a hacksaw. The bone flakes were heated using the oven drying process that aims to reduce the water content in the bones. They were then milled using a blender to obtain coarse bone powder, and followed by refining process of powder using a ball mill and then sieved using a #230 mesh siever. The last step of the process was the process of bone powder calcination that was carried out at a temperature of 800°C with a heating time of 2 hours. For obtaining a high level of calcium, ball milling and calcination processes were conducted four times. The characterization of HA was observed by using SEM and EDX.

Artificial bone prototype was a dog-bone type tensile test specimen that was prepared according to ASTM standard, where the length and diameter of specimens is 36 mm and 6±0.1 mm, respectively as can be seen in Fig. 1.

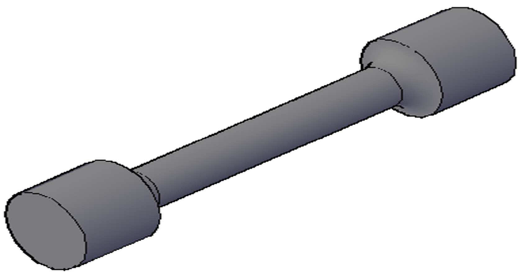

Figure 1: Tensile test specimen of artificial bone

The specimens were fabricated by molding process of the bone powder and polymer resin powder. In the molding process of artificial bone specimens, the content of the resin was varied from 10 to 40% with a composition of 10% resin- 90% powder, 20% resin with 80% powder, 30% resin with 70% powder, and 40% resin with 60% powder. These mixtures was coupled with a 4 ml liquid resin for each composition of the specimen. Furthermore, the molding process of the artificial bones specimens was conducted by mixing process between powder and the solvent (resin). The initial form of the bone powder for main material of the artificial bone is shown in Fig. 2a. Subdsequently, the material that has been mixed uniformly, it was inserted into the mold cavity, and then carried out the compaction by using a finger. The specimen material has been solidified using plaster. It aims to be not the movement of the structure of artificial bone specimen material during the drying process. After 30 minutes, the mold is opened and the results are shown in Fig. 2b. There is 2 samples for each condition in this study.

(a) (b)

Figure 2: (a) Photo HA artificial bone as raw materials, (b) Specimens Photo Artificial Bone

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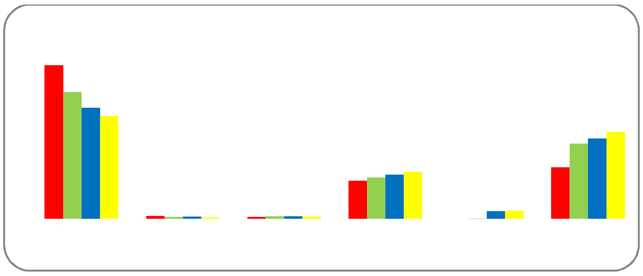
3.0 RESULTS AND DISCUSSION

3.1 Powder Composition

Fig. 3 shows the change of composition for each stages mentioned above. It can be seen that the composition is different for each process. The difference is the result of the process of refining and re-calcination of HA. Calcium content increases rapidly from calcination 1 to calcination 2. On the calcination 4, calcium level reachs 35.07%. At the same time, oxygen level is decreased by increasing calcination stages. This is due to the oxidation process of calcinating powders bones. Oxygen will decompose into air so that the composition of calcium and phosphorus increase with increasing calcination level. In the previous study, the high calcium level is obtained by heating at a longer (over 24h) and higher temperature (above 800^{o}C).



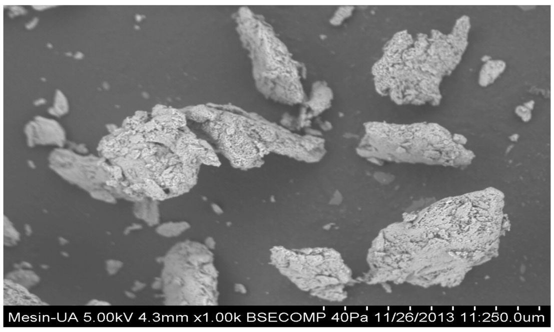
Calcination 1 Calcination 2 + Ball Mill

Calcination 3 + Ball mill Calcination 4 + Ball mill

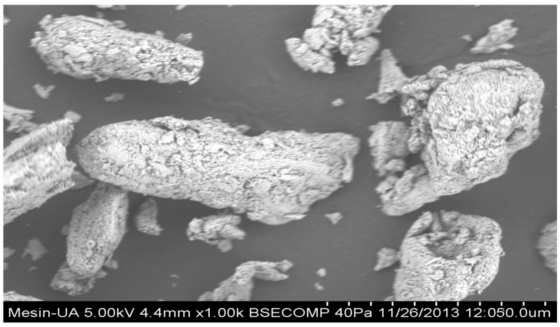
Figure 3: Composition of bone powder for each calcination

3.2 Bone Powder Morphology

SEM examination of the powder shows clearly changes the shape and morphology of powders at each stage of the process (Fig4a). It can be seen that, at an early stage of calcination process, the powder grain is coarse with irregular shape. Such a structure is not so suitable for the manufacture of artificial bones.



