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The Potential of Hydroxyapatite-Alumina Composites for Artificial Bone Implant

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Abstract: The production of hydroxyapatite (HA) from bovine bone has been carried out, to which alumina (Al_2O_3) and ortho resin will be added to form HA-alumina composites. The aim of this research is to obtain HA-Alumina composites which can be used as artificial implants. Bovine bone waste was synthesized by mechanical thermal. Its charaterization was performed by means of X-Ray Fluorescene (XRF) for ratio (Ca:P) analysis, Scanning Electron Microscopy (SEM) for microstructure, X-Ray Diffraction (XRD) to analyze the crystals of HA, Electron Diffraction X-Ray (EDX) was utilized to measure the composition of HA-alumina composites. The mechanical method with calcination at a temperature of 800ºC was conducted on bovine bones to obtain HA. The HA-alumina composite was continued with the addition of 0%, 15%, 25%, and 50% of the total weight of alumina by adding resin as a binder. Analysis with SEM showed irregular round grains with an average size of 42.25µm. The obtained XRF analysis result was a Ca/P ratio of 1.61. The hardness and tensile strength of the composite increased and the percentage of agglomeration area decreased as the alumina composition was added. The addition of 50% alumina resulted in maximum hardness and tensile strength values of 124.8 HB and 86.56 MPa. The addition of alumina increased the mechanical properties of hardness and tensile strength. Hence, HA-Alumina composites could be used as artificial bone implants.

Keywords: Alumina, Bovine Bone Waste, Composite, Hydroxyapatite, Ratio.

1 Introduction

Currently, the need for artificial bones to replace or repair damaged bone tissue in patients is excessive, because these bones are not only functioned as supporting bones for the thigh, knee, and teeth, but also to replace the breastbone, eyes, and ears [1-2]. The number of femurs grafting operations, and arm bone replacement, due to bone damage continues to increase from year to year [3]. Damage to the hard tissues (bones) of the human body can be caused by various factors, including fragility, bone cancer, accidents, natural disasters, and birth defects. There are 3 (three) alternative ways to recover the bone function, including autograft, allograft, and xenograft [4].

In its application, there are still various limitations causing delays in the treatment process. Taken autograft alternative as the example, there is limited bone tissue that can be used. While the allograft alternative has the potential to cause disease derived from the donor, and the xenograft alternative experiences compatibility problems [4]. In line with this, synthetic biomaterials, like bioceramics, are needed to make more economical and compatible artificial bones with human bones. Bioceramics have good biocompatibility properties with body cells compared to polymer or metal biomaterials, are non-toxic, and most widely used to replace the function of human tissues or organs [5].

Hydroxyapatite (HA) with the chemical formula $[Ca_{10}(PO_4)_6(OH)_2]$ has been acknowledged in biomedical fields (orthopedics and dentistry), and bioceramics because it has biocompatible and bioactive properties when integrated into living tissue [6-7]. However, its application is inadequate due to its fragility, so it cannot be used for heavy load-bearing applications such as bone or dentures [8], and only for implant applications with small loads [9].

To make hydroxyapatite meets the standards of bone substitution material, it is necessary to improve its mechanical properties. One of the ways is to composite hydroxyapatite with other materials having higher mechanical properties, resulting implant materials with better mechanical properties. One of the options that can improve the mechanical properties of HA as bone implant material is alumina (Al_2O_3) [10-12]. Alumina is biocompatible, low toxicity, bioinert, resistant to acid or alkali at high temperatures, and its applications have been used in the orthopedic and dental implant fields [13]. Alumina also has antibacterial properties and is used as a support material in composite materials to improve the mechanical and thermal properties of a material [14]. Therefore, the HA-alumina composite is an attractive

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material for artificial bone applications. Synthesizing hydroxyapatite using bovine bone waste (biowaste) is an effort to utilize the abundant bovine bone waste which until now has been suboptimal in their usage [15-17].

Thus, it is necessary to analyze the mechanical properties of HA-alumina composites that are applicable for low loading bone implant materials [9]. So that the need for implants that comply with the standard of bone substitution material is met, and bone waste becomes a useful material with selling value.

