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Biocompatibility of bio-Mg-Zn alloy within bone with heart, liver, kidney and spleen

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A magnesium-zinc alloy rod was implanted into the marrow cavity of the distal femur in New Zealand rabbits. The femur with the implanted alloy was compared with the contralateral femur in which a bone tunnel without implant was formed as a control. Degradation of the magnesium-zinc alloy was analyzed via X-ray, scanning electron microscopy, and element energy spectrum analysis. Serum magnesium, liver and kidney function tests, and myocardial enzymes were measured. Heart, liver, kidney and spleen were sectioned for pathological analysis, and the effects of the implanted material on the histology and function of important organs were analyzed. Magnesium-zinc alloy was resorbed from the bone marrow cavity of the femur; 87% of the alloy was degraded within 14 weeks after the surgery. There were no significant differences in serum magnesium, liver or kidney function tests, or myocardial enzymes before the surgery and after degradation of the magnesium-zinc alloy. Histology of the heart, liver, kidney, and spleen did not change. This study demonstrated that magnesium-zinc alloy can be resorbed in bone, and that the degradation products have good biocompatibility with heart, liver, kidney, and spleen.

magnesium-zinc alloy, bone, blood magnesium, biocompatibility, intraosseus fixation

Metals used in intraosseous fixation include stainless steel, titanium, and cobalt-chromium alloy. The flexibility modulus of these metals is greater than that of human bone; thus, they may cause a stress-shielding effect, which results in decreased bone strength and delay in bone healing. These metals also may induce an inflammatory reaction in the surrounding tissue. In addition, material biocompatibility may decrease through corrosion or abrasion, thus releasing toxic particles, and resulting in bone dissolution. The metal materials used for bone fracture fixation are permanent and require removal by an additional surgical procedure, which results in unnecessary morbidity[1−4].

It is preferable to choose biodegradable materials for fixation of bone fracture. Currently, the main *in vivo* biodegradable materials are polymers and ceramics; No metal-based materials have been utilized. Although the mechanical properties of polymers and the tenacity of ceramics are poor, metals have excellent strength and plasticity. Therefore, the development of resorbable metal implants to impart strength to healing bone recently has become a research focus[5−9].

Magnesium has the advantage of being biocompatible and biodegradable[1], and of having high specific strength and specific stiffness. The elastic modulus is 45 GPa, close to that of human bone. It can effectively reduce the stress-shielding effect, and is in accordance with the requirements for ideal bone plates[1]. In addition, magnesium is a cation, which is the fourth most abun-

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dant cation in the body and the second most abundant intracellular cation. It is an essential nutrient for the human body, and has important influence on the function of nerve, muscle, bone, and heart. Magnesium supplementation has attracted much attention in clinical practice. Adults need a magnesium intake of 300−350 mg/day, suggesting that magnesium is safe for use in biomedical materials.

Current researches on magnesium alloy focus mainly on magnalium alloy and magnesium-rare-earth alloy. Huang found that magnesium alloy had good biocompatibility when magnesium alloy AZ31B was implanted on both sides of rat spine[6]. Witte et al.[4] constructed magnesium-alloy AZ31 and AZ91 rods, and implanted them in the marrow cavity of the guinea pig femur, with the result that a large quantity of new bone formed around the magnesium alloy. Witte et al.[3] added the rare-earth elements to the magnesium, and made magnesium-rare-earth alloys for *in vivo* degradation tests. When magnesium-rare-earth alloys LAE442 and WE43 were compared with magnalium-alloys AZ31 and AZ91, LAE442 had the lowest degradation rate, indicating that the addition of different elements can affect the degradation rate of magnesium alloy. In 2007, Witte et al.[2] mixed magnesium alloy AZ91D and hydroxyapatite (HA) to make metal matrix composite (MMC-HA), on which were cultured human osteoblasts, osteoblast cell line MG-63, and macrophage cell line RAW264.7. These three kinds of cells could survive and proliferate in the degradation layer of magnesium alloy, and had a good adhesion capacity with this alloy, suggesting that MMC-HA has good cell compatibility. Pietak et al.[10] developed calcium-magnesium alloy and magnesium-zinc alloy. When rat bone marrow mesenchymal cells were cultured with magnesium alloy, the differentiation of bone marrow mesenchymal cells into osteoblasts was promoted. This alloy had a promotional effect on adhesion, differentiation, and proliferation of osteoblast-like cells. There was formation of bone-like matrix around the magnesium alloy, which had no toxicity on the bone-like cells *in vitro*.

