Synthesis and In Vitro Testing of Mg-6Zn-xHAp Biocomposites from Beef Bone as Biodegradable Bone Implant Material

Jojor Lamsihar Manalu[1*], Francisca Tjhay[2], Theodora Kristoforus[3], Sovian Aritonang[3]

[1]*Department of Physiology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jl. Jend. Sudirman No.51 Jakarta 12930, Indonesia.*
[2]*Department of Medical Biology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia Jl. Jend. Sudirman No.51 Jakarta 12930, Indonesia*
[3]*Department of Physics, Materials Science, University of Indonesia, Jakarta, Indonesia*
**Corresponding author: jojor.lamsihar@atmajaya.ac.id*

Article history:
Received: 29 September 2024 / Received in revised form: 1 November 2024 / Accepted: 6 November 2024
Available online 11 November 2024

ABSTRACT

This study aimed to develop biodegradable Mg-6Zn hydroxyapatite (Mg-6Zn HAp) biocomposites for potential use in bone replacement applications. The hydroxyapatite (HAp) powders, sourced from cow bone, were synthesized through an eco-friendly and cost-effective process, leveraging bioresources for material sustainability. The Mg-6Zn and HAp powders were mechanically mixed through ball milling for six hours to ensure homogeneity. The resultant powder mixture was then subjected to isostatic pressing at a high pressure of 570 MPa, forming a dense coin-shaped composite with a 1.5 cm diameter. This coin was consolidated in a capsule furnace at elevated temperatures for one hour to enhance material integrity. The Mg-6Zn HAp alloy was thoroughly characterized using X-ray diffraction (XRD) to assess phase formation and crystallographic structure, and Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDX) to examine microstructural features and elemental composition. For composite preparation, varying amounts of HAp (5%, 8%, and 12%) were incorporated into the Mg-6Zn matrix. SEM analyses revealed a uniform distribution of HAp particles along the boundaries of matrix particles, enhancing composite structure and stability. Results demonstrated that with an increase in HAp content, there was a corresponding improvement in the relative density and hardness of the composites. The corrosion rate decreased with higher HAp content, indicating improved biocompatibility and stability in physiological environments. This suggests that the Mg-6Zn HAp biocomposites, with their tailored microstructure and enhanced mechanical properties, hold promise for use in biodegradable bone replacement applications.

Keywords: *Biodegradable biocomposites, bone replacement materials, corrosion resistance, MgZnHAp, microstructural characterization*

I. Introduction

Bone fracture cases are often found, especially in developing countries like Indonesia. Bone fracture management is done by using bone implantation surgery procedures. Biomaterials commonly used in bone fixation are inner biometals such as stainless steel, cobalt-chromium alloys, and titanium alloys [1]. The use of bone implant biomaterials such as titanium and iron has been widely used. The disadvantages of these materials are that they are not cheap, and a second operation is required to remove the implanted material when the bone tissue has recovered. Therefore, a biodegradable implant is needed so that a second operation is not required [2], [3].

DOI: 10.17977/um016v8i22024p410

Biodegradable materials are generally categorized into metals, ceramics, and biodegradable polymers. Biodegradable metals are biomaterials generally applied to bones because they have better mechanical strength than other biomaterials. This metal is expected to corrode gradually in vivo and can be completely dissolved when the supported tissue has healed [4], [5]. Until now, three groups of biodegradable metals have been developed: Mg alloys, Fe alloys, and Zn alloys [6], [7]. The application of biodegradable metal implants began shortly after the discovery of the element magnesium by Sir Humphrey Davy in 1808 [8] and has developed rapidly over the past 15 years [9].

One of the biodegradable materials that is widely used is Magnesium (Mg) [10], because it has high biocompatibility with the human body, is one of the important nutritional elements for the human body with a consumption requirement of 240 - 420 mg/day [7], and also has Young's modulus that is closer to the natural young modulus of bone (3-20 GPa) [7], [8]. But Mg has a weakness, namely low corrosion resistance, which can reduce mechanical integrity before the bone heals completely [11], [12]. Therefore Mg must be combined with other elements so that its mechanical and corrosion properties increase and are by clinical needs. Biodegradable materials that will be applied in the orthopedic field must have mechanical integrity of more than 18 weeks in the bone tissue healing process, a corrosion rate of less than 0.5 mm/year in body fluids of 37 °C, strength higher than 200 Mpa, and flexibility greater than 10% for bone fixation applications [11]-[13].

