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## Study on the Biocompatibility of Mg-4.0Zn-1.0Sr-0.4Ca Alloy with Phosphating Treatment after Implantation in Rats

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# Study on the Biocompatibility of Mg-4.0Zn-1.0Sr-0.4Ca Alloy with Phosphating Treatment after Implantation in Rats

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**Abstract.** The phosphating treatment coating is prepared on Mg-4.0Zn-1.0Sr-0.4Ca alloy surface, and the phosphating coating is consisted with HA and a small amount of calcium hydrogen phosphate hydrate. The hemolysis rate of both phosphated coating and uncoated samples is less than 5 %, showing that both have good hemolytic resistance which meet the requirements of use. It is found through the pathological studies that after implantation Mg-4.0Zn-1.0Sr-0.4Ca alloy for 30 days, for the leg muscle tissue, kidney tissue, liver tissue and bone tissues of rat the inflammation phenomenon is gradually recovered or completely recovered being passed the corrosion peak, while for the rat implanted the phosphating treatment alloy was in the acute stage of inflammation. After implantation Mg-4.0Zn-1.0Sr-0.4Ca alloy and the phosphating treatment alloy for 30 days, all of the brain tissue, myocardial tissue, lung tissue, intestinal tissue are unaffected.

## 1. Introduction

In recent years, the study of magnesium alloys as a novel biomedical planting material is paid more attentions[1-3]. It is well known that magnesium (Mg) and magnesium alloys possess many properties superior to other metallic biomaterials, which one of the important properties is degradability. 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 [4-6].

The surface modification is one of the most effective ways to improve the alloy corrosion resistance, including the phosphating surface treatment can greatly 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-1.0Sr-0.4Ca alloy alloys is designed, and phosphating surface treatment process is optimized to improve the corrosion resistant properties of the alloy, and the biocompatibility of the alloy with and without coating after implantation in rats 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-1.0Sr-0.4Ca (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 Phosphating Treatment Coating Preparation of Mg-4.0Zn-1.0Sr-0.4Ca Alloy

The process of the phosphating treatment coating preparation is that after Mg-4.0Zn-1.0Sr-0.4Ca alloy sheet specimen is prepared, it is placed in phosphating solution (The proportion of phosphating solution: 0.17 mol/LCa(NO<sub>3</sub>)<sub>2</sub> solution, and 0.17 mol/L KH<sub>2</sub>PO<sub>4</sub> solution) soaked for 48h, which the water bath is set at 70°C and the solution is changed every 12h. The surface morphology and composition were observed and analyzed by means of Scanning electron microscopy (SEM) and X-ray diffraction (XRD) respectively.

### 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 cm<sup>2</sup>/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 (20 g/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:

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

where  $D_t$  is the absorbance of the testing specimen;  $D_{nc}$  is the absorbance of negative control groups (10 mL SBF with 0.2 mL diluted blood) and  $D_{pc}$  is the absorbance of positive control groups (10 mL distilled water with 0.2 mL diluted blood).