However, aluminum is neurotoxic, rare-earth elements, such as yttrium, cause liver toxicity, so magnesium-aluminum alloy and magnesium-rare-earth alloy have potential toxicity. In order to avoid the potential hazards of these elements, we replaced them with a magnesium alloy consisting of nutrients only, including magnesium and zinc[11]. Magnesium alloy components consisting of nutrient elements required by the body theoretically should have a good biocompatibility. However, further studies are needed to explore whether they will cause metabolic or structural abnormalities after their degradation *in vivo*.

Current research on magnesium alloyed documents degradation of the materials in simulated body fluids, and observed changes in animal bone after implantation of the alloy. There has been only limited attention to the effects of magnesium alloys on serum magnesium, liver and kidney function, and the function of other vital organs. The present study implanted magnesium-zinc alloy rods into the marrow cavity of the distal femur of New Zealand rabbits so as to analyze the degradation of the alloy in bone, and to study the effects on peripheral blood biochemistry, liver and kidney function, myocardial enzymes, and histological changes of vital organs. The results of this study may provide an experimental basis for the *in vivo* application of the magnesium-zinc alloy.

1 Research Design

1.1 Material preparation

Magnesium-zinc alloy was produced and provided by Shanghai Aoruiji Medical Technology Limited Company. The alloy composition is shown in Table 1. The alloy was formed into Φ4.5 mm ×10 mm rods, which were dried and stored after the ethylene oxide sterilization.

Table 1 Chemical composition of the magnesium-zinc alloy

Materials	Chemical Composition (wt.%)							
	Mg	Zn	Ni	Cu	Al	Mn	Fe	Si
Mg-Zn	Balance	5.6210	0.0005	0.0005	0.0085	0.0004	0.0038	0.0016

1.2 Animal experiments

Experimental animals were 12 healthy adult New Zealand rabbits, each 2.0−2.5 kg. Six were male and six were female. Each animal had a magnesium alloy rod implanted in one femur; the contralateral femur served as the control, in which only a bone tunnel was established, and no materials were implanted. Rabbits were anesthetized with intravenous injection of pentobarbital sodium (30 mg/kg). Under sterile conditions, the skin and the subcutaneous tissue were incised to expose the distal femur. The femur tunnel was established using a 3.5 mm kir-

schner wire to drill into the marrow cavity of the femur. The magnesium alloy rod was implanted into the tunnel and the incision was sutured in layers after saline irrigation. Postoperatively, the animals received intramuscular injection of penicillin 400000 units/ day for three days. Animals were housed unrestrained in individual cages. X-rays were taken at scheduled times after the implantation surgery to observe postoperative bone changes.

1.3 Examination of the peripheral blood

Peripheral blood (5 ml) was drawn one day prior to the surgery. After implantation of the magnesium-zinc alloy in the femur marrow cavity, 5 ml of peripheral arterial blood was drawn one day, three days, one week, and three weeks after the surgery.

Total protein was measured using the Biuret method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S717). Albumin was measured using the bromocresol green method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S718). Alanine aminotransferase (ALT) was measured using the UV-lactate dehydrogenase method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S701). Aspartate aminotransferase was measured using the UV-malate dehydrogenase method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S502). Alkaline phosphatase was measured using the AMP buffer method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S712). Gamma-glutamyl transferase was measured using the γ-GT reagent (Shanghai Kehua Dongling Diagnostics Reagent Ltd. S713). Creatinine was measured using sarcosine oxidase-PAP method (reagent: Shanghai Kehua Dongling Diagnostics Reagent Ltd. S708). Serum magnesium was measured using the chemical method (WAKO, Japan EH357). Urea nitrogen was measured using the urea nitrogen method (reagents: the First Chemical Co., 034 RAB-039RCB). Uric acid was measured using the uricase method (reagents: the First chemical Co., 051 RJA-052RBB). Lactate dehydrogenase was measured using the lactic acid substrate UV method (Reagent: Roche 03002209). Serum creatine kinase was measured using the creatine phosphate substrate UV method (reagent: Roche 12132672). All tests were performed on the automatic biochemical analyzer (Hitachi 7600-020).