2 Research Method

The materials used in this research were bovine bone waste, commercial alumina (Al_2O_3) white color with a size of 20 mesh and a purity of 99.9% from KIMIAPEDIA company, dental resin brand Ortho Resin (quick self-curing, acrylic resin) made in England. While the equipment used were boiling equipment (presto), hand grinder, blender, planetary bowl mill, specimen printing equipment, digital scales, furnace, sieve with size number 60 (250 µm), number 120 (125 µm), number 230 (63 µm). Characterization was conducted by employing Scanning Electron Microscope (SEM), Electron Diffraction X-Ray (EDX), Universal testing machine, Micro Hardness, X-Ray Fluorescence (XRF), and X-Ray Diffraction (XRD).

Research preparation

The research procedure was divided into four stages, namely, the first was the preparation of raw materials for the manufacture of bone powder from bovine bone waste. The second was the manufacture of HA by the calcination process, the third was specimen printing, and the fourth was a physical analysis using SEM-EDX, XRD, and XRF as well as mechanical properties testing on hardness and tensile strength.

First, the tissue and remaining substances on the bone surface were cleaned, then washed with liquid soap. The clean bones were boiled for four hours to remove any remaining marrow and fat. It was then dried in the sun and then cut into 20 x 60 mm sizes, then boiled one more time to remove fat and brittle bones for another four hours in a pressure cooker. The next process was dried in the sun for the second time to reduce the moisture content, and continued heating in the oven for two hours. Finally, the bones were mashed in a blender to obtain bovine bone flour.

The process of making HA began with calcining bovine bone meal at a temperature of 800ºC in an electric furnace for three hours. Then it was cooled in stages with furnace settings starting from 200ºC, 400ºC, 600ºC, and 800ºC with 15-minute time increments on temperature, and 30 minutes holding time until reaching room temperature. Furthermore, the bone flour was mashed with 45 stainless steel bowl mill grains with a diameter of 10 mm, a speed of 200 rpm with three repetitions left and right, and the time for each repetition was 20 minutes. Next, it was sieved using a graded sieve starting at 60 (250µm), 120 (125µm), and 230 (63µm).

The resulting HA was divided into two parts, namely pure and mixed parts. Pure HA was coded A (without the addition of alumina), while mixed HA were coded B, C, and D, with variations of alumina addition of 15%, 25%, and 50%, as in Table 1. The amount of resin added for each sample was 30% of the mass of the mixed material, with the volume of liquid resin set at 4 ml per sample [18]. After the mixture of HA and alumina was stirred evenly, the liquid resin was added and stirred again quickly, after the mixing was complete, it was put into the mold, pressed with a manual pressure, then let it rest for 20 minutes before opening the mold.

Table 1: Composition of the HA-Alumina

Sample code	Percentage of mixture		Mass of component	
	HA (%)	Al_2O_3 (%)	HA (gram)	Al_2O_3 (gram)
A	100	0	20	0
B	85	15	17	3
C	75	25	15	5
D	50	50	10	10

Physical characteristic and mechanical properties

Physical characteristics utilizes Hitachi Horiba S–3400 N SEM-EDX, Japan, in order to observe the microstructure of HA powder and composites. Meanwhile, analysis of the chemical composition of bovine bone powder uses Panalytical Epsilon 3 X-Ray Fluorescence (XRF), and Energy Dispersive X-Ray (EDX) EMAX software. The purity of HA was analyzed by X-Ray Diffraction (XRD-Panalytical, Type PW3040/60). Tensile strength testing takes Galdabini Universal Testing Machine, with max loading of 10 Tons with the testing standard referring to the ASTM E8 [19]. Hardness testing employs Shimadzu Micro Hardness Tester, Type-M, by testing standards based on ASTM 384 [20].

