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Cytotoxicity of Mg-1.6Gd Alloys After Hot Rolling at an 80% Reduction Level as Implant Material

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Abstract. The high number of traffic accidents in Indonesia causes many victims which suffer from broken bones. Treatment which can be conducted is bone implants installation. Furthermore, magnesium is an attractive material for biodegradable bone implants since its physical properties are almost similar to human bone, but in the human body, magnesium experiences rapid dissolution before new tissue grows. Therefore, it needs to be combined with gadolonium in order to delay the solubility of magnesium. Before the Mg-Gd alloy was used as implant material, it is important to conduct a cytotoxicity test by using osteoblast cells. In order to obtain cell viability after it was given treatment, this study used the MTT Assay method. Furthermore, Mg-1,6Gd samples which had rolled with 80% at various temperatures of 400°C, 450°C, 500°C, and 550°C were inserted into plates which contained osteoblast cells. After the samples were incubated for 3, 7, and 14 days, the MTT was dissolved into the plate and it was read with Elisa Plate Reader. The study shows that the sample which has rolled with 80% reduction is non- toxic since the average viability value is above 70%. In addition, samples which had rolled with 80% at a temperature of 400°C have stable viability value which is above 70%.

Keywords: Implant Bone, Magnesium, Gadolinium, Osteoblast Cells, Cytotoxicity

1 Introduction

Magnesium-based metals have attracted attention due to their ability to dissolve in and be absorbed or excreted from the body. The human body requires approximately 375 mg/day of magnesium, which is stored in bone and blood tissues. Magnesium is the lightest metal with a density of 1.738 g/cm3 and an elastic modulus of 42 GPa, which is close to the natural elastic modulus of human bone (10-40 GPa). It can degrade through corrosion that occurs in bodily fluids [2]. However, the high solubility of magnesium is also a weakness, as corrosion can occur rapidly in physiological pH (7.4-7.6) and high chloride environments, resulting in decreased mechanical properties prior to healing and new tissue growth [4]. Adding rare earth elements to magnesium can enhance its mechanical properties and slow down the corrosion rate. Therefore, it would be beneficial to use them in the body during the bone tissue growth process [5]. The addition of Lanthanum (La) and Cerium (Ce) effectively improves corrosion resistance and the plasticity of the resulting alloys. However, in vitro tests

have shown that materials made with La and Ce exhibit higher toxicity compared to those made with other elements. Gadolinium (Gd) and Dysprosium (Dy) have better solubility rates of 23.49% and 25.3% respectively than Neodymium (Nd), making them more suitable as alloying elements. Hort et al.'s research shows that the addition of Gd to Mg results in an alloy with mechanical properties closer to bone and better elongation. Bian et al. also reported that Gd in Mg-1.8Zn-0.2Gd alloy produces low corrosion rates. Due to Gd's good solubility when combined with Mg, it opens up possibilities for the wider use of Mg-Gd alloys [6].

Mg-Gd alloys subjected to rolling deformation exhibit improved mechanical properties such as hardness, tensile strength, yield strength, and elongation. Based on these conditions, further testing is needed to assess the cytotoxicity of Mg-Gd alloys, considering them as implant materials that can be safely used in humans. This study will examine the compatibility of Mg-1.6Gd alloys produced through hot rolling processes with 80% and then the result related to mechanical tests.

2 Literature Review

2.1 Biomaterial as Implant Material

Biomaterial is a material that comes into direct contact with the biological systems of organisms, and its application can be used to replace or restore the function of damaged bone components. One of the uses of biomaterial in healthcare applications is for implants. In the selection of implant materials, they must meet various criteria, such as biocompatibility, which means the material must be able to integrate with the human body, in other words, not be rejected by the human body. Additionally, the material must be corrosion-resistant because the environment inside the human body is highly corrosive, so the implanted material must be durable during the rehabilitation phase [7].

Implant materials must also have mechanical properties similar to bones. This way, the implant can perform its function of replacing damaged joints or bones. As a load-bearing element, when the implant material is implanted into the human body, it is expected to integrate with the body's tissues. The material should also be osteoconductive, meaning it can bond with bone and act as an adhesive. One of the materials that can be used is magnesium [8].

2.2 Magnesium

Magnesium is the fourth most abundant cation in the human body. This element plays a crucial role in the function and structure of the human body. Magnesium deficiency can lead to diseases such as diabetes, hypertension, and atherosclerosis [9]. Magnesium is an essential electrolyte that helps control the movement of substances in and out of cell membranes, convert fats, proteins, and sugars into energy, and assist in controlling blood pH and body fluid balance. Magnesium is estimated to account

for about 1% in the blood, 33% in muscles and soft tissues, and 66% in bones. Magnesium has a tensile strength of 110 N/mm² in cast form [10].