1.4 Histological examination of the viscera

Six rabbits were sacrificed six weeks after the surgery. The heart, liver, kidney, and spleen were prepared for pathological examination, and were stained with hematoxylin and eosin to observe any histological changes in the organs.

1.5 Degradation analysis of magnesium-zinc alloy

Eighteen days before sample collection, animals received an intramuscular injection of tetracycline (30 mg/kg). Fourteen weeks after the surgery, materials were removed for element spectrum analysis (energy dispersive spectroscopy, EDS) and the degradation patterns of the materials were observed using a scanning electron microscope (JSM-6460). The tissue surrounding the implanted material was decalcified, and was conventionally embedded in paraffin and sprayed with gold. The changes in configuration after the interactive reaction between materials and bone tissue were observed using a scanning electron microscope.

1.6 *In vitro* measurement of the pH changes after the material degradation

Three Φ10 mm × 2 mm Mg-Zn wafers were constructed from the magnesium-zinc alloy, and were polished with 1000 # sandpaper. Wafers were placed individually in three 80 ml beakers in 20 ml DMEM high-glucose cell culture medium (Gibco companies, pH 7.44) equilibrated at 37℃ in a heated water bath. The pH was measured using a magnetic PHS-3C precision pH meter at prescribed time intervals.

1.7 Statistical analysis

Statistical analysis was performed using SPSS11.5 (social statistics package) on data derived from measurement of serum magnesium, liver and kidney function tests, and myocardial enzymes. $P < 0.05$ indicates statistically significant difference.

2 Results

2.1 Animal experiments

No infections complicated the implantation of the magnesium-zinc alloy into the distal femur marrow cavity of the New Zealand rabbit. X-rays showed that within three weeks of the surgery, subcutaneous gas had accumulated adjacent to the implant, but that the gas had disappeared within 6 weeks without any treatment. At twelve weeks, the implants became blurred and there was bone resorption around the implant; at 24 weeks, the implant material could not be visualized, suggesting that it might

have been resorbed completely. There was a large amount of new bone formation in the outer cortex of the femur. The tunnel had healed by 3 weeks postoperatively in the control group (Figure 1). After the tetracycline labeling, scanning electron microscopy demonstrated a large gap between the implant and the inner cortex of the femur 6 weeks and 14 weeks after the surgery (Figure 2).

2.2 Examination of the peripheral blood and histological examination of the viscera

Preoperative serum magnesium of the New Zealand rabbit was 1.17 mmol/L. This did not change significantly 3 weeks after the surgery. The serum uric acid increased transiently ($P < 0.05$), and returned to normal by one week after the surgery. The serum creatine kinase increased rapidly and significantly one day after the surgery ($P < 0.05$), but it returned to normal 3 days after the surgery. Albumin and the albumin to globulin ratio were decreased one week after the surgery. Although the values one week after the surgery were significantly different from the values prior to the surgery, the albumin to globulin ratio was not inverted after the surgery. Moreover, there were no significant differences between the preoperative and three-weeks-postoperative values of the total protein, alanine transaminase, or aspartate aminotransferase, suggesting that the liver function was not affected. There were no significant differences between the preoperative and postoperative values of creatinine or urea nitrogen within three weeks after the surgery, suggesting that the renal function was not adversely affected.

The New Zealand rabbit was sacrificed at six weeks after the surgery and the heart, liver, kidney, and spleen were made into pathological sections and were stained with hematoxylin and eosin. By optical microscopy, the cellular structure of the heart, liver, kidney, and spleen did not change morphologically, and there was no infiltration by inflammatory cells (Figure 3). Magnesium-zinc alloy showed degradation three weeks after the surgery, and might have been completely resorbed 24 weeks after the surgery. The dispersal of degradation products did not affect the serum magnesium concentration, or the liver or kidney function tests. Histology of the heart, liver, kidney, and spleen of the New Zealand rabbit did not change significantly.

