Study of Mg-Hydroxyapatite Composite with various composition of Hydroxyapatite which obtained From Cow Bones in Simulation Body Fluid (SBF)

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ABSTRACT— ***The objective of the present study was to synthesize biodegradable Mg-hydroxyapatite (Mg-HAp) composites for bone replacement. Hydroxyapatite (HAp) powders were synthesized from bioresources cow bone in a cost effective and ecofriendly way. Cow bone was converted to hydroxyapatite by a heat treatment method at different temperatures. Hydroxyapatite was characterized by X-ray diffraction (XRD) and scanning electron microscope (SEM), Energy Dispersive X-Ray spectroscopy (EDX) and FT-IR. The results show a uniform distribution of HAp with a crystal size range of 400-800 nm. The Mg–HAp composites were prepared by mixing different amounts of HAp powders to Mg powder and finally the powder was pressed to make samples. The microstructure of Mg–HAp composite was examined by optical microscope (OM).The results show a uniform distribution of HAp particles along the boundary between matrix particles.. The relative densities of the composites initially increased with increase the amount of HA addition. The theoretical and experimental hardness of the composites are compared with the addition of 3 to 9 wt% HAp. Addition of HAp decreases the crystal size of the nanocomposites. The mechanical properties, i.e. hardness is evidently increased with increasing HAp content up to 5 wt%.***

Keywords— Magnesium, hydroxyapatite from cow bone, composites

1. INTRODUCTION

In the recent years there was an intensive effort on reducing cost in the medical sector. Implantation can also regenerate or trigger the formation and development of new bone cells. Generally, three types of metals such as austenitic stainless steel (SS), Co-Cr alloys and pure Ti and its alloys are used as an implant material [1-2]. The third of the implant material have the advantage of good mechanical properties, corrosion resistance is quite good, levels biocompatibility good and the price is not too expensive (for SS and alloy Co-Cr). However, these three materials implants commonly used has several short comings that limit the function of organs, such as the broken arm, the hand movement will be limited, affecting bioactivity body presence of localized corrosion around the area of the implant installed where possible release of ions, or particles of material implant poisonous and also may cause inflammatory cascade that reduces biocompatibility, and cause tissue loss. In addition, the third elastic modulus of the implant material is not in harmony with the natural bone tissue that can lead to stress-shielding effect which cause a reduction in the stimulation of new bone growth and implant stability decreased. Also needed a second operation to remove the implant material while healing occurs. It is necessary to implant material that is not permanent or can decompose in the body (biodegradable). Recently, magnesium alloys have attracted much attention as potential biodegradable bone implant materials due to their biodegradability in the bioenvironment [3–5], and their excellent mechanical properties such as high strength and possessing an elastic modulus close to that of bone [1]. However, the rapid corrosion of magnesium alloys in chloride containing solutions including human body fluid or blood plasma has limited their clinical applications [1,6]. Therefore, in order to use Mg as an effective implants, the corrosion rate of Mg needs to be slowed down. Alloying elements have to be chosen adequately for this kind of application. In order to improve mechanical properties and biocompatibility it could be interesting to reinforce the magnesium alloy with hydroxyapatite (HAp). HAp has proven a very attractive material for bone replacement due to its close resemblance to vertebrate bone mineral and good bioactivity [7-10]. HAp also promotes new bone tissue formation and accelerates the bone growth [11]. The advantages of HAp from natural

sources is inexpensive and uncomplicated. The thermal calcination method is commonly used for the isolation of natural HAp. Micro structural HAp has already been obtained from fish bone by thermal treatment, thermal decomposition, alkaline hydrothermal, sub critical water process from bovine bone, teeth and bones of pig, extracted human teeth and cuttle fish [12-15]. Although much has been learned about HAp isolation from natural sources, the most important parameter, exact isolation temperature, remains poorly understood. In general, it is known that Indonesian cow bone consists of HAp (57%) and gelatinous organic matter (33%). To prevent pollution by this organic matter, cow bone was calcinated. The waste of cow bone has recently become a serious issue in coastal areas of Indonesia; one of the simplest ways to decrease pollution is the selective isolation of HAp from this waste.