### 2.4. Implantation Experiment

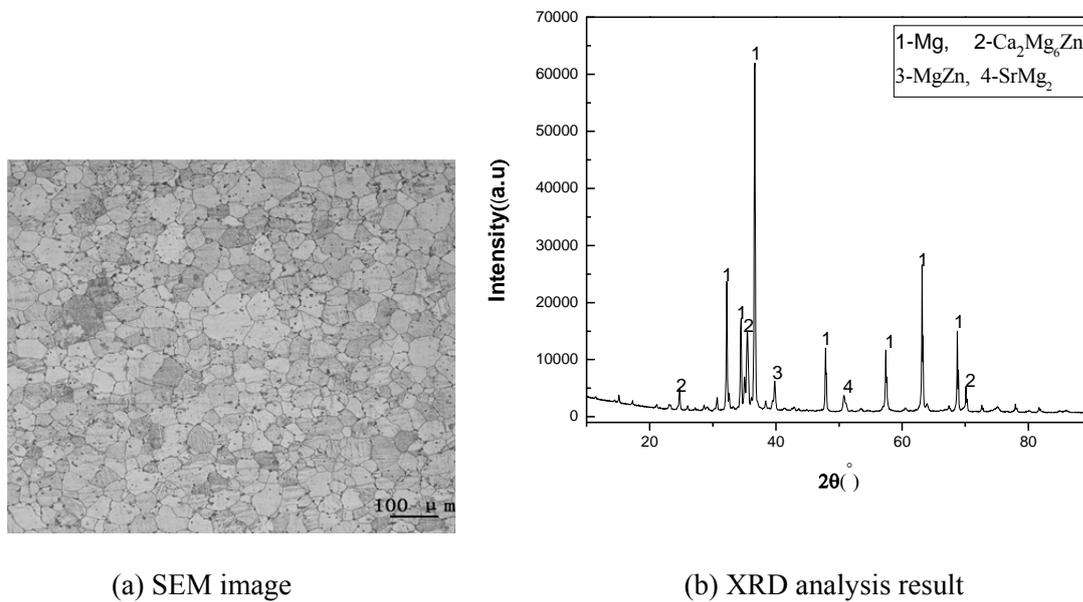
During the experiment, male SD rats aged about 10 weeks were selected whose weight is about 280-300 g. Before the implantation experiment, they should be bred for a period of time so that they can adapt to the hospital environment. The SD rats were anesthetized by abdominal injection of 10 % chloral hydrate before implantation. The anesthetic content varies according to the body weight of SD rats. Generally, SD rats can faint injected in 2 ml anesthetic content.

## 3. Results and Discussion

### 3.1 The Surface Morphology and Microstructure of Mg-4.0Zn-1.0Sr-0.4Ca Alloy with and without Coating of Phosphating Treatment

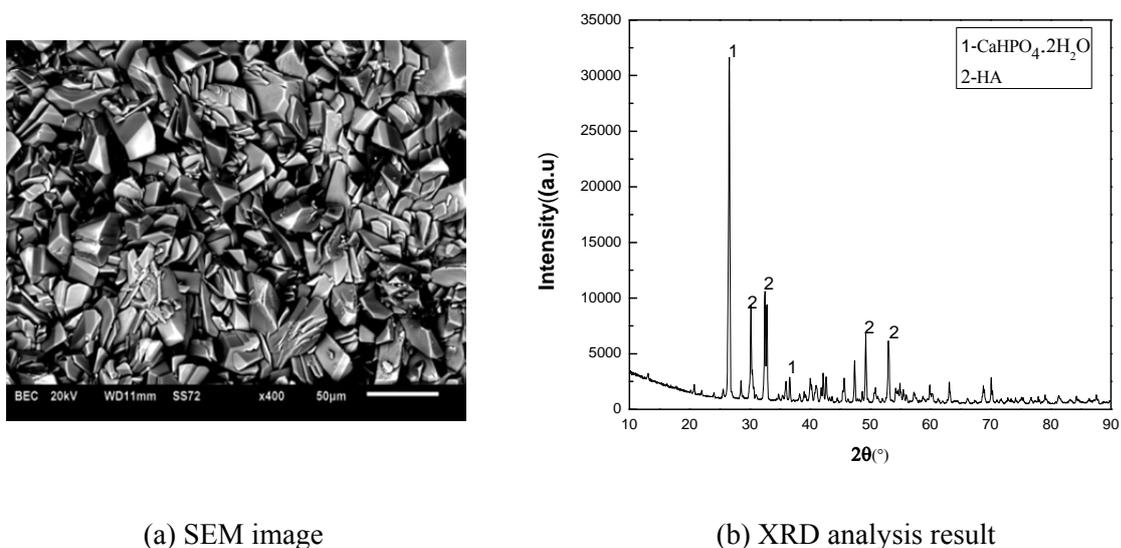
SEM image of the cast structure of Mg-4Zn-1Sr-0.4Ca alloy is shown in figure 1(a). It can be seen that the alloy grains distribution is fine and uniformly. From XRD analysis result of Mg-4Zn-1Sr-0.4Ca alloy shown in figure 1(b), it can be seen that besides the  $\alpha$ -Mg phase, there are also secondary phases such as MgZn<sub>2</sub>, SrMg<sub>2</sub>, Ca<sub>2</sub>Mg<sub>6</sub>Zn<sub>3</sub>, etc. The alloy element Zn plays a role of solid solution strengthening to the alloy, while the element Sr can be refined grains. As well as the addition of certain amount of Ca, the constitutional super-cooling will be generated at the front of the solid-liquid