3. Results and Discussion

Analysis of Hydroxyapatite Morphological

The morphological description of HA powder was obtained from SEM observations with several variations in magnification as shown in Figure 1. Figure 1a-1c shows the uneven globular granules where the fineness is almost uniform with average size of about 42.25 µm in the range between (30.29 -136.4) µm. They were the results from image processing by using the “image J” software as can be seen in Table 2. Meanwhile, Figure 1d-1f shows larger particle size with various shapes. Refers to the results obtained from previous studies, the average size of granules was 63 µm, with an uneven distribution [18]. This fine size of the granules is useful in the process of mixing materials, thereby the distribution of the granules is more even and

affects the mechanical strength of the composite [10-11], [16]. As the finer the grain, the wider the surface, the stronger the bond in the composite [18].

Table 2: HA grain size distribution

No.	Interval Class	Frequency	%
1	30.286-41.600	217	62.536
2	41.601-52.916	84	24.207
3	52.917-64.232	22	6.340
4	64.233-75.548	10	2.882
5	75.549-86.864	10	2.882
6	86.865-98.180	2	0.576
7	98.181-109.496	0	0.000
8	109.497-12.811	0	0.000
9	120.812-132.127	0	0.000
10	132.128-143.443	2	0.576
	Total	347	100

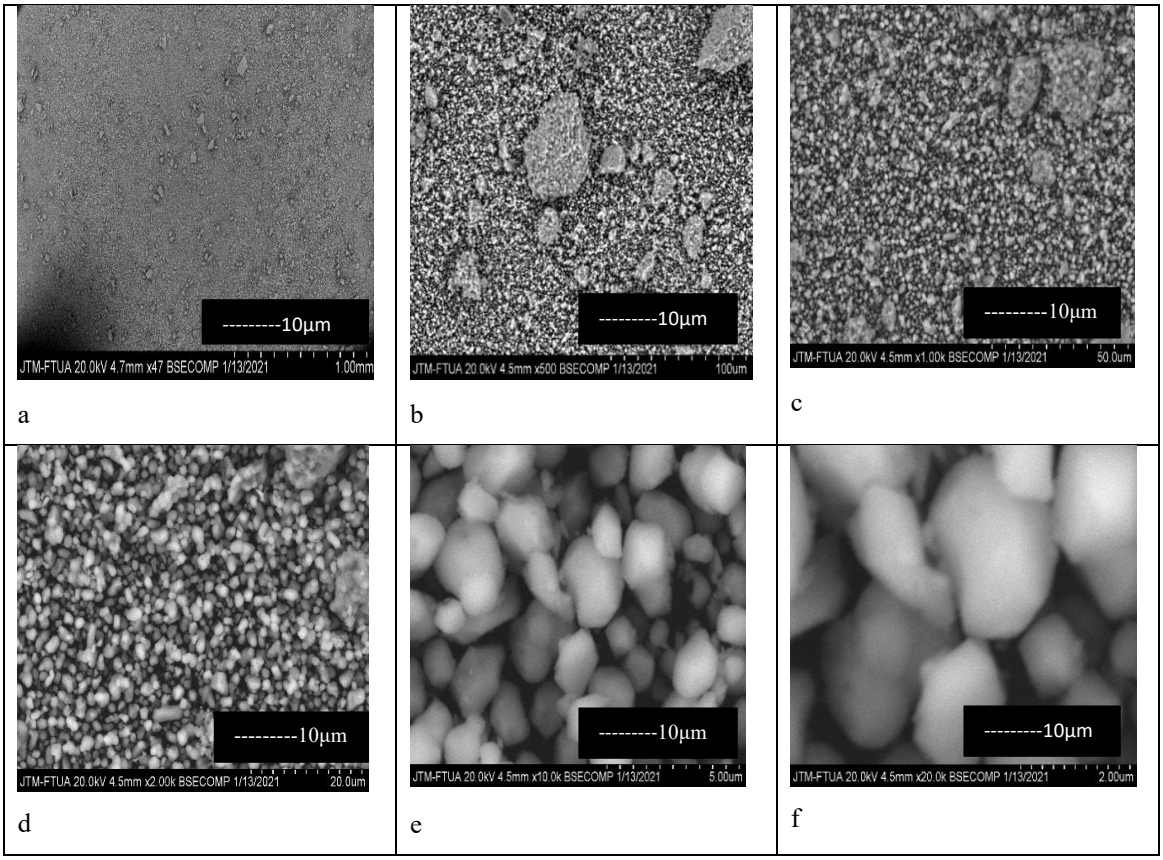

Fig. 1: SEM results of beef bone powder with various magnification

a. 10x, b. 47x, c. 500x, d. 1000x, e. 2000x, and f. 5000

Chemical Composition Analysis of Hydroxiapatite

The results of the HA powder chemical composition testing with XRF and EDX are presented in Table 3 and Table 4. Table 3 indicates the obtained level of Calcium (Ca) and Phosphorus (P) are 37.418% and 23.185%, with the Ca/P ratio is 1.61. Whereas the EDX analysis in Table 4 also obtained a Ca/P ratio of 1.61. It is worth noting that these two tests obtain the same ratio. In the previous studies, HA was synthesized from the bovine bone using the calcination-bowlmill method resulting the highest levels of Calcium (Ca) at 35.07% and Phosphorus (P) at 19.04%, so that a Ca/P ratio of 1.84% was obtained [16, 18]. Wahdah I, (2014) stated that the results of HA synthesis from bovine bones using the wet-precipitation method emerged Ca and P content levels of 38.88% and 18.78% with a Ca/P ratio of 2.06 [21-22].