The research was conducted with the aim to prepare and characterize the hydroxyapatite from cow bones for bone substitution [16]. In the orthopedic applications of biodegradable materials will biodegrade in the body during a healing thereby reducing the phenomenon of the reduction of stress shielding and also not needed a second operation to remove the implant material as is done on the material that is non-degradable implant. A biodegradable material that is being developed is a hydroxyapatite Magnesium alloy (Mg-HAp). It can control the level of degradation. This material does not decompose too quickly, and not easily broken as pure magnesium metal. In addition, the modulus of elasticity of the metal biomaterials are generally used do not correspond to natural bone tissue, which can lead to stress-shielding effect thus reducing the stimulation of new bone growth that causes decreased stability of the implant. While the modulus of elasticity of magnesium approaching the modulus of elasticity of human bone, so the broken bone have enough time to grow back. The addition of HAp from cow bone can reduce the corrosion rate of Mg-HAp composite and the resulting corrosion is not toxic.

In this study, magnesium-hydroxyapatite composites processed by dry blending and heat sintering were investigated. The aim of this research is extended to study the effect of different percentages of hydroxyapatite from cow bone, namely 3, 5, and 9 %, on the corrosion behavior and degradation level of pure magnesium after their immersion in simulated body fluid (SBF). The aim is also to ensure that the biological environment is conductive for cell attachment, proliferation, tissue growth and adequate nutrient flow. To achieve this objective, the study was carried out using corrosion test was performed with a potentiostat EG & G in demineralised water media and media solution artificial body (SBF).

2. MATERIALS AND METHOD

2.1. Sample Preparation

The material used in this study is Magnesium powder obtained commercially (Merck) with 99.9% purity. To obtain hydroxyapatite, bovine bone are soaked in the acetone for an hour to removed collagen, fats and other impurities. Then it washed with distill water and let it dried. The bones were placed in an open silica crucible and heated in an electric furnace under ambient conditions, at different temperatures ranging from 200°C to 1000°C with 5 hours holding time. Then the bones were ground into 200 μm particle size. Hydroxyapatite powder chosen for making composite, is HAp sintered at a temperature 850^0C.

The main sample is a composite of Mg-HAp, wherein HAp used is sourced from bovine bones were sintered at a temperature of 850^0C. The composition used is as follows Mg/3.0 wt% HAp cow bones (850^0C): Mg/5.0 wt% HAp (850^0C), Mg/7.0 wt% HAp (850^0C) and Mg/9.0 wt% HAp cow bone (850^0C) .Then these samples were homogenized using a ball mill. This study used a method of dry blending with horizontal cylindrical grinding tool along with the ball. Ceramic balls and steel balls used for crushing and stirring the mixture with a weight ratio of ball powder and powder 5:1 and the diameter of the ball varies between 1-2 cm. The mixing time performed for 6 hours at a constant speed and at room temperature conditions. Then powder dry grinding results with a weight of 4 g included in the pellet-shaped mold and pressed with a load of 120 MPa, and sintered at a temperature of 550^0C in an atmosphere of argon.

2.2. Characterization

The phase and crystallinity of Mg-HAp were evaluated using X-ray diffractometer (Phillips, PW-3710) Co-Kα radiation with wave length 1.7889 Å and scan 2θ angle from 20° to 80°, step size 0.02, scan speed 4°/min with 40 kV voltage and 30 mA current. The X-RD pattern were compared with literature profile JCPDS 09-0342/1996 to identify the phase. Morphology and chemical elements of Mg-HAp crystals was obtained by field emission scanning electron microscopy (FE-SEM JSM-6700F, JEOL, Japan) equipped with an *in situ* energy dispersive X-ray (EDX) spectrometer.

The density of the sintered samples was measured by the Archimedes method. Theoretical density of the Mg-HAp composites were calculated based on the mixture rule in composite theory with ρ_{HAp} = 3.16 g/cm^3 and ρ_{Mg} = 1.74 g/cm^3.

Corrosion test samples of Mg-HAp were conducted with potentiostat method, using a potentiostat-Galvanostat EG and G. The sample size for the corrosion test is 14 mm in diameter and a thickness of 1-5mm. In this study, the medium used was artificial or simulation body fluid (SBA) with composition show in table 1.

Table 1: Composition of Simulation Body Fluid (SBF)

No	Compound	Amount	No	Compound	Amount
1	NaCl	6.547 g/l	5	$MgCl_2.H_2O$	0.305 g/l
2	$NaHCO_3$	2.268 g/l	6	$CaCl_2.2H_2O$	0.368 g/l
3	KCl	0.372 g/l	7	Na_2SO_4	0.071 g/l
4	Na_2HPO_4	0.124 g/l			

3. RESULTS AND DISCUSSION

3.1. X-Ray Diffraction Analysis

The phase and purity of derived HAp crystals were confirmed with XRD analysis. Figure 1 shows the XRD pattern of HAp from calcined cow bone at 850^0C.