interface, which can improve the nucleation rate and inhibit grain growth, improving the strength and plasticity of magnesium.



**Figure 1.** SEM image and XRD analysis result of the cast structure of Mg-4.0Zn-1.0Sr-0.4Ca alloy

SEM image of the cast structure of Mg-4.0Zn-1.0Sr-0.4Ca alloy with coating of phosphating treatment is shown in figure 2(a). It can be seen that the phosphated coating is uniformly distributed in the form of sheet or hexagonal prism, which the main elements on the surface are P, Ca and O, and other matrix elements are almost covered. XRD analysis result of the cast structure of Mg-4.0Zn-1.0Sr-0.4Ca alloy with coating of phosphating treatment is shown in figure 2(b). It can be seen that phosphating coating is consisted with HA and a small amount of calcium hydrogen phosphate hydrate.



**Figure 2.** SEM image and XRD analysis result of the cast structure of Mg-4.0Zn-1.0Sr-0.4Ca alloy with coating of phosphating treatment

### 3.2 The Results of Hemolysis Test

The concentration of magnesium ions in the leaching solution can reflect the corrosion of the sample in physiological saline. Clearly, the higher the concentration of  $Mg^{2+}$ , the lower will be the corrosion resistance of the sample, and the faster the corrosion rate. It can be seen from table 1 that the concentration of  $Mg^{2+}$  in the leaching solution of the uncoated sample is higher than that of the phosphating treatment sample, which also reflects that the phosphating treatment improves the corrosion resistance of the alloy. From table 2, we can see the different absorbance values of uncoated alloy and phosphated alloy, as well as the calculated corresponding hemolysis rate. The hemolysis rate of uncoated alloy sample is 3.9 %, and that of phosphated alloy sample is 0.3 %, which has better hemolysis resistance. In addition, the hemolysis rate of both phosphated coating and uncoated samples is less than 5 %, showing that both have good hemolytic resistance which meet the requirements of use.

**Table 1.** Magnesium ion concentration (mol/L)

Alloy	concentration
Mg-4.0Zn-1.0Sr-0.4Ca alloy	1.5
The phosphating treatment alloy	0.3

**Table 2.** The comparison of hemolysis rate

Alloy	$D_t$	$D_{nc}$	$D_{pc}$	HR(%)
Mg-4.0Zn-1.0Sr-0.4Ca alloy	0.602	0.512	2.811	3.9
The phosphating treatment alloy	0.519	0.512	2.811	0.3

### 3.3 Pathological Analysis Results after Implantation in Rats

After general anesthesia, the rats were placed on their backs on the experimental table, the leg muscles were passively separated, and the sterilized 2\*2\*13mm column specimen were implanted into the leg muscles of rats as shown in figure 3. Then, the wound was sutured layer by layer and kept for one month. During the feeding process, the state of rats should be observed every day and avoid the disappearance of marked.

The brain, bone tissue, heart, kidney, small intestine, lung and liver of the rats who participated in the implantation experiment for one month were tested to determine the influence of implantation materials on internal organs.