The HA obtained from this recent study is more optimal than the previous ones in terms of Ca/P ratio which is close to the optimal value of Ca/P HA ratio in general at 1.67. The value of Ca/P affects the strength of HA, because the greater the ratio of Ca/P, the more increase the strength is. The maximum level will be reached at the value of Ca/P ~ 1.67 and decrease at the value of the Ca/P ratio > 1.67 [23-24].

Table 3: Chemical HA composition obtained by XRF

Compound	Concentration (%)	Compound	Concentration (%)
Mg	0.764	MgO	0.509
Al	1.611	Al_2O_3	1.865
Si	0.219	SiO_2	0.345

P	23.185	P_2O_5	22.072
Cl	0.124	Cl	0.079
K	0.094	K_2O	0.069
Ca	37.418	CaO	34.124
Ti	0.009	Ti	0.004
Mn	0.006	Mn	0.003
Fe	0.047	Fe_2O_3	0.033
Zn	0.032	Zn	0.016
Sr	0.087	Sr	0.042
Zr	0.003	Zr	0.001
Na	0.658	Na	0.476

Table 4: Recapitulation of elements identified by EDX observation

No	Element content (% weight)	Treatment (HA + Alumina)			
		A	B	C	D
1	Ca	30.21	26.40	17.95	8.34
2	P	18.76	16.41	11.14	5.15
3	O	51.03	52.04	59.86	63.86
4	Al	-	5.18	11.07	22.65

The mixing composition of HA-alumina powder is highlighted in Table 1, and the results of the analysis the microstructure by SEM is in Figure 2. Sample A shows the amount of agglomeration at several points due to incomplete mixing of the materials during stirring, so that the resin is unable to bind HA evenly. Whereas in sample B, the addition of 15% alumina reduces the percentage of agglomeration by 2.37% of the previous total area, with the growing focus of the agglomeration due to the even distribution of bonding between HA-alumina and resin. In samples C and D, it is perceptible that the agglomeration is decreasing, as the alumina increases, and the bond between HA-alumina is getting wider, where the reduction percentage is viewed in Figure 2. By adding 25% and 50% of alumina, it makes the agglomeration decreases, along with the increase in alumina and the wider the bond between HA-alumina, [26]. and the largest percentage reduction is occurred at the addition of 50% alumina of 9.16% as shown in Figure 2.