In recent years, research on combining magnesium with rare earth elements such as Dy, Y, and Gd has been conducted. Based on previous studies, the tensile strength of Mg-10Dy alloy is higher than 131 MPa, the yield strength is higher than 83 MPa, and the corrosion rate is 0.8 mmpy. The corrosion resistance of Mg-8Y obtained through the solidification method of Mg and Y alloy reaches 2.17 mmpy. As for the Gd element in the Mg-Gd alloy, it has a broader potential use in biomedical applications. This is because the Mg-Gd alloy supports better growth of new cells in the body compared to other alloys [11].

2.3 Magnesium Alloys

Magnesium and its alloys have received special attention due to their potential applications in various industrial fields, including orthopedic medicine. Various studies on advanced materials offer options for magnesium and its alloys because they possess suitable chemical, physical, and mechanical properties for bone implant applications, with their low density being biocompatible and having optimal mechanical properties for bones [12]. For instance, magnesium and its alloys have an elastic modulus ranging from 40 to 50 GPa, which is similar to the elastic modulus of human bones, which is about 10-40 GPa [13].

Magnesium alloys that have been widely used as biomaterials include AZ91 (Mg-9%Al-1%Zn) and AZ31 (Mg-3%Al-1%Zn), which contain Zn as an additional element [14]. These alloys have good mechanical properties as implants but can produce Al3+ ions, reducing biocompatibility and resulting in poor corrosion resistance. This can hinder the perfect growth of fractured bone tissues [4].

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2.4 Mg-Gd Alloy

Mg-Gd alloy is considered as a biodegradable implant material. This is because Mg-Gd alloy has mechanical properties that are almost similar to bone, and the dissolution products of this alloy will not cause side effects such as dangerous toxic reactions [15]. In a study by Francesca et al., the effect of Mg release in Mg2Ag, Mg10Gd, and Mg4Y3RE alloys on human cells was tested. According to the test results, the corrosion of Mg10Gd helps human cells to grow and adhere better to the bone.

Due to the non-toxic nature of Mg-Gd alloy, it is expected to exhibit biocompatibility as an implant. According to the International Union of Pure and Applied Chemistry (IUPAC), biocompatibility is the ability of a material to respond to a specific application or interact with cell tissue without causing side effects. Additionally, there are five definitions of biocompatibility, which are as follows [16]:

1. Condition of being nontoxic or not causing side effects of the implant on the body.
2. Material's ability to adapt to the immune response in a physical application.
3. Comparison of the results of the implant material's response with the side effects of the tissue response.
4. Relates to the biomaterial's ability to perform its function without causing local damage and systemic effects on cells.
5. The capacity of the implant prosthesis to adapt within the body, which correlates with its influence on hormones, cells, and tissues without causing changes.

2.5 Cytotoxicity Test

Cytotoxicity refers to the toxic or harmful properties of a compound towards living cells. Cytotoxicity testing is an in vitro evaluation using cell cultures to assess the safety of drugs, food, cosmetics, and other chemical substances. In vitro cytotoxicity testing offers several advantages, such as rapid processing, the ability to condition cells, requiring small sample sizes, and providing a direct insight into cell behavior. Cytotoxicity testing is necessary for chemical compounds to determine their safety limits [17].

There are several methods to determine the results of cytotoxicity tests, including Trypan Blue Staining, Tritium-labeled Thymidine, and MTT assay. Trypan Blue Staining is a simple way to evaluate cell membrane integrity, which can indicate cell death or proliferation. However, this method is less sensitive. The second method, Tritium-labeled Thymidine, uses the radioactive compound tritium labeled on thymidine. The measurement of the amount of radioactive material taken up by the cell is highly accurate, but this method requires more time. On the other hand, the MTT assay is a colorimetric method that measures the reduction of tetrazolium salt to form purple formazan crystals by live cell mitochondria through their metabolism. The purple color formed is measured using an ELISA plate reader [18].

3 Experiments

3.1 Research Scheme

The steps undertaken in conducting this research can be seen in Figure 3.1.