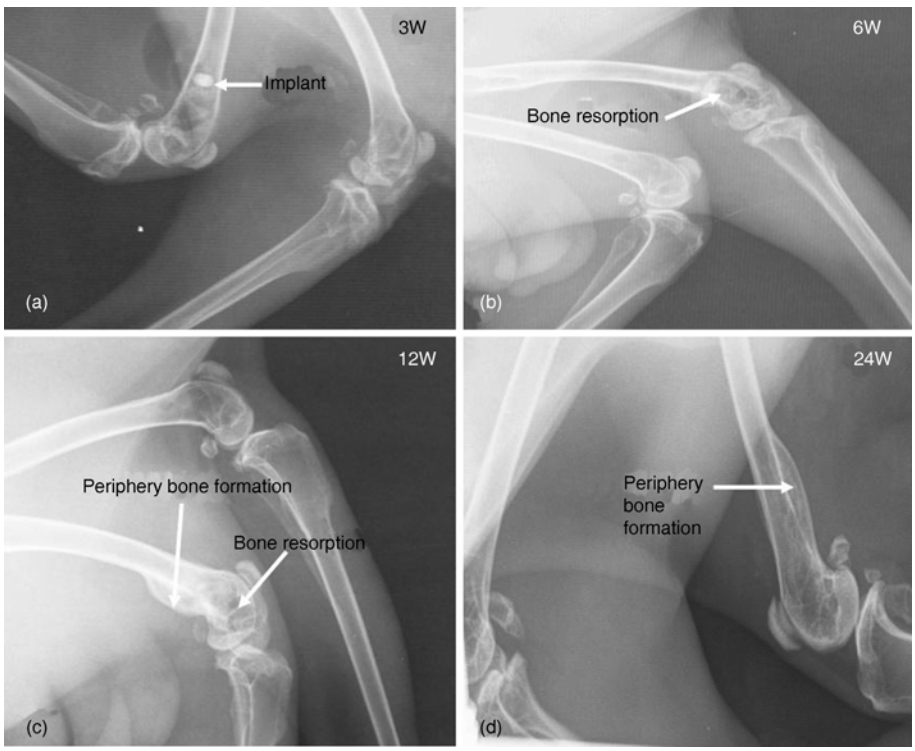

Figure 1 (a) There was gas accumulation around the femur 3 weeks after the implantation of the zinc-magnesium alloy, when the tunnel in the contralateral bone had healed; (b) Bone resorption existed around the implant 6 weeks after the surgery; (c) The image of the material was blurred 12 weeks after the surgery, there was bone resorption around the implant, and bone formation outside of the femur cortex; (d) The material was completely resorbed 24 weeks after the surgery, with extensive formation of new bone tissue outside of the femur cortex.

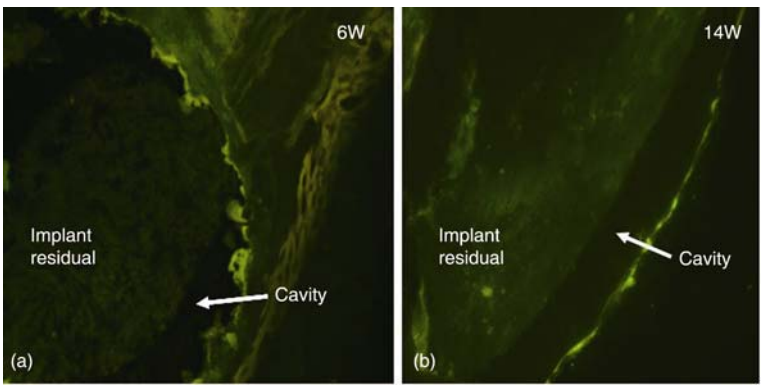

Figure 2 (a) There was a small amount of bone formation around the implant 6 weeks after surgery, and there were cavities between the material and the bone cortex; (b) There was a small amount of bone formation around the implant 14 weeks after the surgery, and cavities remained between the material and the bone cortex.

