Studies on Biocompatibility of Mg-4.0Zn-1.5Sr Alloy with Coated of the Laser Surface Processing Combining Alkaline Treatment

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Abstract. The surface modification of biomaterial Mg-4.0Zn-1.5Sr alloy has been done by means of laser surface processing combining alkaline treatment process, as well as the biocompatibility of Mg-4.0Zn-1.5Sr alloy with and without coatings has been analyzed comparatively. The results indicate that the optimal parameters of laser surface processing are that the power is 3 kW, the current 200A, the width 1mm, the defocus amount 135mm and the scanning speed 1mm/s. The optimal parameters of alkaline treatment are that the solution is NaOH, the concentration 0.5 mol/L, the temperature 80 °C and the time 12 h. After alkaline treatment the surface is smooth and compact. The hemolysis rate of 1 d, 3 d, 5 d for uncoated alloy is 1.04%, 0.8% and 4.56%, respectively, which is less than 5%, and for Mg-4.0Zn-1.5Sr alloys with coated of the laser combining alkaline treatment, the hemolysis rate of 1 d, 3 d, 5 d is 0.62%, 1.24% and 0.83% respectively, which is also less than 5%. Therefore, the phenomenon of hemolysis for the alloy with and without coated will not occur. In addition, HR on coated with the laser combining alkaline treatment have larger decline, and less volatility than that without coated, which is express that surface treatment of the alloy has more application prospects. RGR value with coated of laser combining alkaline treatment is 105.7%, 106.0%, 110.4%, respectively which cultivation for 1 d, 3 days, 5days and slightly higher than that of without coated, which is100%, 108.8% and 101.8%, respectively. According the standard of cell cytotoxicity, for the alloy with and without coated it is zero level cytotoxicity, suitable for used of biomaterial.

1. Introduction
In recent decades, as a novel biomedical planting material, the study of magnesium alloys is paid more attentions [1]. It is well known that magnesium (Mg) and magnesium alloys possess many properties superior to other metallic biomaterials. However, magnesium alloys studied at present are mostly focused on industrial area, usually containing some elements that are harmful to human healthy such as aluminium, manganese or rare earth elements. In addition, the rapid degradation rate and relatively low biological activity restrict its widespread use in clinical applications. Therefore, the research on magnesium-based implant materials which are consisted with all of the nourishment elements and with the lower degradation rate has become an important subject with growing interests [2, 3].

The surface modification is one of the most effective ways to improve the alloy corrosion resistance, including the laser surface treatment can greatly improve the fatigue corrosion resistance, and alkali heat treatment can significantly improve the corrosion resistance. In addition, the biocompatibility of the alloy with surface modification is one of the key factors of application. In this paper, In order to improve the biocompatibility of the alloy, the Mg-4.0Zn-1.5Sr alloy is designed, and
laser surface processing combining alkaline treatment process is optimized to improve the corrosion resistant properties of the alloy, and the biocompatibility of the alloy with and without coating has been studied.

2. Materials and Experimental

2.1. Materials

In the experiment, pure magnesium with a purity of 99.9 % was adopted. The Mg-4.0Zn-1.5Sr (wt. %) alloy ingots was produced by melting with Ar gas protection. The magnesium and its alloy ingots were rolled into sheets in three sequential steps at 350 °C. The final sheet size was 13×13×1mm.

2.2. The Coating Preparation of Mg-4.0Zn-1.5Sr Alloy

By means of YAG laser device to make laser surface treatment, and hot type magnetic stirrer to make alkaline treatment. the optimal parameters of laser surface processing are that the power is 3 kW, the current 200 A, the width 1 mm, the defocus amount 135 mm and the scanning speed 1 mm/s. The optimal parameters of alkaline treatment are that the solution is NaOH, the concentration 0.5 mol / L, the temperature 80 °C and the time 12 h. The surface morphology and composition were observed and analyzed by means of Scanning electron microscopy (SEM) and X-ray diffraction (XRD).