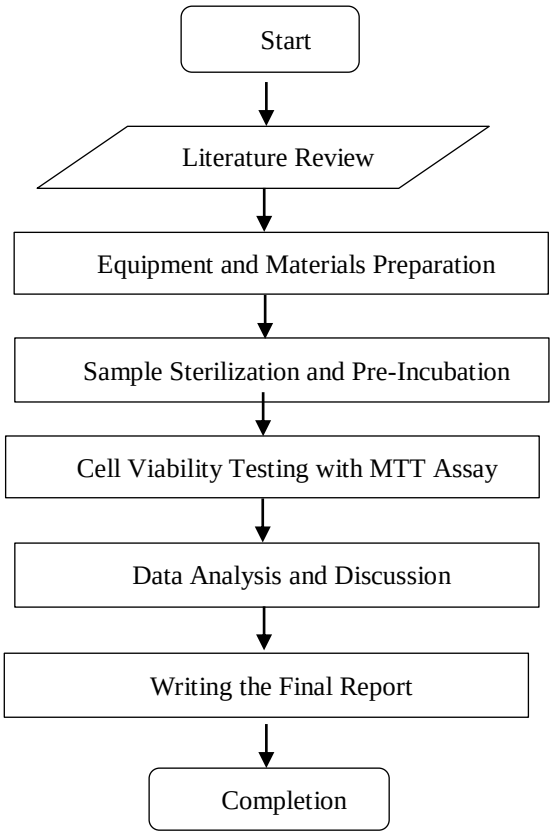


Figure 3.1 Research Diagram

3.2 Research Procedure

3.1.1 Literature Review

A literature review was conducted to gather relevant materials related to the research. This literature review was obtained from previous studies, books, scientific journals, the internet, and consultations with the advisor.

3.1.2 Equipment and Materials Preparation

The Mg-1.6Gd material initially came in sheet form, resulting from rolling with an 80% reduction and a thickness of 1 mm. The material was cut using a hand grinder and saw as auxiliary tools to obtain sample dimensions required for testing.

3.1.3 Sample Sterilization and Pre-Incubation

After the cutting process, there might be particles adhering to the sample. Hence, a cleaning step was performed using an ultrasonic cleaner. In this cleaning process, the sample was immersed in a methanol solution with a neutral pH of 7.33. Subsequently, ultrasonic waves were utilized to remove any remaining particles. Before cell seeding, the sample was pre-incubated to form a protective degradation layer. The sample was stored at a temperature of 37°C in a 5% CO_2 atmosphere for 12 hours.

3.1.4 MTT Assay Testing

To determine cell viability, MTT assay was conducted. Briefly, osteoblast cells were added to well plates containing the previously incubated samples. The cell media was replaced daily throughout the experiment. After 24 hours, 7 days, and 14 days, an MTT solution was added to the cell media. The cells were then incubated in the dark for 6 hours at 37°C. Subsequently, the cell media was discarded, and the cells were lysed using 0.004 NHCl in isopropanol. After cell lysis, the absorbance of the cells was read using a Microplate Reader.

4 Result and Discussion

4.1 Analysis of Biocompatibility Testing of Mg-1.6Gd Alloy

The biocompatibility of a material can be assessed using cytotoxicity methods. In this study, the Microtetrazolium Assay was employed to observe the cellular response to the material. The samples used for biocompatibility testing were MG-1.6Gd with extracts obtained from Ringer's solution after 3, 7, and 14 days of incubation for a duration of 24 hours. The data obtained from the biocompatibility testing of Mg-1.6Gd can be seen in the following tables, Table 4.1

Table 4.1 Data Results of MTT Assay for Mg-1.6Gd Sample with 80% Rolling Reduction.

Temperature	Cell Viability after 3 days (%)	Cell Viability after 7 days (%)	Cell Viability after 14 days (%)
400°C	77,11	85,55	94,35
450°C	30,89	17,11	78,14
500°C	30,67	34,14	78,27
550°C	44,71	36,03	103,43

