Cytotoxicity and Mechanical Properties of New Mg-Zn-Y-Nd-Ca Biodegradable Magnesium Alloys

Yuelai Dai* and Jiang Zhang

Ningbo Sub-academy of the National Weapons Science Research Academy, Ningbo, Zhejiang, 315103, China

*Corresponding author’s e-mail: daiyuelai_1988@126.com

Abstract. The new Mg-Zn-Y-Nd-Ca magnesium alloys are used as the research object, and its components are designed to evaluate the cytotoxicity of magnesium alloys with different component elements. The cytotoxicity test of fibroblast L-929 and osteoblasts MC3T3-E1 indicates that Mg-Zn-Y-Nd-Ca, as a biodegradable material, can meet the needs of cytotoxicity and promote the proliferation of osteoblasts MC3T3-E1. The mechanical properties test shows that the new magnesium alloys have good mechanical property, tensile strength is about 290Mpa and elongation can reach 15%.

1. Introduction

When the external material is implanted into the organism, there is the possibility of various biological, physical and chemical reactions. Biocompatibility refers to the material implanted into the organism, which can remain relatively stable with the organism, without obvious damage and rejection. When a new material can be implanted into the human body, the first step is to verify whether the material would cause toxic effects on various tissues of the human body. Therefore, it is necessary to evaluate the biocompatibility of new materials by conducting in vitro cytotoxicity tests. The amount of magnesium ions in human cells is second only to potassium ions. Magnesium alloys have negative charge on the surface and have low thrombotic and good biocompatibility [1]. In addition, magnesium also has the effect of reducing acute stent thrombosis, anti-arrhythmia and inhibiting endothelin angioconstriction reaction [2]. X Gu et al. [3] studied the cytotoxicity of binary magnesium alloys and found that Mg-1Al, Mg-1Si, Mg-1Sn, Mg-1Y, Mg-1Zn and Mg-1Zr had no obvious cytotoxicity. F Feyerabend et al. [4] studied the effect of Mg-RE alloy on primary cells, in which Mg-RE alloy contains rare earth elements (Y, Nd, Pr, Gd, La, Ce, Eu), and found that the ionic radius of rare earth elements is an important influencing factor. Y Zheng et al. [5] found that Mg-1Ca and Mg-5Ca alloy had no obvious cytotoxicity, while Mg-10Ca reduced cell activity by about 40%. E Zhang studied Mg-1Zn-1Mn magnesium alloy, which showed no obvious cytotoxicity [6].

2. Sample preparation

In the melting process of rare earth magnesium alloys, the addition of rare earth elements is mainly in the form of magnesium - rare earth intermediate alloys. Magnesium intermediate alloys are mainly Mg-20% Y, Mg-20% Nd and Mg-12% Zn. Calcium elements with grain refinement are added in the form of Mg-30% Ca. Table 1 shows Mg-Zn-Y-Nd-Ca magnesium alloys designed with different components. The magnesium alloys of various components are cut into Φ 22 mm x 3 wafers. After sandpaper grinding and diamond polishing, wafers are polished by magnesium oxide powder solution.
Table 1. Composition of Mg-Zn-Y-Nd-Ca alloy (wt.%)  

| Sample | Zn | Y | Nd | Ca | Mg |
|--------|----|---|----|----|----|
| A      | 0.5| 3.5| 2.4| -  | Balance |
| B      | 0.5| 3.5| 2.7| -  | Balance |
| C      | 0.5| 4.2| 2.4| -  | Balance |
| D      | 0.5| 4.2| 2.7| 0.6| Balance |

3. Experimental method  
The cultured cells include fibroblast L-929 and osteoblasts MC3T3-E1. The environmental conditions are 37°C and 5% CO2. The samples are ultrasonic cleaned in anhydrous ethanol for 30 minutes. The culture solution is extracted with medium containing serum. The incubation time is 1, 3 and 5 days respectively.

The relative growth rate (RGR) of the cells is calculated as follows:

$$RGR = \frac{OD(\text{experimental group})}{OD(\text{negative control group})} \times 100\%$$

The correlation between relative proliferation rate and cytotoxicity is shown in table 2, which can be used to classify the toxicity of materials.