(a)



(b)

(c)

(d)

Figure 4: SEM micrograph of the powder for a) first stage, b) 2nd stage, c) 4th stage, and 4th stage of calcinations

In Fig. 4b is the second stage calcination process that is interspersed by ball mill porocess. It can be seen that there is unification granules and compaction after reheating, but the grain size is not too much change. This is due to uneven heating of the powder, the microstructure starts heading solid form, such a structure has been able to come together and binding during molding. For better results, it is performed smoothing with a higher level ball mill than before and calcination process is carried out at the same temperature. In the calcination stage 4 are shown in Figure (4d) has uniformly fine grain size of 3 calcination process in Figure (4c). The structure is in globular form and distributed uniformly. Such a microstructure is possible to increase the strength.

3.3. Tensile Properties of Artificial Bone

Fig. 5 is a photograph of fracture tensile test specimens. Testing of specimens was using a Com-ten tensile testing machine. It can be seen that the specimen is broken in the middle of gauge length. There is alsmost no reduction in area of the specimen indicating that the specimen is quite brittle.

Figure 5: Fractured Tensile Test Specimen of Artificial Bone

Fig. 6 shows the strength of the specimen as a function of bone powder (HA) compositition. The composition of 30% resin has the highest strength of 13.66 MPa followed with 20% (11.84 MPa), 40% (9,56MPa), 10% (5,57MPa). The tensile strength increases width addition of resin up to 30%. But, it the strength decreases with addition of more than 30% resin. In the previous studies, M. Wang et al. 1997, [10], their use as ingredients in HA Polyethylene resin composites (HAPEXTM), whereas in this study using self acrylic resin curing of Ortho resin. HA type used in the previous study is P88 and P81B HA (Plasma Biotal Ltd, UK),with P88 and P81B HA particle size of 4.14 μm and 7.32 μm, respectively [10]. Both types of HA used is much smaller than than present study (63 m). Moreover, the content of resin in both study is much different where in present study less than 40% resin, while, in the case of previous study, is 50% resin and above.

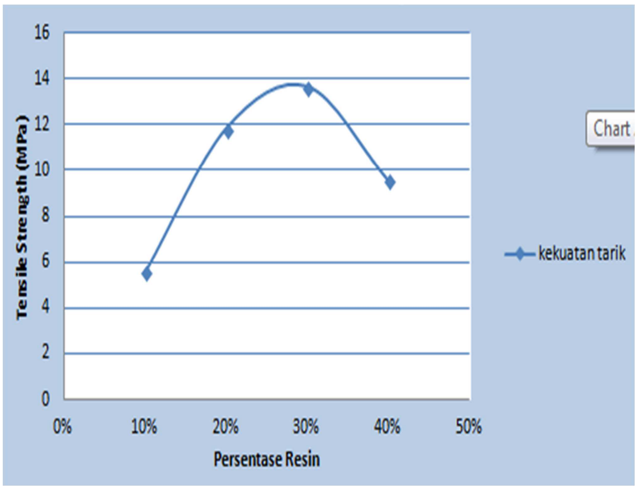


Figure 6: Tensile sstrength as a function of resin content

A comparison of tensile strength previous work [10] and present study is shown in Fig. 7. It can be seen the considerable influence of the change in the composition of the resin to the tensile strength. While the study of Wang [10] shows that the amount of resin is only slightly affect the tensile strength. The differences of the results may be due to different resin used in both study. Previous study has been used Polyethylene resin composites (HAPEXTM) as a mixture of the bone powder.