Zinc (Zn) is one of the elements that can improve Mg's mechanical properties and corrosion rate. Zn is a metal with better mechanical properties than magnesium, which positively impacts Mg's corrosion resistance and strength [16], [17]. Zn is also an important element in the human body because it is included in various aspects of cellular metabolism. It also plays a role in improving enzyme function, supporting immune function, supporting protein and DNA synthesis, and supporting wound healing [16], [17]. Mg-Zn alloys show sufficient mechanical properties to be applied to bones [15], [18], but it is necessary to have material that increases the corrosion resistance and bioactive properties of implants [16]. Therefore, this study will composite Mg-6Zn alloy with hydroxyapatite (HAp), a biodegradable ceramic material widely applied in orthopedics [19], [20]. HAp commonly used is commercial HAp, which has good biocompatibility but still has toxicity properties; therefore, in this study, HAp that will be used is HAp from bovine bone. The addition of HAp from bovine bone is intended to increase corrosion resistance and bioactive properties and reduce the toxicity of implants [21].

This study aims to create biodegradable bone implant materials by compositing Mg-6Zn alloy with HAp bioceramic material from bovine bone and then observing the effect of HAp composition on the mechanical properties, corrosion rate, and cytotoxicity properties of the resulting implant.

II. Material and Methods

1. Materials

The materials used in this study were pure Mg and Zn powder, 99.9% Zn obtained commercially (Aldrich), and HAp powder sourced from cow bones sintered at 900 °C.

2. Synthesis of Mg-Zn MgZn alloy

Synthesis of Mg-Zn MgZn alloy was obtained by mixing Mg powder and Zn powder with a composition of 94:6. Mixing was done by ball milling with a ratio of 5:1. The time used was 6 hours at constant speed and room temperature. Then, the resulting powder was composited with HAp with variations in HAp composition of 5%, 8%, and 12% using the high energy milling (HEM) method.

Milled Mg-6Zn HAp powder was then filled into 1.5 mm diameter dyes and compacted with a uniaxial compaction machine with a pressure of 570 MPa to obtain pellets with a diameter of 1.5 mm. The pellets were then sintered in a capsule and carried out in an argon environment to avoid oxidation. After that, the samples were characterized using scanning electron microscope (SEM), energy dispersive spectroscopy (EDS), and X-ray diffraction (XRD). Density, corrosion, hardness, and toxicity tests were also carried out on the samples.

3. Material Characterization

The implants' surface morphology and metal composition were analyzed using a SEM with an EDS (JSM-6510LA, JEOL) manufactured by JEOL Ltd a Japanese company. This setup allows detailed observation of the implant microstructure. Iron and stainless steel implants were polished using sandpaper up to #2000 grid and etching using Nital (2% Nital Etch, USA). SEM analysis was performed at an acceleration energy of 20 keV. X-ray diffraction was also performed using CuKα radiation (Empyrean, PANalytical) a company based in the Netherlands after further polishing the implants to grid #1500 for phase identification.

