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In vitro of Mg-1.6 Gd alloys after hot extruded for biomaterial application

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Abstract. Mg-1.6Gd (wt%) alloys have attracted interest in biodegradable implant materials because of the potential to eliminate secondary surgeries. The purpose of this study was to evaluate the *in vitro* degradation performance of the alloys after the thermo-mechanical process (hot extruded and hot rolled) and to determine whether the materials are sustainable for further investigation. The performances of the Mg-1.6Gd alloys was using cell viability (MTT) test during the experiment. The results showed that verified viable the osteoblast cell on all the different thermo-mechanical process and no obvious toxic effect within the various time.

1. Introduction

Binary Mg-Gd alloys are under investigation as degradable materials because of their promising properties for biomedical applications such as vascular stents or orthopedic implants. Current metallic implants as orthopedic devices present problems as they require secondary surgery for removal. A large portion of elective orthopedic operations for patients with bone fractures need the removal of fixation devices [1]. The Revision surgery can increase the medical costs as well as the health risks for patients. The development of the degradable implants materials as orthopedic devices aims to lower these medical expenses, and evidence has shown that total medical expenses were lower when using absorbable (polymer) orthopedic devices compared with traditional non-absorbable metallic devices [2-5].

An ideal biodegradable orthopedic implant must have the adequate mechanical support that adapts the process of bone healing and progressive resorbs. Therefore, magnesium alloys have been intensively investigated in recent years due to their potential as a new generation of degradable material with excellent properties, such as low density, high strength, creep resistance, inherent biocompatibility, and an elastic modulus of 45 GPa that is close to that of human bone [6-13].

Recently, Susanti *et al.* reported that certain Mg- 1,6Gd alloys exhibit values of mechanical properties, in detail tensile yield strength (TYS), ultimate tensile strength (UTS), and elongation through the thermo-mechanical process (hot extrusion) with the different temperature. The effect of microalloying addition Gd (1.6 wt%) in magnesium can improve strength due to precipitation hardening and at the same time can increase the elongation. The extruded alloy exhibits the recrystallized grain size and

excellent mechanical properties. In this case, a tensile strength between 187 and 232 MPa and a tensile yield between 127 and 142 MPa can be observed. The mechanical properties were associated with the change in the grain size. This discussion already explained in the paper before [14].
Susanti *et al.* also showed that the mechanical properties of Mg- 1.6Gd alloys could be improved by hot rolled at high deformation ratio [15]. The binary Mg-1.6Gd alloy was hot rolled with a total reduction of 95 % in the range of the recrystallization temperature. The mechanical properties of hot rolled samples prepared by two different methods were studied comparatively. The maximum tensile strength and yield strength observed for UR samples were 197 MPa and 157 MPa, respectively and for CR samples, the values were 164 MPa and 107 MPa, respectively. Further, the maximum elongation for UR samples was 26 % and, 17 % for CR samples.

2. Experimental

Cells were used osteoblast cells NHOS (Normal Human Osteoblast) (Lonza). Primary NHOS was cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Invitrogen, Jakarta, Indonesia) containing 10% serum bovine fetal (FBS), 100 U / ml penicillin, 100 mg / ml streptomycin at 37 ° C with 5% CO2, atmospheric conditions are incubated (INCO 2 Memmert) for about 4 days to allow for cell development. Correspondingly, to produce extracts of the Mg-1.6Gd alloy prepared according to ISO 10993-5: 1999 standards , where the sample was dissolved in Ringer's solution (1 ml / 0.2 gr sample)with the variation time of 3, 7 and 14 days. After that, osteoblast cells are planted in 96 cell culture plates with 5x103 sell / 100μL per well and incubated for 24 hours to allow the attachment to the base of the well. Some wells which are not mixed with Mg-1.6Gd alloy extract can be used as negative controls. Medium or each well then added with 25 μL of Mg-1.6Gd alloy extract solution and incubated for 1 day, then followed by giving MTT solution as much as 50 μL added on each well (incubation for 3 hours). After that, 100 μL of Acidified Isopropanol solution was added which was intended to differentiate the color of the growing and dead cells, then incubated for 1 hour. Spectrophotometric absorbance of each good measure cell density (OD) was used as a microplate reader (Bio-Rad 680) with a wavelength measurement of 570 nm.
The results were read by the number of living osteoblast cell can endurance. The negative controls were considered as normal osteoblast cell proliferation rates, the results obtained in the treatment group had a higher proliferation rate than the control group through viability reading.
Cell relative growth or the cell relative growth rate (RGR) is calculated using the formula:

$$RGR = \left(\frac{OD\ sample}{OD\ negative} \right) \times 100\% \qquad (2.1)$$

RGR = Relative Growth Rate (%) = Viability
OD = Optical density

3. Results and Discussion

Figure 2.1 shows that the hot extruded of the Mg-1.6 Gd alloys extract was not cytotoxic at all the different temperature and times. On the immersion for 3 days, the extract of the 400 °C temperature shows the cell viability of 90 %. The cell viability increase with the increase of the extrusion temperature and the viability for extrusion samples on 550 °C temperature increases significantly at about 126 %.