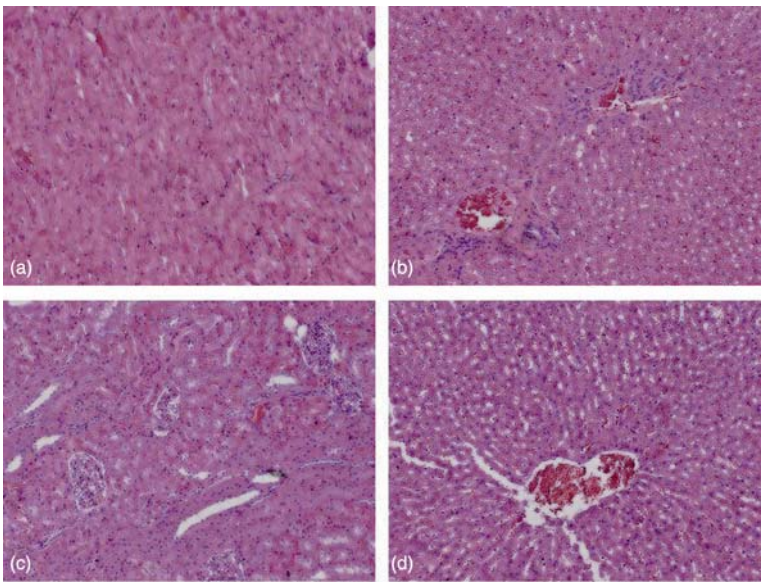

Figure 3 Pathological sections showing normal structure of (a) heart cells (×100); (b) liver tissue (×100); (c) kidney with normal renal tubules; (d) spleen tissue (×100).

2.3 Degradation analysis of magnesium-zinc alloy

The magnesium-zinc alloy was removed 14 weeks after the surgery. Its weight was only 13% of the original weight, suggesting that 87% of the material had been resorbed. At visual inspection, the surface of the magnesium alloy had lost its original metallic luster, and its edge was not distinct. After drying, the surface was coated with some loose white material. Cava as well as a black substance was seen within the femur marrow cavity. These results suggested that the magnesium-zinc alloy had poor biocompatibility with bone in the marrow cavity of the femur (Figure 4).

Visible under the scanning electron microscope was a layer of degradation products on the surface of the magnesium-zinc alloy, with a rough surface and irregular cracks[5]. EDS analysis showed that the main components of the degradation products were oxygen, magnesium, zinc, calcium, and phosphorus (Table 2). The

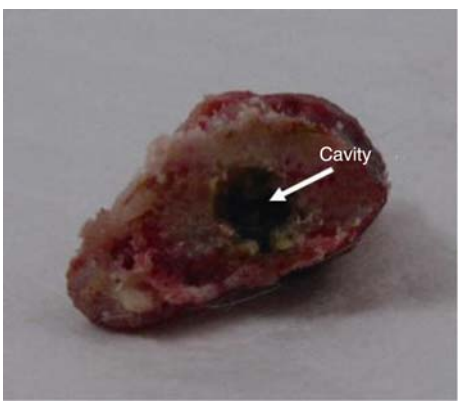

Figure 4 The cavity around the implant 14 weeks after surgery.

