Available online at www.sciencedirect.com
ScienceDirect
Trans. Nonferrous Met. Soc. China 25(2015) 824−831
Transactions of Nonferrous Metals Society of China
www.tnmsc.cn

Effects of chitosan coating on biocompatibility of Mg−6%Zn−10%$Ca_3(PO_4)_2$ implant

Jun ZHAO[1], Liang-jian CHEN[2,3], Kun YU[1,2], Chang CHEN[3], Yi-long DAI[1], Xue-yan QIAO[1], Yang YAN[1], Zhi-ming YU[1]

1. School of Materials Science and Engineering, Central South University, Changsha 410083, China;
2. State Key Laboratory of Powder Metallurgy, Central South University, Changsha 410083, China;
3. The Third Xiangya Hospital, Central South University, Changsha 410083, China

Received 16 April 2014; accepted 31 October 2014

Abstract: A Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite with a chitosan coating was prepared to study its in vivo biodegradation properties. The chitosan dissolved in a 0.2% acetic acid solution was applied on the surface of Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite specimens and solidified at 60 °C for 30 min to form the coating. The cytotoxicity evaluation of chitosan coated specimens is at level 0, which indicates that such coating is safe for cellular applications. The in vivo tests of chitosan coated composite show that the concentration of metal ions from the composite measured in the venous blood of Zelanian rabbits is less than that from the uncoated composite specimens. The chitosan coating impedes the in vivo degradation of the composite after surgery. The in vivo testing also indicates that the chitosan coated composite is harmless to important visceral organs, including the heart, kidneys and liver of the rabbits. The new bone formation surrounding the chitosan coated composite implant shows that the composite improves the concrescence of the bone tissues. And the chitosan coating is an effective corrosion resistant layer that reduces the hydrogen release of the implant composite, thereby decreasing the subcutaneous gas bubbles formed.
Key words: biocompatibility; magnesium composite; chitosan; cytotoxicity

1 Introduction

Magnesium alloys have the potential to serve as biomedical implants because their elastic modulus matches that of cortical bone tissue, allowing them to avoid the stress shielding effect induced by a serious mismatch between the modulus of the natural bone and implants [1,2]. Mg-based implants corrode and degradate with the body fluid in the electrolytic physiological environment [3,4]. Therefore, Mg alloys are candidates for biodegradable implants for repairing bone fractures [5]. Biodegradable Mg alloys can provide good mechanical properties and produce non-toxic corrosion products in physiological systems [6,7]. However, some disadvantages to the biodegradation of Mg alloys are perceived, including a rapid corrosion rate, hydrogen gas emissions and a high environmental pH value of corrosion products [5,8]. Since rapid corrosion is an intrinsic response of magnesium to the human body's fluid or plasma, controlling or decreasing the corrosion rate of magnesium in body fluid is a significant issue in the development of magnesium implants [9]. Alloying can significantly slow down the corrosion rate of magnesium in body fluids. For example, magnesium alloyed with Al can reduce dissolution and hydrogen evolution rates [10]. But the presence of Al ions in the human body is undesirable for human health. Some other alloying elements in the magnesium matrix such as cadmium, manganese or rare earth metals have also been proposed in order to improve the alloy's corrosion resistance, but these elements can barely be tolerated because they are poisonous to the human body [11−13]. Therefore, there are a limited numbers of elements that can be employed in magnesium alloys used for biomaterials.

One of the alternatives for adjustment of the corrosion rates of Mg alloys to meet the requirement of bone repair is the application of Mg-based metal matrix composites (MMCs) and their surface coating treatments [14,15]. Previous studies showed that Mg−6%Zn−10%$Ca_3(PO_4)_2$ exhibits good synthetic properties as a

Foundation item: Project (2014) supported by the Open Fund of the State Key Laboratory of Powder Metallurgy, China
Corresponding author: Kun YU; Tel: +86-731-88879341; E-mail: yukun2010@csu.edu.cn
DOI: 10.1016/S1003-6326(15)63669-X

biomaterial [16]. A suitable addition of hydroxyapatite or tricalcium phosphate can decrease the corrosion rate of the magnesium matrix and increase its biocompatibility. An anodizing or micro-arc oxidizing surface treatment of Mg alloys has been reported to improve their corrosion resistance. Such surface treatments of magnesium alloys have been employed for industrial applications but have seldom been explored for using in a bio-environment because the surface of the implanted magnesium must be non-toxic to the human body [17−19]. The surface treatment of the implanted magnesium must be non-toxic to the human body. Chitosan has been proven to be biocompatible with the human body [20]. Therefore, in the present investigation, a chitosan coating is applied to the surface of l Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite in order to adjust its biodegradable behavior and to study the effects of the chitosan coating on the in vivo degradation characteristics of the magnesium implant.