Physical Properties of HA-alumina Composite

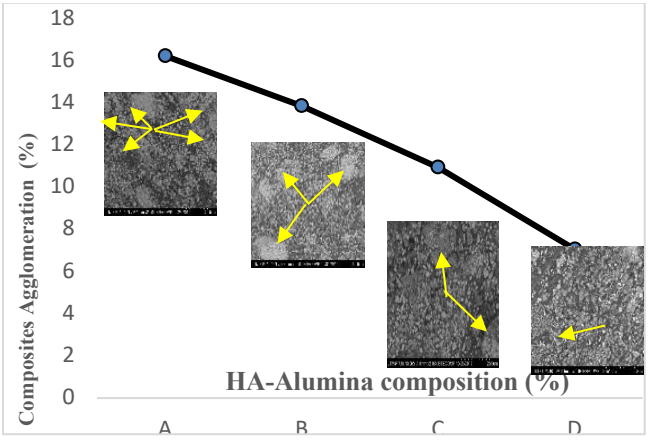

Fig. 2: Microstructure and percentage of agglomeration of HA-Alumina composites

Characterization of Bovine Bone Hydroxyapatite

To examine the purity of the bovine HA, the crystalline phase characterization was performed using XRD for each particle size group and mixture. The X-ray diffractometer was conditioned to use cupper radiation as an anode (CuKa, λ = 1.54060 Å, 2θ = 10–100) at 40 kV and 30 mA. The scan time in the step was 7.14 s

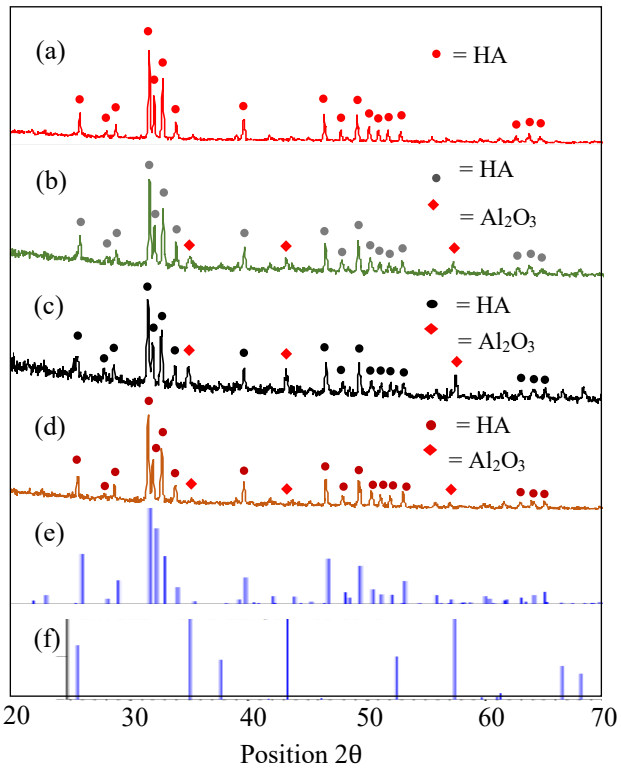

Fig 3: XRD patterns of HA-Alumina after sintering at 1000 °C for three hours. (a). A, (b). B, (c). C, (d). D, (e). HA

reference 96-900-2215, and (f). Alumina reference 96-100-0018

In XRD analysis results, a change is identified in the intensity of the peaks on the diffractogram. High crystallinity diffractograms will have peaks with high intensity [24]. These peaks are established in the presence of diffractogram of HA powder without alumina mixture and HA with alumina added in varied alumina concentration, i.e., 15%, 25%, and 50%, respectively. The results as illustrated in Table 5 and Figure 3 indicates that the main peaks from the 2-theta position of HA powder are identified at the angles of 31.717, 32.155, 32.843, 34.019, and 39.725. The main peaks of the 2-theta position for bovine HA and for the other 3 blends are close to these angles. The XRD analysis results demonstrate that the phase formed is hydroxyapatite, in accordance with the results obtained in previous studies [24-25]. XRD identification of alumina shows, the main peaks of the 2-theta position are at the angles of 35.139, 43.337, and 57.480. These values are in line with powder diffraction pattern from the International Centre Data Diffraction with a reference code of HA reference 96-900-2215, Alumina reference 96-100-0018, and chemical formula of $Ca_{10}(PO_4)_6(OH)_2$.