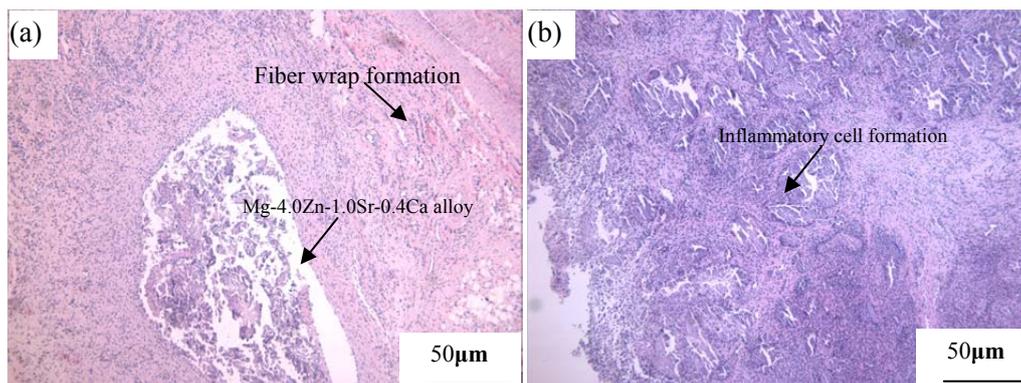


**Figure 3.** The implantation of the specimen in rat

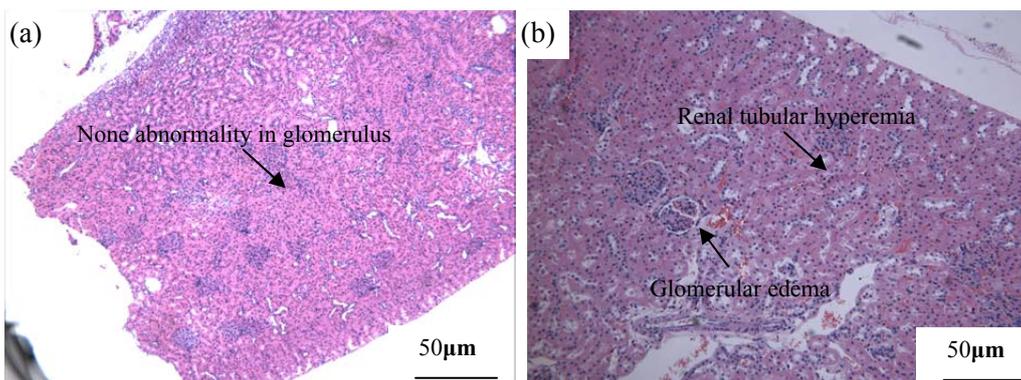
The leg muscle tissue of rat A after implantation the alloy for 30 days is shown in figure 4(a). It can be seen that there are magnesium alloy fragments and inflammatory cell infiltration phenomenon, which indicates that inflammation occurs in the legs of rat A, as well as there are a large number of fiber packages formed, indicating that the rat A are already in the recovery period. The leg muscle

tissue of at B after implantation the phosphating treatment alloy for 30 days is shown in figure 4(b). It can be seen that there are a large number of magnesium alloy fragments and a large number of inflammatory cell infiltration. Only a small amount of fiber was formed, which indicated that the rats B were in the acute stage of inflammation. Because the existence of phosphating treatment coating, the corrosion rate of the sample at the initial stage of corrosion is decreased. It means that the phosphating treatment coating alloy reached the corrosion peak after implantation in rat for 30 days, while the rat A implanted with uncoated sample passed the corrosion peak and the inflammation phenomenon gradually recovered.