3. Toxicity test

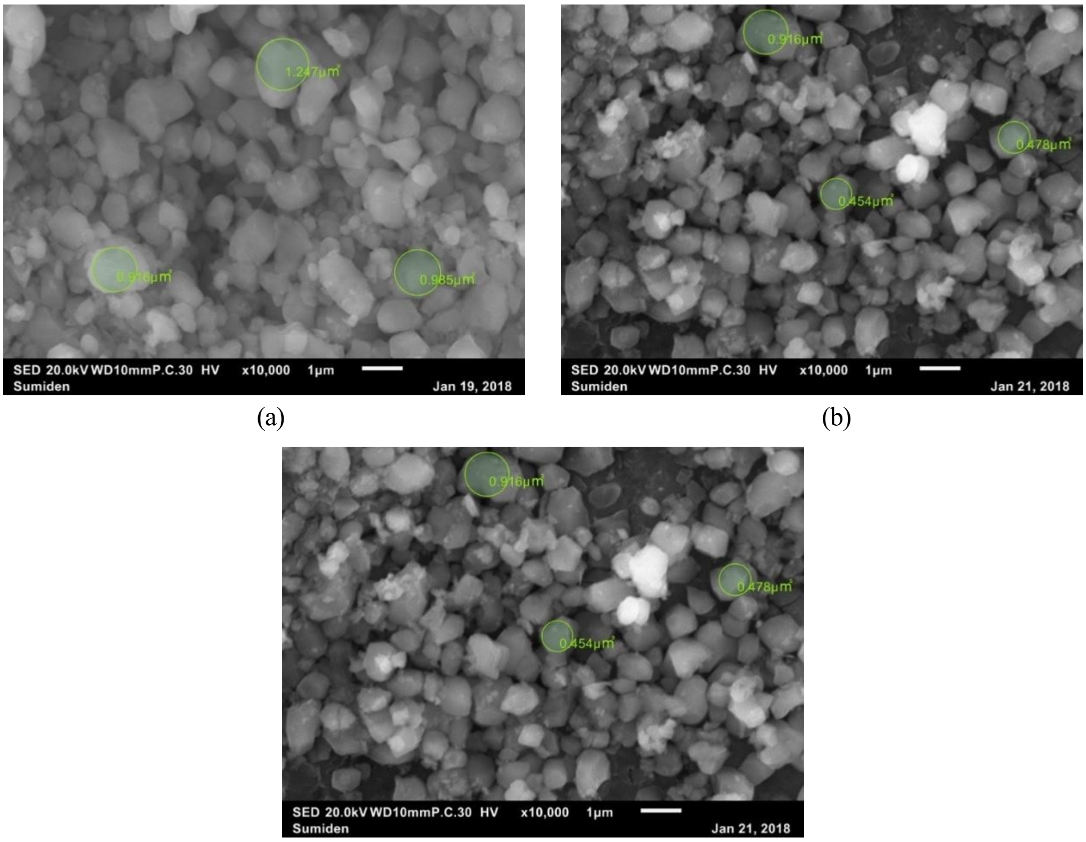
A toxicity test was conducted in vitro using CPAE (Cattle Pulmonary Artery Endothelial) cell culture media. This test was also performed in the microbiology and immunology laboratory of the Primate Animal Study Center, Bogor Agricultural University. The method was the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method with 1 type of sustainable cell in one plate with each sample, namely Mg-6Zn, with HAp variations of 5%, 8%, and 12%. After that, the samples were tested for toxicity in vitro by placing them in a CPAE cell culture with 50% confluence (24 hours of cell culture age).

III. Results and Discussions

1. Microstructure Analysis of Mg-6Zn HAp Biocomposite

Figure 1 shows the SEM results of Mg-6Zn-HAp composites with varying HAp concentrations. Mg particles appear larger than Zn particles, and after the mixing process, Zn particles are distributed homogeneously in the matrix. HAp particles are difficult to see in the microscopic structure because their size is much smaller than Mg and Zn, but their distribution can be observed with increasing HAp concentration. In composites with higher HAp concentrations, HAp particles are located at the grain boundaries, forming some agglomerations that help strengthen the material structure. This indicates that the addition of HAp in the composite contributes to the strengthening of mechanical properties through interactions at the grain boundaries. When its percentage increases, the presence of HAp becomes increasingly evident in the microstructure picture, as shown in Figure 1c. HAp occupies the grain boundaries and forms several agglomerations in its microstructure.

HAp can form strong bonds with the magnesium-zinc matrix. This bonding increases the cohesion between the material phases, contributing to the biocomposite's tensile and compressive strength. The proper microstructure between Mg-6Zn and HAp can help regulate the porosity level, which affects the biocomposite's ability to support tissue growth and its mechanical properties. Evenly distributed HAp can reduce the potential for corrosion by forming a protective film that facilitates direct contact between the metal and the environment.



(a) (b)