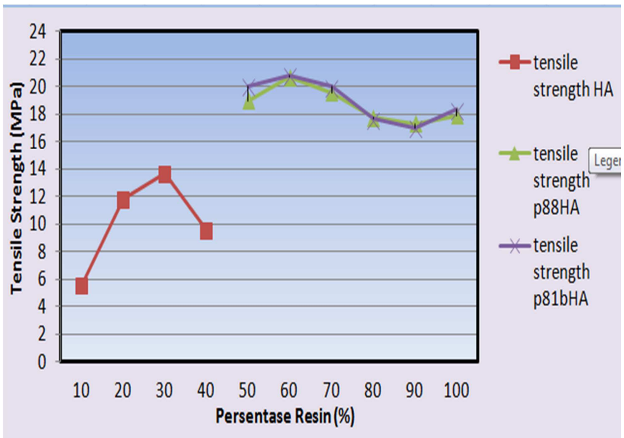


Figure 7: Tensile Strength vs Content of Resin

Other difference is in terms of the amount of the composition of the HA. Present study focuses on HA as the main material, and a resin as an additional to bind HA. While study focuses on the Polyethylene resin composites (HAPEXTM) as the main ingredient and the HA as an additional mix. In this case, it can be seen that the use of resin as the main ingredient, demonstrated a higher tensile strength than the HA as the main ingredient. Current study focuses on the use of HA as a main component, because the original purpose of the study is to get HA with high quality, while the HA is a kind of bioceramic that is highly susceptible weak against tensile stress. Nainar work [11] shows a great tensile strength and fracture surface morphology form . In this study using this type of Synthetic Hydroxyapetite commercial HA (HA, Fluka 21223) powder from Sigma-Aldrich Fluka 21223 with grade (particle size: 10-20 microns, Mw: 502.24 g / mol). HA is used as an ingredient in Poly-lactic acid (PLA resin, Specific gravity: 1:24, grade3051D from NatureWorks®, China). The process of making sample was using a twin process screwextruder and injection molding. Composite composition named hydroxapatite and Poly (lactic) acid (HA / PLA) is a pure PLA, 10% HA (HA / PLA10), 20% HA (HA / PLA20), 30% HA (HA / PLA30) [11].

The results of tensile testing of composite HA / PLA in Fig. 8, shows a reduction in the amount of resin and addition of HA. The tensile strength is decreased by 7% of the composition of HA / PLA10 (52.5 MPa) to HA / PLA 30 (45.5 MPa). From these results can be compared to the present study, the additional increase in the tensile strength of the resin of the addition of 10% (5.57 MPa) to 30% (13.66 MPa), an increase of 8.09%, but also decreased with the addition of resin 40% (9.56 MPa) by 4.1%. From the results of this study can be seen two different types of resin used, the composition of HA, HA type, shape (model and dimensions) Specimen and processes used in the manufacture of specimen. In the study, Wang et al and SM Nainar already using sophisticated processes, while the author is still use conventional processes in the molding of artificial bone specimen, but it is already getting relatively good results.

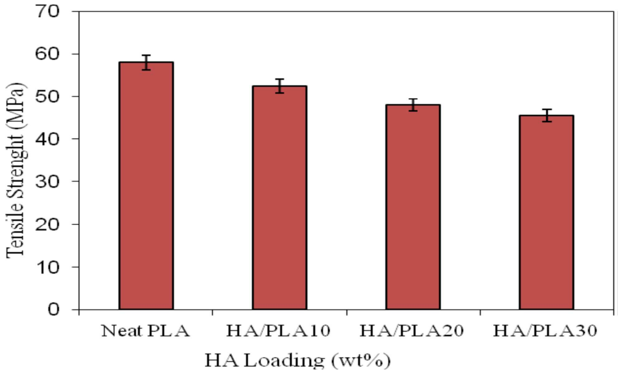
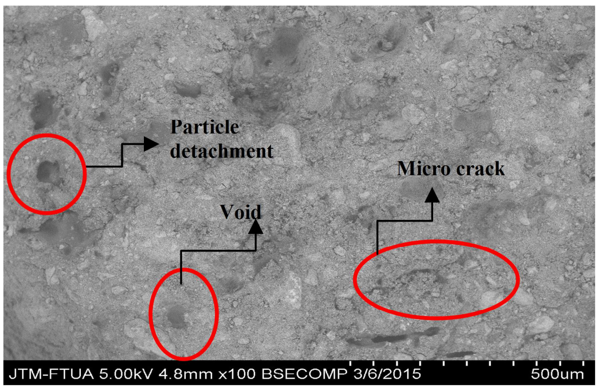
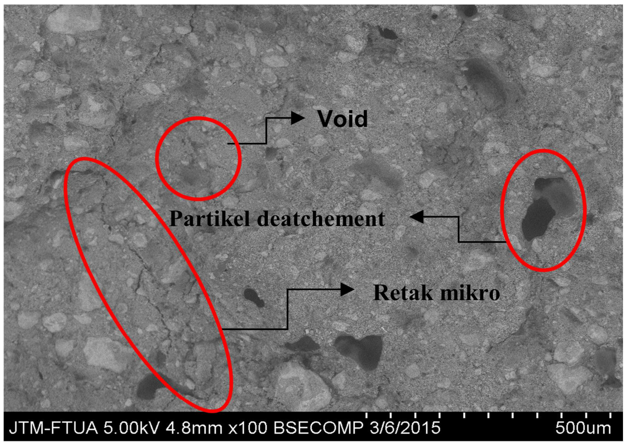