2 Experimental

2.1 Material production and measurement

The composite specimens were prepared with Mg, Zn and $Ca_3(PO_4)_2$ powders and sintered at 620−640 °C for 1 h in a vacuum furnace under argon gas protection. The average particle diameter of the Mg and Zn powders was 23.0 μm, and the average particle diameter of the $Ca_3(PO_4)_2$ powder was 7.85 μm. The specimens for testing were cut from the sintered composite billets. The specimens for chitosan coating were firstly abraded using sand paper and treated with a 40%H_3PO_4+H_2O solution as a pre-treatment. Chitosan, with a relative molecular mass of 300 k, was mixed in a 0.2% acetic acid solution to form the coating mixture. The coating mixture was then smeared on the surface of the composite specimens and solidified at 60 °C for 30 min. All of the experimental results for in vitro corrosion testing and in vivo measurements were obtained from the chitosan coated composite specimens. The uncoated Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite was utilized experimentally as the control specimen. The microstructures of the experimental MMCs were observed with a JEOL JSM-5600Lv scanning electron microscope (SEM).

2.2 Cytocompatibility assessments

The cytotoxicity of the experimental composites was measured via indirect contact testing according to ISO 10993-5:1999. The composite specimens were immersed in a 10% fetal bovine serum in a humidified incubator with 95% relative humidity and 5% CO_2 at 37 °C. L-929 cells were cultured in Dulbecco's modified Eagle's medium (DMEM). The culture medium was replaced by 100% extraction media or by 50% or 10% dilutions. The DMEM acted as a negative control, and a sample of the DMEM medium containing phenol acted as a positive control. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was then dissolved in a phosphate-buffered saline (PBS) solution at a concentration of 5 mg/mL. The samples were incubated for 4 h after adding 10 μL of the MTT solution. Subsequently, 100 mL of the formazan solution was added to each sample, and the optical density (OD) was measured using a spectrophotometer. The cell relative growth rate (R) was calculated as follows:

$$R=(D_{test}/D_{negative})\times 100\%$$

where R is the cell relative growth rate; D_{test} and $D_{negative}$ are the test and negative optical density, respectively.

2.3 In vivo biodegradation testing

All animal experiments, including anesthetic, surgical and post-operative treatments, were performed by ISO 10993-5:1999 and approved by and fulfilled the requirements of the Ethics Committee of the Xiangya Third Hospital and complied with the animal welfare legislation of the Chinese government. Eight adult male Zelanian rabbits weighing between 2.0 and 2.5 kg were randomly assigned to two groups. One group received uncoated Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite implants, while the others received the chitosan coated specimens. The rabbits in both groups were measured at the times of 2 weeks, 4 weeks, 8 weeks and 12 weeks. In the animal experiments, a 12 mm × 5 mm × 2 mm sized splint from the two types of experimental composites was fastened to the animal's pre-broken femoral shaft. A 5 mm× 5 mm × 2 mm size composite sample was implanted into the dorsal muscle of the animals. All rabbits were anaesthetized with amyl-barbiturate (30 mg/kg) for the surgery. Venous blood samples from the rabbits were phlebotomized at different times from 1 d to 12 weeks to detect the variations in the concentration of Mg^{2+}, Zn^{2+}, and Ca^{2+} in the blood with the LT-I Biochemical Analyzer. The animals were euthanized 12 weeks after the surgery. The muscle tissue around the implanted composite and the tissue from the heart, liver and kidneys of the rabbit were also stained with hematoxylin and eosin (HE) for histological analysis and in order to detect whether the degradation of the composites harmed these important visceral organs. Micro-computed tomography devices were used to observe the in vivo degradation process of the composite and the pre-broken bone healing process after the implant fixation.

3 Results

3.1 Morphology and microstructure characterization

The surface morphology of the Mg−6%Zn−10%$Ca_3(PO_4)_2$ composite without chitosan is shown in