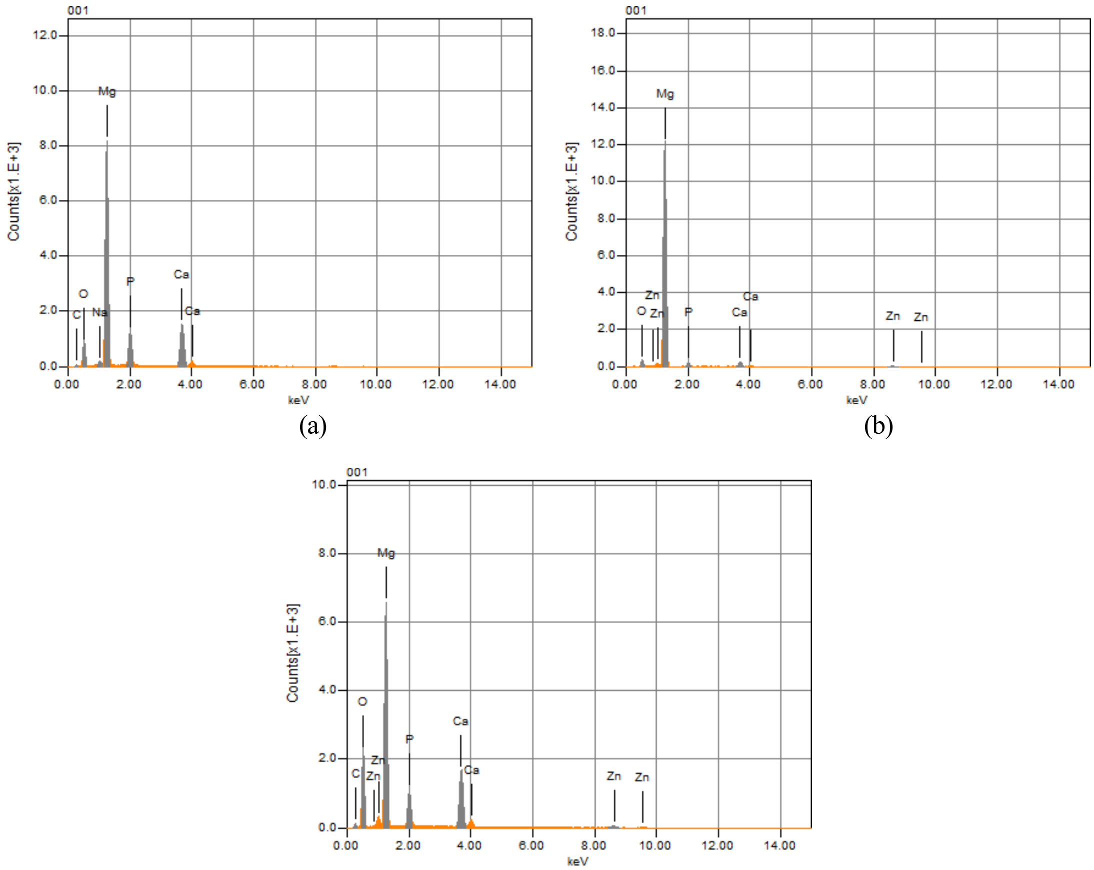
(c)

Fig. 1. SEM results on Mg-6Zn HAp biocomposite: (a) Mg-6Zn HAp 5%; (b) Mg-6Zn HAp 8%; (c) Mg-6Zn HAp 12%

2. EDS Analysis of Mg-6Zn HAp Biocomposite

Figure 2 shows the EDS results of the Mg-6Zn HAp biocomposite used in this study. HAp particles are almost indistinguishable in the mixture because their size is much smaller than Mg and Zn. However, the EDS results in several spots in Figure 2 show that the HAp element has been distributed in the mixture. The presence of HAp is increasingly evident in the microstructure image when the percentage is greater, as shown in Figure 1. The EDS results of the MgZn-8HAp Biocomposite in Figure 2b show only Mg elements in the grains (dark colour, 1b), while Zn and HAp are at the grain boundaries (light colour). The alloying elements at the grain boundaries can usually strengthen the material's properties. Therefore it can be said that the Mg-6Zn-xHAp biocomposite is stronger than the MgZn alloy.

Zn detected in the grains without visible phase differences in its microstructure indicates that some Zn has dissolved into Mg. The solubility limit of Zn in Mg alloy is 6.2% by mass at 340 °C. However, the EDS results at several spots in Figure 2 indicate that the HAp element has been distributed in the mixture.



(a) (b)

(c)

Fig. 2. EDS results on Mg-6Zn HAp biocomposite: (a) Mg-6Zn HAp 5%; (b) Mg-6Zn HAp 8%; (c) Mg-6Zn HAp 12%

3. Phase Analysis of XRD Results of Mg-6Zn HAp Biocomposite

The diffraction pattern of Mg-6Zn biocomposite with HAp variations sintered with Furnace is shown in Figure 3. Diffraction pattern of Mg-6Zn HAp 5% (black colour), Mg-6Zn HAp 8% (red colour), and Mg-6Zn HAp 12% (blue colour). Phase analysis was carried out by observing the positions of the peaks found in the diffraction patterns of biocomposites with different HAp compositions. Based on the diffraction pattern in Figure 3, it can be seen that no new peaks appear after the sintering process, meaning that no new phases are formed after the sintering process. However, when viewed in more detail, there is an increase in the intensity of all Mg peaks along with the addition of HAp and a decrease in the intensity of the Zn peak.