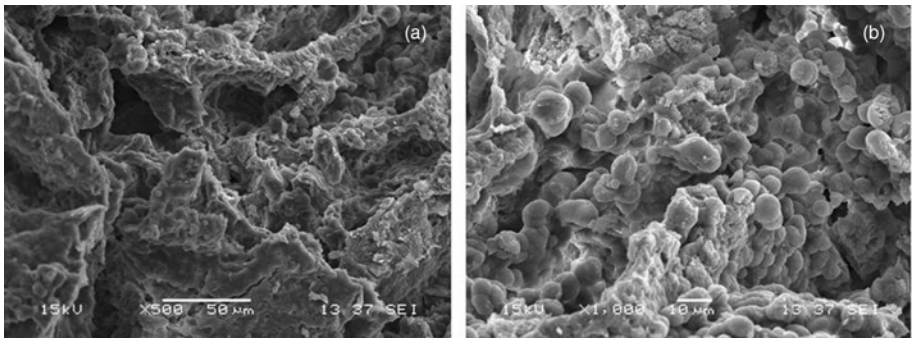

Figure 5 (a) Degradation patterns of the magnesium alloy using the scanning electron microscope (×500). The surface of the magnesium alloy was rough with gully-like texture; (b) Scanning electron micrograph of magnesium alloy (×1000), showing rough surface with copious off-white degradation products.

magnesium-zinc alloy was placed in the DEME cell culture medium, and the changes of the pH are shown in Figure 6.

Table 2 Element energy spectrum analysis of the degradation components on the surface of the magnesium-zinc alloy

Elements	Wt %	At %
O	20.64	35.33
Mg	23.22	26.15
Zn	26.96	11.29
P	9.4	8.31
Na	6.19	7.37
Ca	7.52	5.13
N	1.57	3.06
Cl	2.71	2.09
K	1.8	1.26

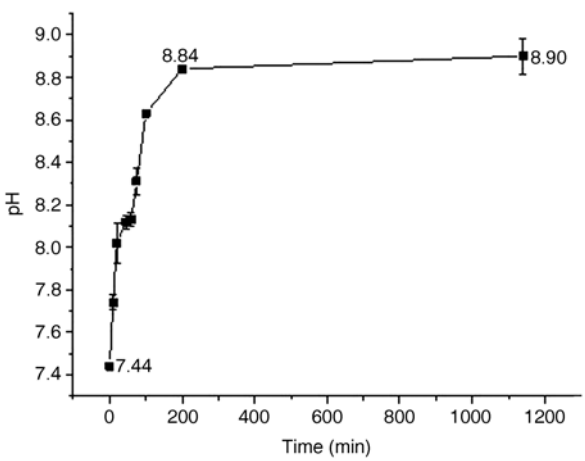

Figure 6 The pH increased rapidly for 120 min; subsequently the increase was more gradual. It took 200 minutes for the pH to rise from 7.62 to 8.84, but it took 1000 min for the pH to increase from 8.84 to 8.90.

3 Discussion

The magnesium-zinc alloy developed and tested in this study consists of the nutrients, which can be resorbed after being implanted into the marrow cavity of the distal femur in New Zealand rabbits. X-ray images of the material were fuzzy 12 weeks after the implantation. At 24 weeks, the images of the material had completely disappeared, suggesting that the materials might have been completely resorbed. In the implant-side femur there was gas accumulation three weeks after the surgery, which disappeared by 6 weeks, suggesting that the gas from the material degradation can be resorbed. However, there was bone resorption around the material while at the same time the tunnel in the contralateral femur had already healed.

Twelve weeks after the surgery, bone resorption still was evident around the material, but there was new bone formation in the outer cortex of the femur with material implantation. The material might have been resorbed completely at 24 weeks postoperatively, accompanied by formation of new bone in the outer cortex of the femur. The magnesium-zinc alloy was powder-like at 14 weeks after the surgery, at which time the absorption had reached 87%, and there existed cavities at the location of the implant in the femur marrow cavity. After tetracycline labeling, scanning electron microscopy revealed a large gap between the implant and the inner cortex of the femur, suggesting that magnesium-zinc alloy has poor biocompatibility with the bone in the femur marrow cavity. The energy spectrum analysis of the remaining materials showed that oxygen, magnesium, zinc, calcium, and phosphorus were the main degradation products.

The fact that magnesium, zinc, calcium, and phosphorus can promote bone formation[1] raises the question of why there was bone resorption around our implants. We speculate that the magnesium-zinc alloy degraded

quickly, with extensive formation of magnesium hydroxide. Consequently, the pH of the local environment increased, resulting in a poor environment for the surrounding cells. Song and Simaranov[8,12] reported that corrosion of pure magnesium in a NaCl solution leads to local alkalinization of the solution in the vicinity of the sample. Even in an acidic solution of pH 4, the pH near the sample was still 10 or even greater. This rapid increase of pH is unfavorable to the cell growth *in vitro*.