2.3. Hemolysis Test

In order to investigate the dynamic change of hemolysis of the experimental material during degradation, the samples were immersed in SBF for 3 days, and their hemolytic rates were evaluated. The hemolysis test in vitro was conducted as follows: the samples (20 mm×20 mm×1 mm) were immersed in SBF for different times with extraction ratio of 1.25 cm²/mL at 37 °C and incubated for 24 hours. 10 mL of fresh human arterial blood from a healthy donor containing 0.5 mL potassium oxalate (20g/L) anticoagulant was added into the SBF solution which was kept at 37 °C for 60 minutes and then centrifuged at 3000 rpm for 5 minutes. At last, the optical density (OD) of the supernatant solution was measured using a spectrophotometer (722, Shanghai Precision Science Instrument Co., Ltd., Shanghai, China) at the wavelength of 545 nm. The hemolytic rate (HR) was obtained by the following equation:

$$HR(\%) = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}}$$  \hspace{1cm} (1)

where Dt is the absorbance of the testing specimen; Dnc is the absorbance of negative control groups (10mL SBF with 0.2mL diluted blood) and Dpc is the absorbance of positive control groups (10mL distilled water with 0.2 mL diluted blood).

2.4. Cytotoxicity Test

The samples were washed with distilled water, cleaned with ethanol, dried in air, and then sterilized by use of ethylene oxide. Then the samples were separately placed into a RPMI1640 culture medium and incubated at 37 °C for 24 h with a ratio of sample surface area to RPMI1640 medium volume at 1 cm²/10mL. After incubation, the media containing the extracts of the samples were collected for testing with cells. L929 fibroblasts cells were used as the model cells to test cytotoxicity. Healthy fibroblasts were digested by 0.25 % pancreatin to prepare a cell suspension with a cell density of 1x10⁴ cells per mL. The 200 μL of the cell suspension were pipetted into each well of a 96-well culture plate. Total of three 96-well plates were incubated under standard cell culture conditions (that is, a sterile, humidified, 37 °C, 5 %CO₂ + 95 % air environment). After 24 hours of incubation, the culture media were discarded and the wells of the 96-well plates were washed twice by phosphate buffered saline (PBS). The RPMI media containing the extracts of the alloy samples were added into the three 96-well plates. RPMI1640 culture medium without the extracts was used as a negative control. This experiment was performed in triplicate for all testing samples. One 96-well plate was taken out for characterization after 3 days of culture. First, the morphology and membrane integrity of
the cells were observed and imaged under an inverted microscope. Then, the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) cytotoxicity test is conducted. The 20 μL of 5% MTT was added into each well of 96-well plates and incubated for 4 hours under standard cell culture conditions. After 4 hours, the media were removed from the well, the 150 μL DMSO was added into the wells, and the plates were gently agitated for 10-15 minutes to fully dissolve the crystals. A standard enzyme detector was used to measure the absorbance at 490nm. Finally, the relative growth rate (RGR %) of the cells was calculated by the relation below:

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RGR\% = \frac{\text{Average absorbance value of the testing group}}{\text{Average absorbance value of the negative contrast group}} \times 100\%
\]

3. Results and Discussion

3.1. The Surface Morphology and Microstructure of Mg-4.0Zn-1.5Sr Alloy with Coating of Laser Combining Alkaline Treatment

The SEM image of the cast structures of Mg-4.0Zn-1.5Sr alloy is shown in Fig.1 (a). It can be seen that the second phase shows mainly the point and the chain which has a good dispersion. The cast microstructure of Mg-4.0Zn-1.5Sr alloy is mainly composed of the phases of α-Mg, MgZn2, Mg17Sr2 and small amount of MgZn. The metallographical image of Mg-4.0Zn-1.5Sr alloy after laser surface processing combining alkaline treatment is shown in Fig.1 (b). It can be seen that after alkaline treatment the surface is smooth and compact.

The XRD analysis result of Mg-4.0Zn-1.5Sr after laser combining alkaline treatment is shown in Fig.2. It can be seen that after alkaline treatment, there are only two phases of Mg (OH) 2 and magnesium matrix. In addition, the large amount of Mg (OH) 2 generated the surface can improve the corrosion resistance of the alloy.