Kidney tissue structure after implantation for 30 days is shown in figure 5. Comparing the kidneys of the rats after the two implanted materials, it is found that different degrees of pathological changes have taken place. From figure 5(a), it can be seen that the glomerulus is basically normal, but some renal tubules are hyperemia, showing mild nephritis symptoms. It can be seen from figure 5(b) that most glomeruli are edematous, renal capsule disappears, and congestion occurs in renal tubules, showing obvious nephritis symptoms. By comparing figure 5(a) and figure 5(b), the nephritis symptoms in figure 5(a) has been recovered, which shows that the coating increases the corrosion resistance of the sample in rat body and delays the corrosion rate. The corrosion rate of Mg-4.0Zn-1.0Sr-0.4Ca alloy shown in figure 5(a) which the inflammation has been recovered is faster than that of the phosphating treatment alloy shown in figure 5(b). While, the corrosion rate of the phosphating treatment alloy is relatively slow shown in figure 5(b), which is at the stage of inflammatory attack.



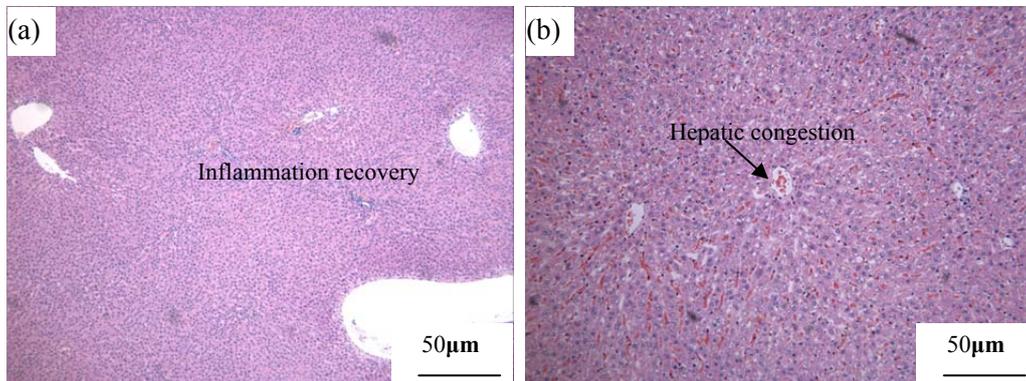
**Figure 4.** The leg muscle tissue after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy



**Figure 5.** Kidney tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy

Through the analysis of liver tissue implanted into the rats, the liver tissue of rat A implanted Mg-4.0Zn-1.0Sr-0.4Ca alloy shown in figure 6(a) is normal, while the liver tissue of rat B implanted the

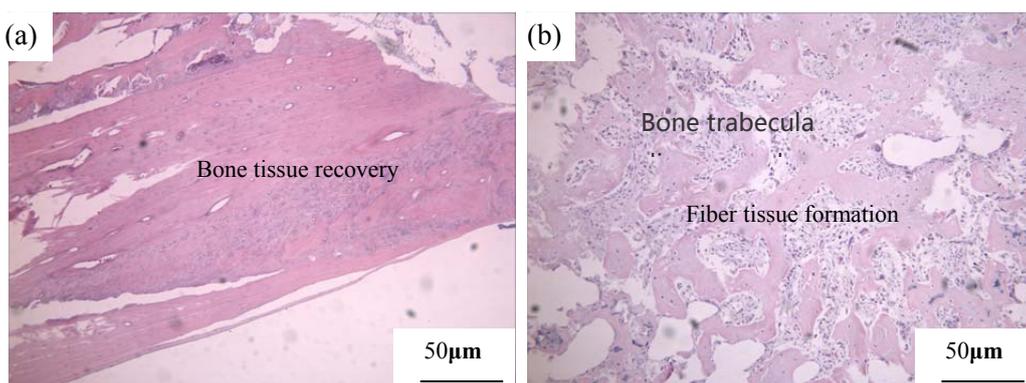
phosphating treatment alloy shown in figure 6(b) shows liver congestion and vacuolar degeneration in different degrees.