The dissolution of Zn into Mg is also confirmed by the decrease in the intensity of the Zn peak. The shift of the Mg peak and the decrease in the intensity of the Zn peak in the diffraction pattern after the sintering process indicate that the Mg-6Zn HAp biocomposite has been successfully synthesized by the sintering process. The resulting biocomposite does not form a new phase but forms a solid solution of the Mg-6Zn HAp biocomposite. The HAp peak is not clearly visible in the diffraction pattern of the Mg-6Zn HAp biocomposite in Figure 3 even though it has been added up to 12%. This is because the position of the highest HAp peak (31.773°) almost coincides with one of the high Mg peak positions (32.194°) but when highlighted in a narrower 2θ range (25°-40°) and a lower intensity range (300-1100) several HAp peaks appear with increasing intensity with increasing HAp percentage. The HAp peaks that appear are on the (211) lattice plane at position 31.7°, the (002) lattice plane at 25.8° and the (112) lattice plane at 32.2°.

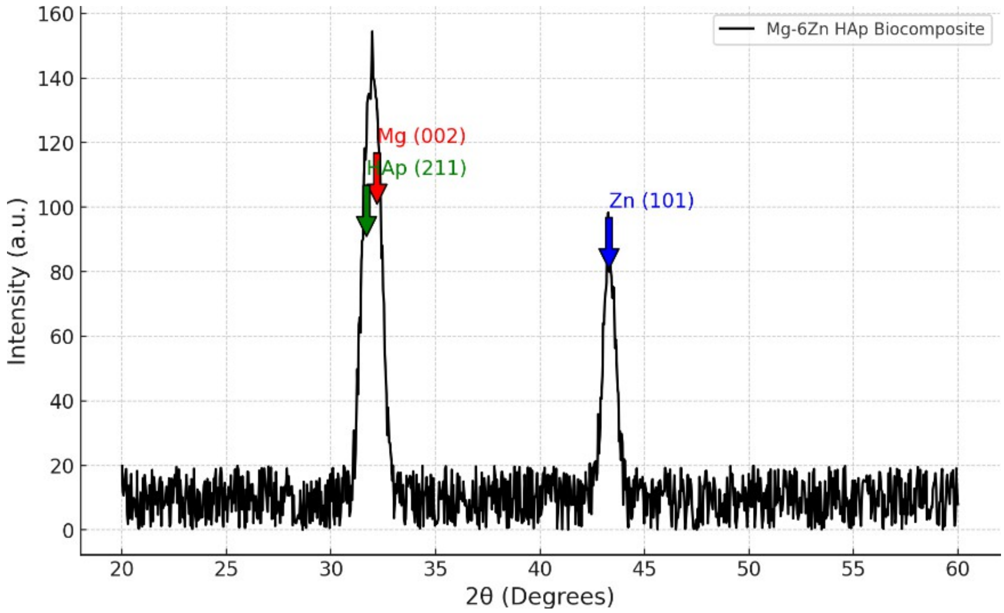


Fig. 3. Diffraction pattern of Mg-6Zn HAp biocomposite

4. Density Analysis

Table 1 presents the density measurements of the Mg-6Zn composite with varying additions of HAp at 5%, 8%, and 12%. The results show that density increases with higher HAp percentages in the composite. This is consistent with the theoretical density values, as HAp, having a higher density than the Mg-6Zn matrix, contributes to an increase in the composite's relative density.

Table 1. Comparison of HAp addition with corrosion level on Mg-6Zn-HAp biocomposite

No	Composition	Density measure (gr/ml)	Density theory (gr/ml)	Density relatively
1	Mg-Zn-HAp 5%	1.9218	2.12030	90.60
2	Mg-Zn-HAp 8%	1.9859	2.15408	92.19
3	Mg-Zn-HAp 12%	2.1218	2.19912	96.48

From the density measurement, it was found that the density increased with the addition of HAp from bovine bone to the Mg-6Zn HAp biocomposite; this is by the theoretical density value [20]. The Mg-6Zn HAp biocomposite has an increase in density with the addition of HAp; this is due to the addition of HAp, which has a higher density than the Mg-6Zn HAp alloy [20], [21]. And the relative density calculated based on the measured density and calculated density has increased.

5. Hardness Analysis

The hardness of pure Magnesium is 32.5 HVN [22]. In this study, the hardness value of 38.2 HVN was produced in the Mg-6Zn HAp biocomposite of 5% bovine bone, increasing to 39.00 HVN or at a HAp percentage of 8% wt. At a rate of Hydroxy apatite from the bovine bone of 12% or Mg-6Zn-HAp biocomposite of 12%, the hardness obtained was 53.84 HVN; this value is still higher than the hardness value of the commercial Mg-HAp composite, which is 45 HVN (theory), 42 HVN [23], or 50 HVN [24] can be seen in Table 2.