Figure 1. The SEM image of (a) the cast structures of Mg-4.0Zn-1.5Sr alloy; (b) Mg-4.0Zn-1.5Sr with coated of laser combining alkaline treatment

Figure 2. The XRD analysis result of Mg-4.0Zn-1.5Sr after laser combining alkaline treatment
3.2. The Experimental Result of Hemolysis Rate

HR values of the Mg-4.0Zn-1.5Sr alloys with and without coated the laser combining alkaline treatment is shown in Fig. 3. It can be seen that for uncoated alloy, the hemolysis rate of 1 d, 3 d, 5 d is 1.04%, 0.8% and 4.56%, respectively, which is less than 5%, so the phenomenon of hemolysis will not occur according to the standard of HR [4]. For Mg-4.0Zn-1.5Sr alloys with coated of the laser combining alkaline treatment, the hemolysis rate of 1 d, 3 d, 5 d is 0.62%, 1.24% and 0.83% respectively, which is also less than 5%, the phenomenon of hemolysis will not occur. In addition, HR on coated with the laser combining alkaline treatment have larger decline, and less volatility than that without coated, which is express that surface treatment of the alloy has more application prospects.

The change of pH values of the Mg-4.0Zn-1.5Sr alloys with and without coated of laser combining alkaline treatment is shown in Fig.4. It can be seen that pH of Mg-4.0Zn-1.5Sr alloys without coated is 12.42, 13.02, 12.94 immersed in SBF for 1day, 3days and 5days, respectively. After coated by laser combining alkaline treatment, it is 11.56, 12.02, 11.9 immersed in SBF for 1day, 3days and 5days, respectively. As well as, pH value of the alloy after treated by laser combining alkaline is slightly lower, which is more suitable for the growth of cells.

![Figure 3](image3.png)

**Figure 3.** HR values of the Mg-4.0Zn-1.5Sr alloys with and without coated of laser combining alkaline treatment

![Figure 4](image4.png)

**Figure 4.** The change of pH values of the Mg-4.0Zn-1.5Sr alloys with and without coated of laser combining alkaline treatment
The dyed morphologies of L929 mice fibroblasts of Mg-4.0Zn-1.5Sr alloys with coated of laser combining alkaline treatment cultivated for the different times are shown in Fig.5. It can be seen that the normal cells form and grow well, which there are the existence of cell apoptosis and growth shown in Fig.5 (a). For the dyed morphologies of L929 mice fibroblasts of Mg-4.0Zn-1.5Sr alloys with coated of laser combining alkaline treatment immersed in SBF, there is the normal cell growth, and compared with the control group, no obvious phenomenon of cell death, expressing cell toxicity is very small. The RGR values of the Mg-4.0Zn-1.5Sr alloy with coated of laser and alkaline treatment is shown in Fig.6. It can be seen that RGR value with coated of laser combining alkaline treatment is 105.7%, 106.0%, 110.4%, respectively which cultivation for 1 d, 3 days, 5 days and slightly higher than that of without coated, which is 100%, 108.8% and 101.8%, respectively. According the standard of cell cytotoxicity [5], for both materials with and without coated it is zero level cytotoxicity, suitable for used of biomaterial.

Figure 5. The dyed morphologies of L929 mice fibroblasts of Mg-4.0Zn-1.5Sr alloys with coated of laser combining alkaline treatment cultivated for (a) the control group; (b) one day; (c) three days; (d) five days

Figure 6. The RGR values of the Mg-4.0Zn-1.5Sr alloy with coated of laser and alkaline treatment
4. Conclusion
The optimal parameters of laser surface processing are that the power is 3 kW, the current 200A, the width 1mm, the defocus amount 135mm and the scanning speed 1mm/s. The optimal parameters of alkaline treatment are that the solution is NaOH, the concentration 0.5 mol/L, the temperature 80 °C and the time 12 h. After alkaline treatment the surface is smooth and compact. The hemolysis rate of 1 d, 3 d, 5 d for uncoated alloy is 1.04%, 0.8% and 4.56%, respectively, which is less than 5%, and for Mg-4.0Zn-1.5Sr alloys with coated of the laser combining alkaline treatment, the hemolysis rate of 1 d, 3 d, 5 d is 0.62%, 1.24% and 0.83% respectively, which is also less than 5%. Therefore, the phenomenon of hemolysis for the alloy with and without coated will not occur. In addition, HR on coated with the laser combining alkaline treatment have larger decline, and less volatility than that without coated, which is express that surface treatment of the alloy has more application prospects. RGR value with coated of laser combining alkaline treatment is 105.7%, 106.0%, 110.4%, respectively which cultivation for 1 d, 3 days, 5days and slightly higher than that of without coated, which is100%, 108.8% and 101.8%, respectively. According the standard of cell cytotoxicity [8], for the alloy with and without coated it is zero level cytotoxicity, suitable for used of biomaterial.

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6. References
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