**Figure 6.** Liver tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy

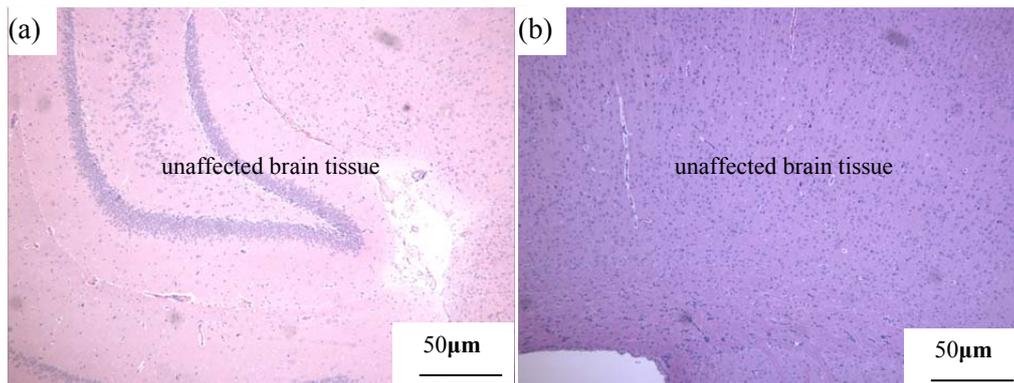
These phenomena indicate that rats have symptoms of hepatitis, which may be caused by the alloy implanted into the rats, resulting in different degrees of pathological changes in the body. From rat A shown in figure 6(a), it can be seen that liver tissue has returned to normal. However, from rat B shown in figure 6(b), it is in the acute phase, which can be explained as the sample of rat B is in the corrosion peak, while rat A has passed the corrosion peak.

Bone tissue structure after implantation for 30 days is shown in figure 7. Comparing the bone tissues of rat A implanted in Mg-4.0Zn-1.0Sr-0.4Ca alloy shown in figure 7(a) and rat B implanted in the phosphating treatment alloy shown in figure 7(b), it can be clearly seen that the bone tissues and trabeculae of rats A are normal, while the bone trabeculae of rats B is disappeared, with a large number of inflammatory cells infiltrating and fiber wrapping forming, which is in the period of osteoarthritis. However, there are also a large number of fiber wrapping forming in rat B, which the bone tissues are returning to normal and forming callus. This phenomenon is just same as that of kidney and liver, and also proves that the coating improves the corrosion resistance of Mg-4.0Zn-1.0Sr-0.4Ca alloy.