Table 2. Comparison of HAp addition with Hardness on Mg-6Zn-HAp biocomposite

No	Composition	Hardness (HVN)
1	Mg-Zn-HAp 5%	38.2
2	Mg-Zn-HAp 8%	39.0
3	Mg-Zn-HAp 12%	53.84

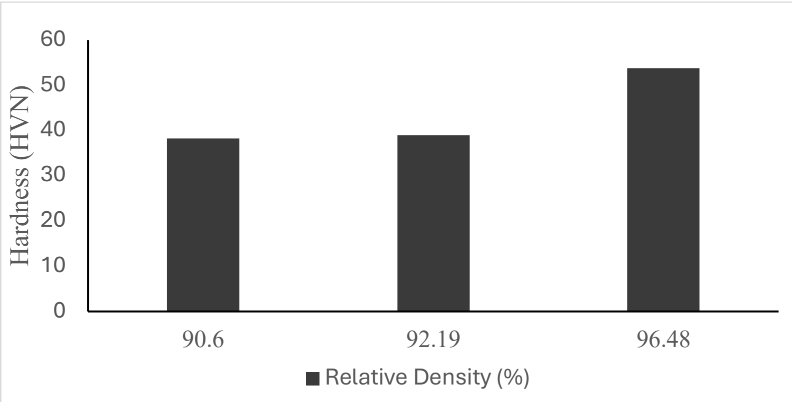


Fig. 4. Graph of the relationship between relative density and hardness

Figure 4 shows the relative density and hardness graphs of the Mg-Mg composite Xwt.% HAp, and the relative density of the composite increases with the increasing amount of HAp addition. We can see that the relative density of the sintered sample reached as high as 96.48 % in biocomposite containing 12 % HAp from bovine bone. This result is based on the theoretical density value in Figure 4, where increasing the weight percentage of HAp in Mg-HAp composite will increase the relative density of the biocomposite.

The hardness measurements showed an increased microhardness value with an increasing weight percentage of HAp. This indicates reinforcement into the magnesium matrix to a certain level according to the addition of bovine bone HAp. The presence of fragmented HAp in the grain boundary region increased relative density and hardness, which can significantly improve the mechanical properties of Mg.

6. Corrosion Analysis

The results of comparison of HAp addition with corrosion level on Mg–6Zn-HAp biocomposite are shown in Table 2. Corrosion testing using a potentiostat showed that the MgZn-xHAp biocomposite implant material from bovine bone is degradable, with a higher corrosion rate than that of SS316L [23]. The more Zn dissolved in Mg, the smaller the corrosion rate. This is because Zn has a smaller oxidation potential than Mg [25], which is more corrosion-resistant than Mg. MgZn-xHAp biocomposite from bovine bone at all HAp percentages from bovine bone has better corrosion resistance than commercial MgZn-xHAp biocomposite as indicated by a smaller corrosion rate value, where the corrosion rate value of commercial MgZn-xHAp biocomposite is 0.279 - 0.565 mpy [26].

Table 3. Comparison of HAp addition with corrosion level on Mg –6Zn-HAp biocomposite

No	Composition	Corrosion (may)
1	Mg-6Zn-HAp 5%	0.238
2	Mg-6Zn-HAp 8%	0.166
3	Mg-6Zn-HAp 12%	0.0417

The corrosion resistance of the MgZn-xHAp biocomposite implant material derived from bovine bone improved across all test solutions with increased HAp composition. Compared to the findings in [27], the corrosion rate of the MgZn biocomposite with bovine-derived HAp was lower than that of commercial HAp.

7. Toxicity Analysis

The results of the toxicity test on the sterile Mg-6Zn HAp biocomposite samples of bovine bone calcined at a temperature of 900 °C were shown by the percentage of CPAE cell viability (Table 4). Using the concentration of Mg-6Zn HAp biocomposite extract of bovine bone calcined at a temperature of 900 °C. Cell viability was very good at a HAp percentage of 5%, namely up to 103.8% at 0.15 ppm.

Table 4. Viability of CPAE cells

Sample Mg-6Zn HAp 5%	Test			Average	Cell Viability Reduction (%)
ppm	ODI	DII	DIII		
10	0.337	0.460	0.544	0.447	5.56
1	0.264	0.397	0.511	0.391	17.46
0.15	0.371	0.476	0.627	0.491	-3.80
Cell control	0.481	0.440	0.499	0.473	0.00
Sample Mg-6Zn HAp 8%	Test			Average	Cell Viability Reduction (%)
ppm	ODI	DII	DIII		
10	0.211	0.191	0.270	0.224	52.68
1	0.177	0.285	0.252	0.238	49.72
0.15	0.192	0.182	0.192	0.189	60.14
Cell control	0.481	0.440	0.499	0.473	0.00
Sample Mg-6Zn HAp 12%	Test			Average	Cell Viability Reduction (%)
ppm	ODI	DII	DIII		
10	0.211	0.191	0.270	0.224	52.68
1	0.177	0.285	0.252	0.238	49.72
0.15	0.192	0.182	0.192	0.189	60.14
Cell control	0.481	0.440	0.499	0.473	0.00