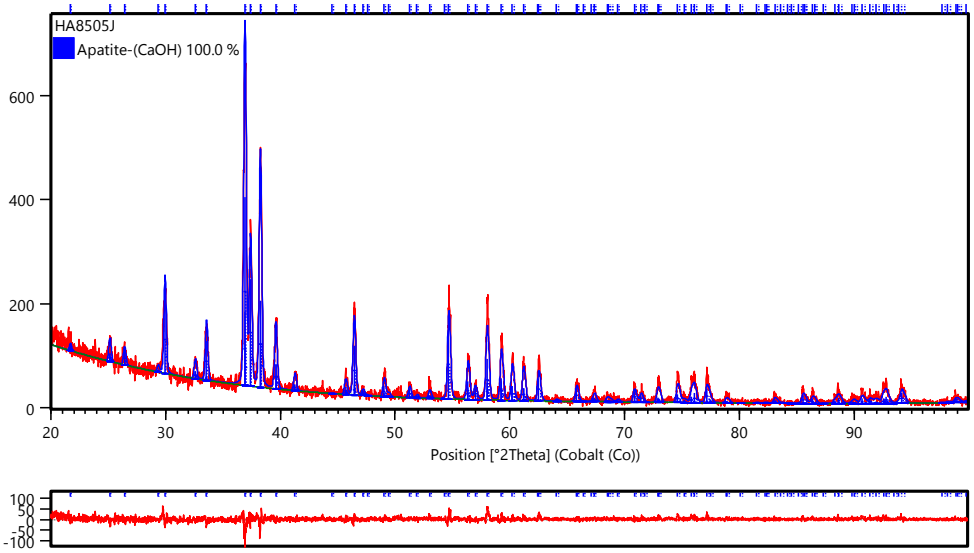

Figure 1. The XRD pattern of Bone , calcined at temperatures 850^0C

The crystalline composition of calcined cow bone was found to be similar to that of HAp (JCPDS-09-0432/1996) when calcined at 850°C. It is well known that as the temperature increases the intensity of the peak increases with a decrease in the peak width. The intensity of the calcined cow bone at 850°C was found the subsequent peaks were highly intense and sharp, indicating the removal of organic portion [16]

Table 2.shows 2θ angle, d-spacing (estimated by Bragg law), and relative intensity at the strongest peak in the XRD spectra. The obtained d-spacing lines, 2θ angle, relative intensity at the different temperatures, have been compared with standard HAp (JCPDS-09-0432/1996). The peaks were found to be closest to standard HAp. The 2θ angles varied a little in comparison to standard HAp, which might be due to the trace removal of OH radicals. Dehydroxylation of the HAp phase would cause a small degree of peak shifting in the XRD curve.

In the present work, it was found that XRD 2θ positions of the bone samples sintered at 850°C shifted by total error of 0.031, respectively, thus indicating that the HA lattice has contracted due to loss of OH radicals. From these results, it is evident that HAp derived at temperatures 850°C is very close to the standard HAp.

Table 2. : The 2θ angle spacing , d-planar and relative intensity of HAp sintered at 850°C (experimental) compared with the standard HAp (JCPDS-09-0432).

hkl	2θ angle		d-planar spacing(A^0)		relative intensity (%)	
	JCPDS	Exp	JCPDS	Exp	JCPDS	Exp
002	29.9	29.9	3.47	3.46	40	36.50
211	37.2	36.9	2.81	2.82	100	38.90
112	37.8	37.4	2.78	2.79	55	56.60
300	38.5	38.2	2.72	2.73	60	45.60
202	39.8	39.6	2.64	2.64	25	13.01
310	46.5	46.4	2.26	2.26	20	26.20
213	57.9	58.0	1.84	1.84	30	9.17

The XRD pattern of the milled composite powder showed at figure 2. The HAp are found as minor phases in case of Mg–HAp composites. It can be seen that the width of the diffraction peaks become wider than before milling suggesting decreasing in crystalite size. As was measured by Scherrer formula, the crystal size of the samples containing 3, 5, 7 and 9 wt% HAp were 23 nm, 30 nm, 42 and 68 nm, respectively. Compared with the XRD profiles of pure Mg-HAp, the Mg phase was the main constituent phase and even there are several peaks corresponding to HA could be observed in the milled sample due to the lower weight fraction of the reinforcement material. There is no new crystalline phase formed during the milling indicating that no chemical reactions between Mg and HAp occurred.