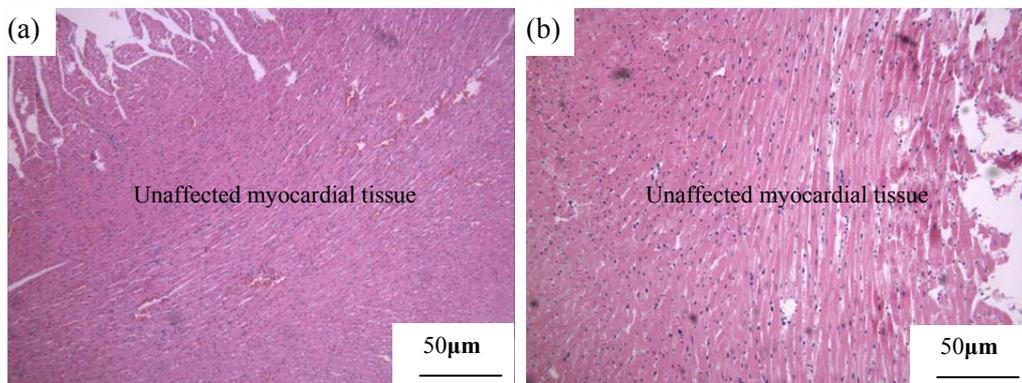


**Figure 7.** Bone tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy

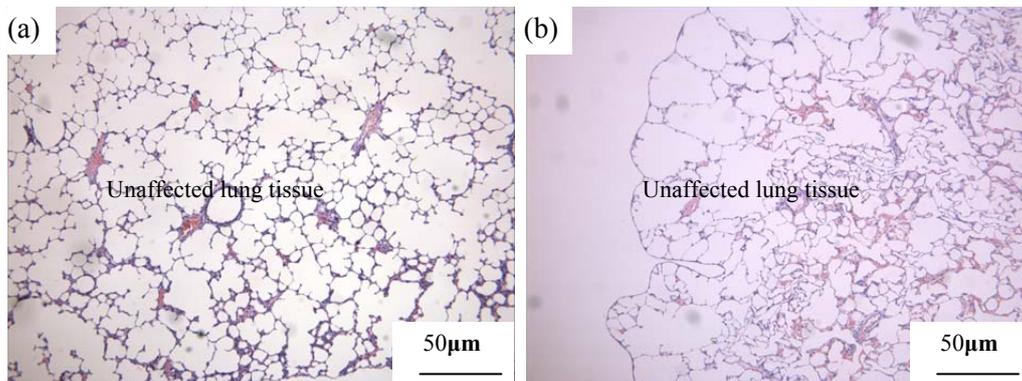
After implantation of Mg-4.0Zn-1.0Sr-0.4Ca alloy and the phosphating treatment alloy for 30 days, it can be seen that myocardial tissue shown in figure 8, brain tissue shown in figure 9, lung tissue shown in figure 10 and intestine tissue shown in figure 11 are all normal, meaning that the implanted materials have no adverse effects on them.



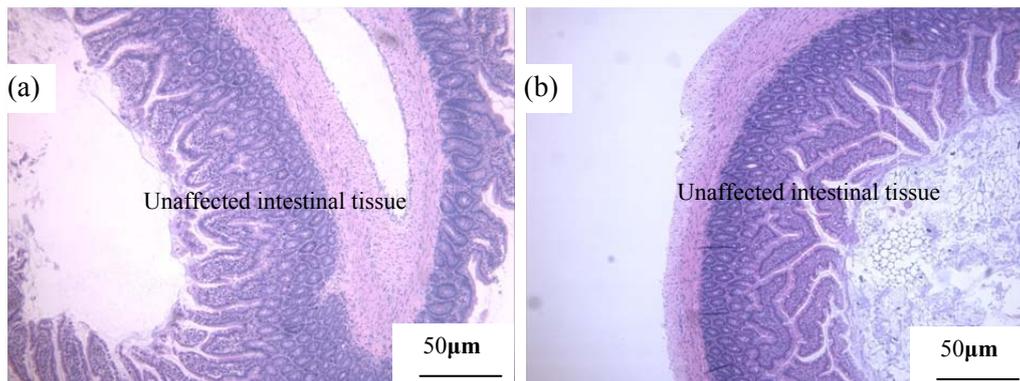
**Figure 8.** Brain tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy



**Figure 9.** Myocardial tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy



**Figure 10.** Lung tissue structure after implantation for 30 days  
(a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy



**Figure 11.** Intestinal tissue structure after implantation for 30 days (a) Mg-4.0Zn-1.0Sr-0.4Ca alloy; (b) The phosphating treatment alloy

#### 4. Conclusion

The phosphating coating is consisted with HA and a small amount of calcium hydrogen phosphate hydrate. The hemolysis rate of both phosphated coating and uncoated samples is less than 5 %, showing that both have good hemolytic resistance which meet the requirements of use. It is found through the pathological studies that after implantation Mg-4.0Zn-1.0Sr-0.4Ca alloy for 30 days, for the leg muscle tissue, kidney tissue, liver tissue and bone tissues of rat the inflammation phenomenon is gradually recovered or completely recovered being passed the corrosion peak, while for the rat implanted the phosphating treatment alloy was in the acute stage of inflammation. After implantation Mg-4.0Zn-1.0Sr-0.4Ca alloy and the phosphating treatment alloy for 30 days, all of the brain tissue, myocardial tissue, lung tissue, intestinal tissue are unaffected.

#### 5. Acknowledgments

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