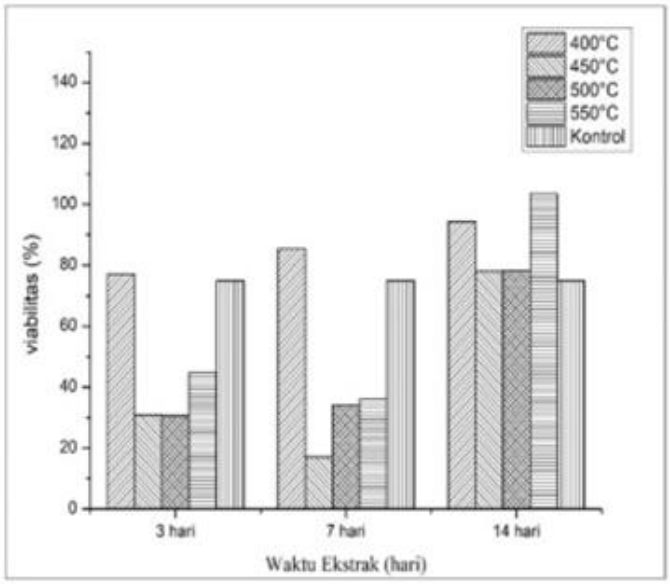
In Table 4.1, the viability values of Mg-1.6Gd with 80% rolling reduction at a temperature of 400°C on day 3 were 77.11%, on day 7 it was 85.55%, and on day 14 it was 94.35%. At a temperature of 450°C, the viability on day 3 was 30.89%, on day 7 it decreased to 17.11%, and on day 14 it increased to 78.14%. At 500°C, the viability on day 3 was 30.67%, on day 7 it increased to 34.14%, and on day 14 it further increased to 78.27%. At 550°C, the viability on day 3 was 44.71%, on day 7 it decreased to 36.03%, and on day 14 it unexpectedly increased to 103.43%.

According to the ISO 10993-5 standard, if the percentage of live cells is less than 75%, it indicates that the Mg-1.6Gd material may be toxic. If the percentage of live cells is above 100%, it indicates that the Mg-1.6Gd material is not toxic to the test cells, i.e., osteoblast cells. From Table 4.1,

it can be observed that the Mg-1.6Gd sample with 80% rolling reduction is not biocompatible as its viability values are below 75%, indicating its toxicity to live cells. However, the sample with the rolling process performed at a temperature of 400°C appears to be non-toxic as its viability values consistently remain above 75%. Furthermore, from Table 4.2, the Mg-1.6Gd sample with 95% rolling reduction shows non-toxic behavior, as its viability values are above 75%.

4.2 Comparison of Viability Test Results for Mg-1.6Gd Alloy

Biocompatibility testing of the Mg-1.6Gd alloy was conducted using the MTT Assay method. The data obtained from the biocompatibility testing using the MTT Assay method yielded viability graphs for each rolling condition. These graphs can be seen in the image below.



(a)

Figure 4.1 Cell viability of Mg-1.6Gd alloy in 3, 7, and 14-day extracts in osteoblast cell solution for 80% rolling

Figure 4.1 shows the cell viability for Mg-1.6Gd alloy with 80% In the 3-day extract, the viability values for the 80% rolling samples were below 70% except for the sample rolled at 400°C. On the other hand, the viability values for the 95% rolling samples were ,above 70%. On day 7, the average viability of the 80% rolling extract showed a significant decrease, while the 95% rolling extract showed an increase. For the 14-day extract, the viability of the 80% rolling samples increased above 70%, whereas the viability of the 95% rolling samples decreased, although still above 70%.

In Figure 4.1 (a), the sample rolled at 400°C exhibited good viability as its values were above 70%. The viability on day 3 was 77.11%, which increased by 8.44% on day 7 to reach 85.55%, and further increased to 94.35% on day 14. For the sample rolled at 450°C, the viability on day 3 was 30.89%, which decreased significantly to 17.11% on day 7. However, on day 14, the viability increased to 78.14%.

The sample rolled at 500°C had a viability of 30.67% on day 3, slightly increased to 34.14% on day 7, and significantly increased to 78.27% on day 14. For the sample rolled at 550°C, the viability was 44.71% on day 3, decreased to 36.03% on day 7, and remarkably increased to 103% on day 14.

From the data above, it can be concluded that the 95% rolling reduction had higher viability compared to the 80% rolling reduction. The viability was also consistently good for the samples rolled at 400°C, making them suitable for implant materials. This is because the percentage of viability always increased and remained above 75%.

Furthermore, for the samples rolled with 80% reduction, the viability ranged from 17.11% to 103.43%, indicating some samples were toxic in the 3-day and 7-day extracts, indicating non-toxic behavior. This finding aligns with Wen Yafeng et al., who reported that Mg-1Zn-1Sn alloy exhibited good biocompatibility and even promoted new bone cell growth [7].

5 Conclusion

The conclusions that can be drawn are as follows:

1. Biocompatibility testing of the samples with 80% reduction showed toxicity as their viability values were below 75%. On the other hand, the samples with 95% reduction had an average viability above 75% and were considered non-toxic.
2. Samples rolled at a temperature of 400℃ showed stable and increased viability values, indicating their suitability as bone implant materials, both for those with 80%

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