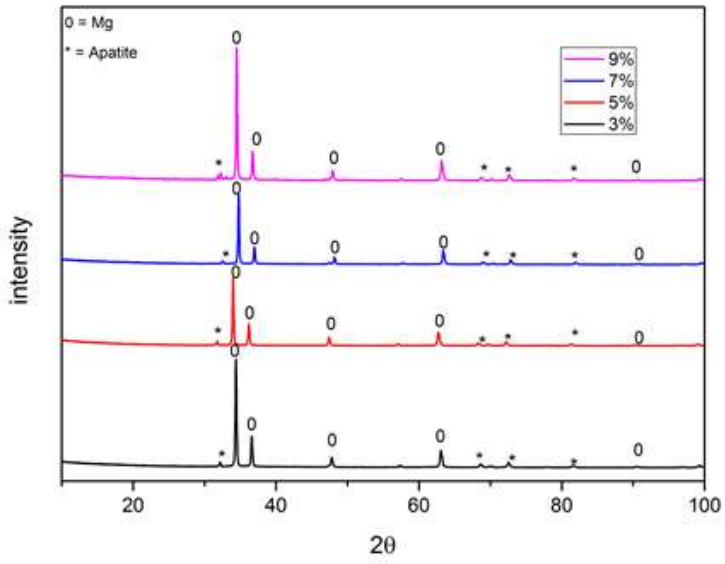

Figure 2. The XRD pattern of Mg-Hap (from cow bone) composites

32..Scanning Electron Microscope (SEM) Analysis

Figure 3 shows the SEM micrographs and EDX analysis of the milled composites. It can be observed that each Mg particle whether it is a small or large is coated with HA particles. EDX analyses clearly showed the presence of Ca, P and O of the HA and Mg (fig 3b, 3c and 3d), except in figure 3a (Mg/3.0 wt% HA) the presence of Ca, P and O of the HA was not clear.

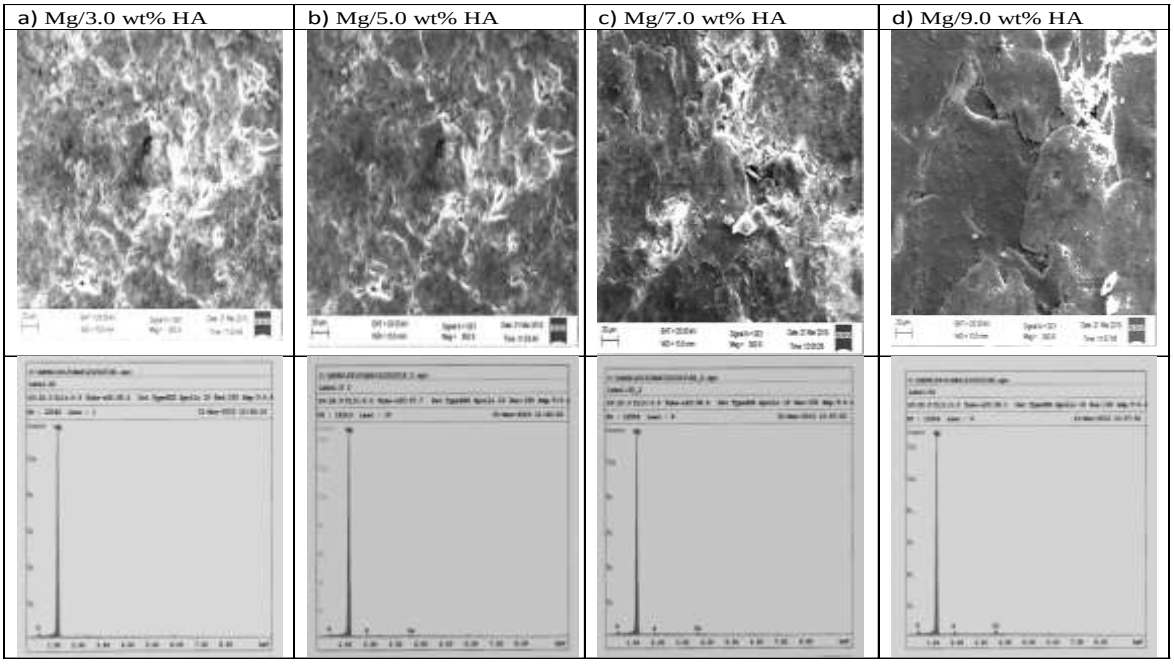

Figure 3. SEM micrographs and EDX analysis of (a) Mg / 3.0 wt % HAp (b) Mg / 5.0 wt % Hap (c) Mg / 7.0 wt % HAp (d) Mg / 9.0 wt % HAp