Table 5: Main peaks of bovine HA powder and HA + Alumina Composites from XRD analysis

Sample code								**HA reference code 96-900-2215**	
A		**B**		**C**		**D**			
Position [2θ]	Intensity [%]	Position [2θ]	Intensity [%]	Position [2θ]	Intensity [%]	Position [2θ]	Intensity [%]	Position [2θ]	Intensity [%]
31.6868	100	31.8767	100	31.7201	100	31.7530	100	31.717	100
32,8612	79.31	32.9069	84.83	32.1558	52.57	32.9178	65.64	32.155	72.5
33,9937	22.52	34.0268	37.25	32.8775	63.05	34.0513	21.78	32.843	45.8
35.4291	5.98	35.1483	22.41	35.1403	24.62	35.4641	4.67	34.019	16.3
39.6756	17.29	39.7925	35.17	39.8049	17.46	39.8256	26.01	39.725	25.6

Analysis of Mechanical Properties of HA-Alumina Composites

The results of the mechanical properties analysis of the hardness of the 4 samples are shown in Table 6. The table illustrates that the highest hardness value is found in specimen D and the lowest is in A confirming that the composite hardness rate increases with increasing percentage of alumina in the mixture. Earlier investigations pointed out that the highest composite hardness gain was 38.8 MPa with the addition of 50% alumina after sintering at 1200°C as shown in Table 7 [26]. On the other hand, the addition of 30% zirconia to HA from bovine bone resulted in a hardness value of 34.3 MPa [23], and the addition of 20% resin to HA from processed bovine teeth obtained a hardness value of 40.39 MPa [23].

Table 6: The average value of tensile strength and hardness of HA-Alumina composites

No	**HA-Alumina Composition (%)**	**Tensile Strength (MPa)**	**Hardness (HB)**
1	A	18.76±1.32	20.20±1.20
2	B	31.83±1.10	67.20±1.17
3	C	66.51±1.21	85.00±1.14
4	D	86.56±1.08	124.80±1.27

Table 6 shows that the highest tensile test value is found in specimen D, while the lowest is in specimen A. Likewise the results of the hardness test, adding 50% of alumina will produce the highest composite tensile value compared to composites without the addition.

Based on the results of Paskarino's (2015) research as shown in Table 7, the maximum tensile test for HA composites were 13.66 MPa using 30% resin [18]. This result seems to be different with the results of this recent study which is higher at 18.78 MPa as a consequence of different values of the Ca/P ratio from the two preliminary studies in terms of their HA synthesis's results [18]. In this study, the Ca/P ratio is 1.61 which is close to the optimal ratio value of 1.67. On the other hand, the ratio from previous research was 1.84, which was greater than the optimal Ca/P ratio, namely 1.67. An increase in the value of the Ca/P ratio > 1.67 is one of the causes of a decrease in composite strength [22], although there are still other variables that influence composite strength, namely powder shape, powder size, and powder size distribution [3, 15].

Table 7: The average value of tensile strength and hardness of HA-Alumina composites [18], [27]

No	**HA-Alumina Composition (%)**	**Tensile Strength (MPa)**	**Hardness (HB)**
1	100/0	5.57±1.20	18.70±1.18
2	90/10	11.84±1.35	33.50±1.21
3	70/30	13.66±1.11	36.30±1.19
4	50/50	9.56±1.150	38.80±1.07

4 Conclusion

HA-Alumina composites have the potential composites to be used as artificial bone implants. The addition of alumina to the HA composite can improve the mechanical properties and reduce the agglomeration percentage. The HA used is

derived from the synthesis of hydroxyapatite from bovine bone waste by the mechanical-thermal method. The HA Ca/P ratio of beef bone produced was 1.61, which was close to the optimal HA Ca/P ratio of 1.67. Adding 50% of alumina to the HA composite and acrylic resin binder can escalate the average hardness value of 124.8 HB along with the average tensile strength value of 86.56 MPa.

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6 Conflict of interest

The authors declare that there is no conflict regarding the publication of this paper.

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