The toxicity test results with MTT show whether the composite matrix produced is safe and does not cause toxicity. In this test, an Elisa reader instrument (Thermo Scientific) with a wavelength of 620 nm was used. The toxicity criteria are as follows: the material is declared non-toxic if the measurement result reading is more than 60%, meaning the percentage of living cells is more than 60%.

Table 4 shows that the extract of Mg-6Zn HAp biocomposite from bovine bone does not inhibit the growth of CPAE cells at a HAp percentage of 5%, but when HAp percentages of either 8% or 12% are added to the Mg-6Zn HAp biocomposite, cell inhibition increases, and cell viability decreases.

IV. Conclusions

This study successfully synthesized Mg-6Zn-HAp composite from bovine bone as a candidate for biodegradable bone implant material through high-energy ball milling and sintering processes. Based on characterization using XRD and SEM/EDX, it was found that the addition of HAp did not create a new phase but formed a solid solution that strengthened the Mg-6Zn matrix. The results showed that the addition of HAp up to 12% gave the best results, marked by an increase in hardness reaching 53.84 HVN and a decrease in corrosion rate to 0.0417 mpy. This HAp composition of 12% provides an optimal combination of mechanical strength and corrosion resistance, making it suitable for implant applications that require sufficient durability until the bone healing process is complete. Comparison with related literature shows that the ball milling method is effective for homogeneous particle distribution, increasing hardness, and corrosion resistance, thus allowing better integration between HAp and the Mg-6Zn matrix. The use of HAp from bovine bone also provides additional advantages in terms of bioactivity and biocompatibility, making it a safe material that supports bone regeneration. Thus, the Mg-6Zn composite with 12% HAp from bovine bone has high potential as a biodegradable bone implant material that is superior to commercial Mg-HAp materials on the market.

References

[1] S.G. Sukaryo, A. Purnama, and H. Hermawan, "Structure and properties of biomaterials," *Biomater. Med. Devices a Perspect. from an Emerg. Ctry.*, pp. 1–22, 2016, doi: 10.1007/978-3-319-14845-8_1.

[2] Q. Chen and G.A. Thouas, "Metallic implant biomaterials," *Mater. Sci. Eng. R Reports*, vol. 87, pp. 1–57, 2015.

[3] M. Hussain, S. Ullah, M.R. Raza, N. Abbas, and A. Ali, "Recent developments in Zn-Based biodegradable materials for Biomedical Applications," *J. Funct. Biomater.*, vol. 14, no. 1, p. 1, 2022.

[4] Y. Liu, X.-H. Chen, J.-A. Yang, H. Pan, D. Chen et al., "Fundamental theory of biodegradable metals—definition, criteria, and design," *Adv. Funct. Mater.*, vol. 29, no. 18, p. 1805402, 2019.

[5] H. Kabir, K. Munir, C. Wen, and Y. Li, "Recent research and progress of biodegradable zinc alloys and composites for biomedical applications: Biomechanical and biocorrosion perspectives," *Bioact. Mater.*, vol. 6, no. 3, pp. 836– 879, 2021.

[6] T. Zhang, W. Wang, J. Liu, L. Wang, Y. Tang, and K. Wang, "A review on magnesium alloys for biomedical applications," *Front. Bioeng. Biotechnol.*, vol. 10, p. 953344, 2022.

[7] F. Witte, "The history of biodegradable magnesium implants: a review," *Acta Biomater.*, vol. 6, no. 5, pp. 1680–1692, 2010.

[8] Y. Chen, J. Yan, Z. Wang, S. Yu, X. Wang, Z. Yuan et al., "In vitro and in vivo corrosion measurements of Mg–6Zn alloys in the bile," *Mater. Sci. Eng. C*, vol. 42,

pp. 116–123, 2014.

[9] F. Živić, N. Grujović, G. Manivasagam, C. Richard, and J. Landoulsi, "The potential of magnesium alloys as bioabsorbable/biodegradable implants for biomedical applications," *Tribol. Ind.*, vol. 36, no. 1, p. 67, 2014.

[10] Y. Chen, Z. Xu, C. Smith, and J. Sankar, "Recent advances on the development of magnesium alloys for biodegradable implants," *Acta Biomater.*, vol. 10, no. 11, pp. 4561–4573, 2014.