The weight (%) of Mg in the synthesis products ranged between 86.29 wt% and 95.17 wt%. In general, EDS mapping indicated that CaP was uniformly dispersed in the samples.

3.3. Density

Table 3. show the density of the composite Mg-3–9wt % HAp from cow bones.

. **Table 3**: Calculated and measurement density of Mg-HAp composite

Sampel	Calculated	Measurement	Relative Density(%)
Mg/3.0 wt% HAp	1.764	1.617	91.67
Mg/5.0 wt% HAp	1.780	1.688	94.83
Mg/7.0 wt% HAp	1.796	1.746	97.21
Mg/9.0 wt% HAp	1.812	1.796	99.11

The relative densities of the composites initially increased with increase the amount of HAp addition. The relative density of the sintered samples reached as high as 99.11 % in the composite containing 9.0wt% HAp.

3.4.Hardness test

Table 4 show the hardness of the composite. Hardness measurements revealed an increase in microhardness values with increasing weight percentage of HAp from cow bone reinforcement into the magnesium matrix to a certain level and then decreased. The hardness of Mg reinforced with 3 to 5 wt% HAp 51.44 HV, it's higher then the theory hardness value as show at table 4. Remarkably, with increasing HAp content more than 5 wt% HAp, the microhardness decreased. Decreasing in microhardness of Mg-HAp composites with increasing HAp from cow bone content can be attributed to two reasons. First, some degree of HAp from cow bone clustering takes place. Second, the sintering temperature of pure HAp is 850^0 C, with increasing HAp content in the Mg matrix, 550^0C the sintering temperature become not enough for densifications [12]. Notably, increasing sintering temperature is not possible due to low melting temperature of Mg matrix. The hardness values for all of the Mg-HAp composite samples have narrow distribution ranges, indicating a homogeneous distribution of the HA phase in the Mg matrix.

. **Table 4**: Hardness Vicker (HV) Kg/cm^2

Composition	Measured Hardness
Mg/3.0 wt% HAp	50.56
Mg/5.0 wt% HAp	51.44
Mg/7.0 wt% HAp	50.14
Mg/9.0 wt% HAp	49.70

3.5 Corrosion test

Table 5 show Corrosion parameters for Mg-Hap composite with various composition in the Simulated Body Fluids SBF solutions.

. **Table 5:** Corrosion test of Mg-HAp composite with various compostion

No	**Sampel**	**E Corr (mV)**	**I Corr (µA/cm^2)**	**Corr Rate (MPY)**
1.	Mg/3.0 wt% HAp	-1552.71	0.6	0.5479
2.	Mg/5.0 wt% HAp	-1254.28	0.25	0.2292
3.	Mg/7.0 wt% HAp	-1525.38	0.94	0.6083
4.	Mg/9.0 wt% HAp	-1543.70	0.76	0.6927

The corrosion mechanism is important, particularly with regard to the biomechanical properties of medical implant devices. Mg-alloys, in general, tend to pitting corrosion, especially close to chloride ions. The results show that corrosion rate are all good. The presence of HAp from cow bones increase the resistance corrosion of magnesium against uniform corrosion and also decreases the probability of localized corrosion . The localized corrosion here might not occur due to the HAp particles enclose magnesium particle and have higher noble potential than that for magnesium.

4. CONCLUSION

We could produced biodegradable Mg–HAp composite. Hydroxyapatite (HA) was synthesized from biosources bovine bone. The phase, purity and crystallinity of calcined HA powder were analysed. It confirms that material prepare from biosources cow bone is hydroxyapatite indeed. The natural HAp obtained by calcining at 850 °C shows the desired quality. The HAp obtained from cow bone powders might be are used as reinforcing agent for Mg–HAp composites. Corrosion test show the acceptable value for Biodegradable materials = 0.2292 mpy

5. ACKNOWLEDGEMENT

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