For immersion of 7 days, all the extrusion temperature show the cell viability increase compared with the immersion of 3 days. the cell viability of 400 °C temperature has the cell viability of 99 %, and the increase is about 132 % at the highest temperature (550 °C). The cell viability reaches the highest at 202 % for immersion of 14 days. It clearly is showed in **Figure 1**.

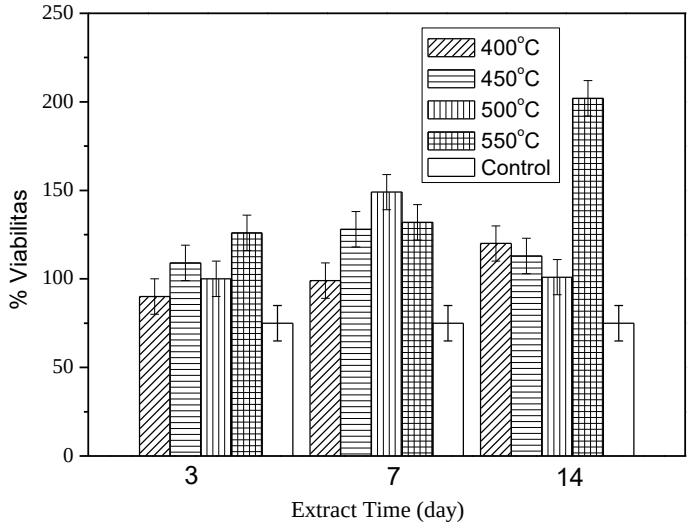

Figure 1. The viability of the osteoblast cell in 3 days, 7 days and 14 days immersion extract of The Mg-1.6Gd

The comparison of visualization cell between the cell control and the extruded alloys cell is shown in **Figure 2.** Both of the osteoblast cell development shows no different results.

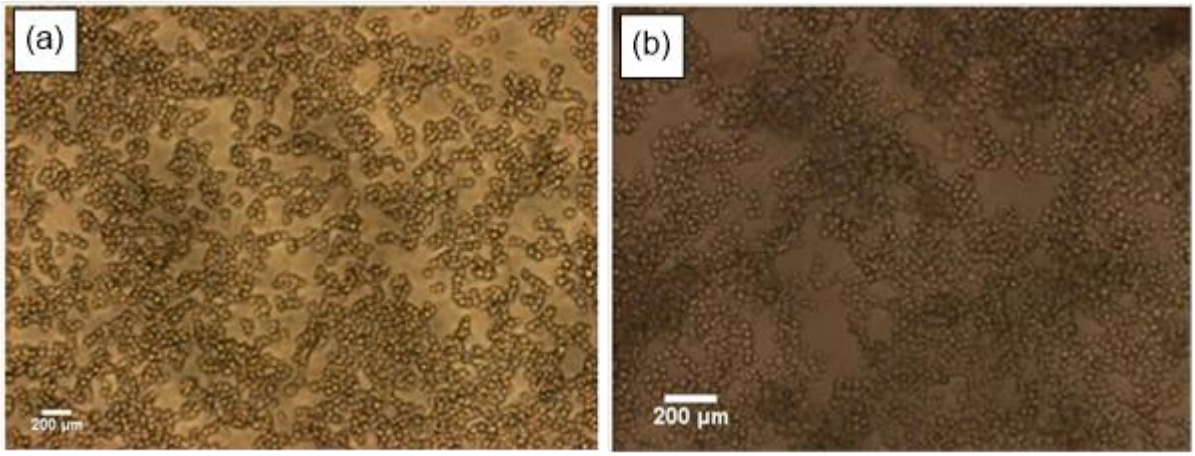

Figure 2. visualization of osteoblast cells for one of the extrusion samples, where the cells develop and grow compared to control cells

4. Conclusion

The cytotoxicity of the extruded Mg-1.6Gd alloys at different temperatures was investigated. The cell viability showed above the standard (75 %) for all the temperature of the hot extruded. That means which the extruded Mg-1.6Gd alloys show no obvious toxicity.

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