[11] X. Gu, Y. Zheng, Y. Cheng, S. Zhong, and T. Xi, "In vitro corrosion and biocompatibility of binary magnesium alloys," *Biomaterials*, vol. 30, no. 4, pp. 484–498, 2009.

[12] H. Hermawan, "Biodegradable metals: state of the art," *Biodegrad. Met. From Concept to Appl.*, pp. 13–22, 2012.

[13] A.H.M. Sanchez, B. J. Luthringer, F. Feyerabend, and R. Willumeit, "Mg and Mg alloys: how comparable are in vitro and in vivo corrosion rates? A review," *Acta Biomater.*, vol. 13, pp. 16–31, 2015.

[14] T.B. Matias, G.H. Asato, B.T. Ramasco, W.J. Botta, C.S. Kiminami, and C. Bolfarini, "Processing and characterization of amorphous magnesium based alloy for application in biomedical implants," *J. Mater. Res. Technol.*, vol. 3, no. 3, pp. 203–209, 2014.

[15] D. Vojtěch, J. Kubásek, J. Šerák, and P. Novák, "Mechanical and corrosion properties of newly developed biodegradable Zn-based alloys for bone fixation," *Acta Biomater.*, vol. 7, no. 9, pp. 3515–3522, 2011.

[16] E.M. Salleh, S. Ramakrishnan, and Z. Hussain, "Synthesis of biodegradable Mg-Zn alloy by mechanical alloying: effect of milling time," *Procedia Chem.*, vol. 19, pp. 525–530, 2016.

[17] E.M. Salleh, H. Zuhailawati, S. Ramakrishnan, and M.A.H. Gepreel, "A statistical prediction of density and hardness of biodegradable mechanically alloyed Mg–Zn alloy using fractional factorial design," *J. Alloys Compd.*, vol. 644, pp. 476–484, 2015.

[18] M.B. Kannan, "Hydroxyapatite coating on biodegradable magnesium and magnesium-based alloys," in *Hydroxyapatite (HAp) for biomedical applications*, Elsevier, 2015, pp. 289–306.

[19] M. Alizadeh-Osgouei, Y. Li, and C. Wen, "A comprehensive review of biodegradable synthetic polymer-ceramic composites and their manufacture for biomedical applications," *Bioact. Mater.*, vol. 4, pp. 22–36, 2019.

[20] J.L. Manalu, B. Soegijono, and D.J. Indrani, "Study of Mg-Hydroxyapatite composite with various composition of hydroxyapatite which obtained from cow bones in simulation body fluid (SBF)," *Asian J. Appl. Sci.*, vol. 4, no. 4, 2016.

[21] S. Zhang *et al.*, "In vitro and in vivo corrosion and histocompatibility of pure Mg and a Mg-6Zn alloy as urinary implants in rat model," *Mater. Sci. Eng. C*, vol. 68, pp. 414–422, 2016.

[22] S. Biswas, S.S. Dhinwal, and S. Suwas, "Room-temperature equal channel angular extrusion of pure magnesium," *Acta Mater.*, vol. 58, no. 9, pp. 3247–3261, 2010.

[23] A.K. Khanra, H.C. Jung, S.H. Yu, K.S. Hong, and K.S. Shin, "Microstructure and mechanical properties of Mg-HAP composites," *Bull. Mater. Sci.*, vol. 33, pp. 43–47, 2010.

[24] K.A. Khalil, "A new-developed nanostructured Mg/HAp nanocomposite by high frequency induction heat sintering process," in *IOP Conference Series: Materials Science and Engineering*, 2012, vol. 40, no. 1, p. 12031.

[25] M. Mandal, A.P. Moon, G. Deo, C.L. Mendis, and K. Mondal, "Corrosion behavior of Mg–2.4 Zn alloy micro-alloyed with Ag and Ca," *Corros. Sci.*, vol. 78, pp. 172–182, 2014.
[26] M. Silalahi, H. Sitompul, J.L. Manalu, K. Dahlan, D. Noviana, and A. Dimyati, "Novel technology on sinthesizing Mg-Zn biomaterial using arc plasma sintering," *Asian J. Appl. Sci.*, vol. 5, no. 3, 2017.
[27] J. Henny, A. Vassault, G. Boursier, I. Vukasovic, P.M. Brguljan, M. Lohmander et al., "Recommendation for the review of biological reference intervals in medical laboratories," *Clin. Chem. Lab. Med.*, vol. 54, no. 12, pp. 1893–1900, 2016.