Figure 8: Tensile Strenth of Composite HA / PLA [11]

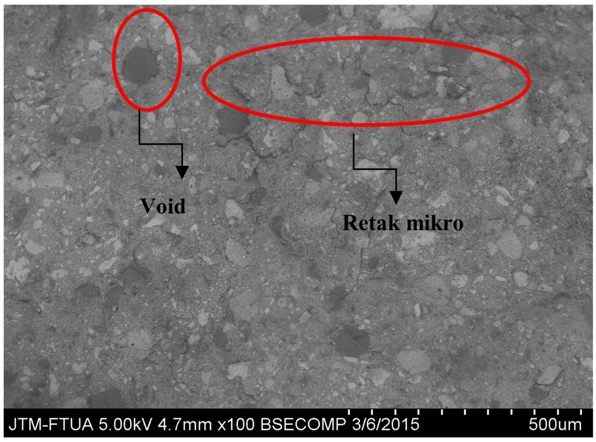
Fig. 9 shows the microstructure of the artificial bone specimens with different composition of resin. It can be seen that the microstructure of the specimens shows micro cracks, voids, detachement particles, and agglomeration with different extent depend on the fraction of the powders. At 10% resin specimens are apparent resin composition for binding HA. HA is still less than the compounds, this has resulted in low strength of the connective on the specimen. In addition, it is also seen HA particle fineness uneven and still looks rough. While, at 30% resin specimens only visible voids and micro cracks, which distinguishes this 30% resin specimens with other specimens. This happens because the resin composition of the specimen is more perfect than the resin composition on other specimens.



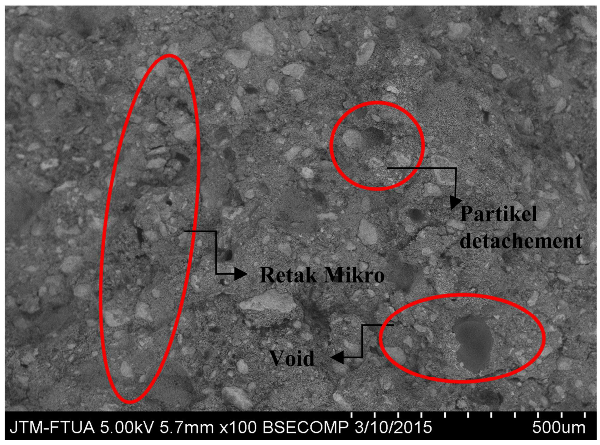
a). 10% Resin



b). 20% Resin



c). 30% Resin



d). 40% Resin

Figure 9: SEM micrograh of the artificial bones with various content of resin.

5.0 CONCLUSION

In order to obtain bovine bone powder with high content calcium (Ca)-phosphorus (P) coumpound (mainly HA), a combination process of ball milling and heating treatment was conducted. The bone powder was then mixed with resin in various composition for manufacturing protoype of artifial bone in the form of dog-bone type tensile test specimen. The following result is obtained:

1. Size of bone powder decreases with increasing stage of process calcination and ball milling. The avarage grain size of the powder is 63 µm.
2. The content Ca and P increases with increasing number processes with the highest level of Ca and P is 35.07% and 19.04%, respectively.
3. The tensile strength of artificial bone varies according to the bone powder and resin composition. The strength increases with increasing content of resin from 10% up to 30%, and then decreases with increasing resin content above 30%. The strength of the artificial bone is 5,57 MPa for composition 10% resin and 90% bone powder, 11.84 MPa for 20-80, 13.66 MPa for 30-70, and 9,56 MPa for 40-60. Thus, the composition 30% resin and 70% powder has the highest strength. However, the tensile strength of these artificial bones is lower than that of original bovine bones (101±36 MPa).
4. The microstructure of the specimens shows micro cracks, voids, detachement particles, and agglomeration with different extent depend on the fraction of the powders. The microstructure of the 30-70 arficial bone shows relatively lower amount of micro cracks and smaller size of void than those of other composition.

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