Table 2. Correlation between relative proliferation rate and cytotoxicity  

| relative proliferation rate /% | cytotoxic grading | toxicity | results |
|-------------------------------|-------------------|----------|---------|
| >100                          | 0                 | non-toxic| qualified|
| 75-100                        | 1                 | very mild toxicity | qualified|
| 50-74                         | 2                 | mild toxicity | qualified|
| 25-49                         | 3                 | moderate toxicity | unqualified|
| 1-24                          | 4                 | severe toxicity | unqualified|

4. Cytotoxicity of new Mg-Zn-Y-Nd-Ca magnesium alloys on fibroblast L-929  
Table 3 shows the toxicity of L-929 cells cultured in 100% new magnesium alloys extract for 1, 3 and 5 days. The results show that the cytotoxicity of the new magnesium alloys to fibroblast L-929 is very mild, which meets the requirements of the material for cytotoxicity.

Table 3. Cytotoxicity of new Mg-Zn-Y-Nd-Ca magnesium alloys on fibroblasts L-929  

| Sample | Time/d | RGR/% | Cytotoxicity Classification | Conclusion |
|--------|--------|-------|----------------------------|------------|
| A      | 1      | 88.2  | 1                          | Qualified  |
|        | 3      | 79.5  | 1                          | Qualified  |
|        | 5      | 75.8  | 1                          | Qualified  |
|        | 1      | 102.5 | 0                          |            |
| B      | 3      | 85.2  | 1                          | Qualified  |
|        | 5      | 79.3  | 1                          | Qualified  |
|        | 1      | 95.4  | 1                          |            |
| C      | 3      | 89.6  | 1                          | Qualified  |
|        | 5      | 85.7  | 1                          | Qualified  |
|        | 1      | 99.5  | 1                          |            |
| D      | 3      | 81.9  | 1                          | Qualified  |
|        | 5      | 94.1  | 1                          |            |

5. Cytotoxicity of new Mg-Zn-Y-Nd-Ca magnesium alloys on osteoblasts MC3T3-E1  
Table 4 shows the toxicity of MC3T3-E1 cells cultured in 100% new magnesium alloys extract for 1, 3 and 5 days. The results show that the new Mg-Zn-Y-Nd-Ca magnesium alloys have no cytotoxicity on MC3T3-E1 cells and promote the proliferation of cells.
Table 4. Cytotoxicity of new Mg-Zn-Y-Nd-Ca magnesium alloy on osteoblasts MC3T3-E1

| Sample | Time/d | RGR/%  | Cytotoxicity Classification | Conclusion |
|--------|--------|--------|-----------------------------|------------|
| A      | 1      | 98.1   |                             | Qualified  |
|        | 3      | 108.5  | 0                           |            |
|        | 5      | 107.3  | 0                           |            |
|        | 1      | 99.7   | 1                           |            |
| B      | 3      | 109.8  | 0                           | Qualified  |
|        | 5      | 105.2  | 0                           |            |
|        | 1      | 97.6   | 1                           |            |
| C      | 3      | 107.3  | 0                           | Qualified  |
|        | 5      | 105.5  | 0                           |            |
|        | 1      | 96.1   | 1                           |            |
| D      | 3      | 106.9  | 0                           | Qualified  |
|        | 5      | 103.4  | 0                           |            |

6. Mechanical properties of Mg-Zn-Y-Nd-Ca magnesium alloy

The sample is processed with the design component of sample D. Tensile properties are tested on the SHIMADZUAG-100KNA material tester at a tensile speed of 1 mm/min. As shown in figure 1, the tensile strength of Mg-Zn-Y-Nd-Ca is about 290MPa and its elongation can reach 15%, which is higher than commercial AZ31 and WE43 magnesium alloys.

![Figure 1. Tensile curves of new Mg-Zn-Y-Nd-Ca magnesium alloys at room temperature](image)

7. Conclusion

The cytotoxicity of new Mg-Zn-Y-Nd-Ca degradable magnesium alloys with different components on different series of cells is studied. New Mg-Zn-Y-Nd-Ca degradable magnesium alloys have very slight cytotoxicity on fibroblast L-929, meeting the requirements of biological materials for cytotoxicity. New Mg-Zn-Y-Nd-Ca degradable magnesium alloys can promote the proliferation of osteoblasts MC3T3-E1. As a biodegradable material, Mg-Zn-Y-Nd-Ca can meet the requirements of cytotoxicity. Compared with traditional commercial magnesium alloys AZ31 and WE43, its mechanical properties are better.

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