We placed the magnesium-zinc alloy used in the experiment into the DMEM cell culture medium, and found that the pH increased sharply within 2 h, and increased gradually thereafter. It took 3 h and 20 min for the pH to rise from 7.62 to 8.84, but it took approximately 17 h for the pH to increase from 8.84 to 8.90. Our *in vitro* experiment further confirmed that the pH of the surrounding environment would increase rapidly when the material was degraded. Because blood flow in bone is too slow to compensate for the alkalinity, the pH around the implant remains high for a relatively long time, resulting in unsuccessful attachment of the bone cells, and thus diminishing the cell growth around the material.

This study also found copious formation of new bone in the outer cortex of the femur with metal implantation at 12 and 24 weeks after the surgery. It is well known that bone has a great capacity for remodeling. Bone resorption in the marrow cavity of the femur may have been compensated by bone formation on the outer surface of the femur. This may represent a host response to the intervention, to restore mechanical homeostasis and maintain bone strength, but elucidation of the specific mechanism would require further studies.

The serum chemistry one day prior to the material implantation and at one day, 3 days, and 1 and 3 weeks after the surgery showed that there was no significant difference in the concentration of magnesium, or liver or kidney function tests before and after the material degradation. The serum uric acid increased rapidly on the first and the third days after the surgery, which was significantly different from the preoperative value ($P < 0.05$). This suggests that there was transient metabolic imbalance, the serum uric acid was resolved one week after the surgery.

On the first day after the surgery, there was a rapid increase in the serum creatine kinase ($P < 0.05$), which returned to the preoperative level on the third day after the surgery, suggesting that the material implantation might have transient effects on the heart. Alternatively, this transient increase might reflect surgical trauma, since the index returned to the preoperative level on the third day after the surgery. Both albumin and the albumin and globulin ratio decreased one week after the surgery. Although the preoperative and postoperative values were significantly different ($P < 0.05$), the albumin and globulin ratio was not inverted after the surgery. Moreover, the levels of total protein, alanine transaminase, and aspartate aminotransferase were at the preoperative levels, suggesting that the liver function was not affected.

Radiographic images showed that the metal was completely resorbed at 24 weeks. Histology of heart, liver, kidney, and spleen was normal, and there was no infiltration by inflammatory cells. These results suggest that the degradation products of the magnesium-zinc alloy have good biocompatibility with heart, liver, kidney, and spleen.

Magnesium-zinc alloy can be completely resorbed when implanted into the femur marrow cavity of the New Zealand rabbit. Degradation of this material has no significant effect on serum magnesium or on liver or kidney function tests. Creatine kinase increased transiently, but returned to the preoperative level on the third day after the surgery. There were no significant histologic effects on the heart, kidney, liver, or spleen six weeks after the surgery, suggesting that the material and its degradation products have good biocompatibility with the heart, liver, kidney, and spleen of the New Zealand rabbit.

4 Conclusion

(i) A magnesium-zinc bar (Φ4.5 mm ×10 mm) was implanted into the distal femur marrow cavity of the New Zealand rabbit for 14 weeks. The material degraded approximately 87%, suggesting that the magnesium-zinc alloy can be resorbed from the femur marrow cavity.

(ii) The implantation of magnesium-zinc alloy in the animal femur marrow cavity had no measurable effect on serum magnesium, or on liver or kidney function tests. The serum uric acid and creatine kinase increased transiently after the surgery, but returned to the preoperative levels one week after the surgery. There were no changes in the histology of the heart, liver, kidney, or spleen of the New Zealand rabbit postoperatively. These observations suggest that magnesium-zinc alloy has good biocompatibility with vital organs.

In summary, magnesium-zinc alloy is resorbable when implanted in bone. It has no effect on the chromaticness, structure or function of heart, liver, kidney, or spleen. Further studies on this magnesium alloy will inform the selection of new material for inraosseous fixation.

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