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Enhanced antimicrobial properties, cytocompatibility, and corrosion resistance of plasma-modified biodegradable magnesium alloys

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ABSTRACT

Magnesium alloys are potential biodegradable materials and have received increasing attention due to their outstanding biological performance and mechanical properties. However, rapid degradation in the physiological environment and potential toxicity limit clinical applications. Recently, special magnesium–calcium (Mg–Ca) and magnesium–strontium (Mg–Sr) alloys with biocompatible chemical compositions have been reported, but the rapid degradation still does not meet clinical requirements. In order to improve the corrosion resistance, a rough, hydrophobic and ZrO_2-containing surface film is fabricated on Mg–Ca and Mg–Sr alloys by dual zirconium and oxygen ion implantation. Weight loss measurements and electrochemical corrosion tests show that the corrosion rate of the Mg–Ca and Mg–Sr alloys is reduced appreciably after surface treatment. A systematic investigation of the in vitro cellular response and antibacterial capability of the modified binary magnesium alloys is performed. The amounts of adherent bacteria on the Zr–O-implanted and Zr-implanted samples diminish remarkably compared to the unimplanted control. In addition, significantly enhanced cell adhesion and proliferation are observed from the Zr–O-implanted sample. The results suggest that dual zirconium and oxygen ion implantation, which effectively enhances the corrosion resistance, in vitro biocompatibility and antimicrobial properties of Mg–Ca and Mg–Sr alloys, provides a simple and practical means to expedite clinical acceptance of biodegradable magnesium alloys.

1. Introduction

Stainless steels, titanium and titanium alloys are the most widely used artificial surgical biomaterials. However, on account of their non-degradability and the mismatch of their mechanical properties with those of human bones, long-term adverse effects or stress shielding may occur after surgery. This may lead to bone loss and a higher risk of implant failure. To avoid this problem, implants made of biodegradable materials are used as alternatives. Among the various biodegradable materials, magnesium alloys have unique biodegradability in the physiological environment, have stimulatory effects on new bone formation and have an elastic modulus similar to that of human bone [1–4]. The major obstacle hampering their clinical use is their rapid degradation inside the human body. Human body fluids and blood plasma contain chloride ions that accelerate corrosion of magnesium alloys, producing hydrogen gas and localized basification [5]. Even though the corrosion resistance of some magnesium alloys can be enhanced by adding aluminum, rare earth elements and heavy metals, the alloyed materials may be not be suitable for biomedical applications due to potential toxicity and pathopoiesis of elements. Since the mechanical properties and corrosion resistance of pure magnesium are unsatisfactory, novel Mg–Ca and Mg–Sr alloys have recently be proposed as alternatives [6,7], but the anti-corrosion properties are still not ideal.

Proper control of the degradation process on magnesium alloys is therefore imperative. Protective coatings have been deposited to inhibit corrosion, and other surface modification techniques have been evaluated as well [8,9]. Ion implantation is an excellent surface modification technique and has been used on various materials to improve their surface properties [10,11]. Compared to conventional coating techniques, ion implantation can introduce a suitable number of ions into the near surface of the materials

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to alter the surface properties without creating an abrupt interface so that film delamination is typically not serious. Metals such as titanium and aluminum have been implanted to control the degradation rate of magnesium and its alloys [12–14]. In metal ion implantation, improvement in the corrosion resistance is often related to the formation of a corrosion-resistant metal oxide and dramatically reduced magnesium content in the near surface [15]. In many cases, after metal ion implantation, it is still necessary to modify the sample surface further to enable optimal formation of the metal oxide, and oxygen co-implantation by means of plasma immersion ion implantation (PIII) can yield the optimal effects.

While issues related to rapid surface degradation must be solved, good antibacterial properties and acceptable cytotoxicity are also necessary. In principle, if cytocompatible and antibacterial elements are introduced, the resulting materials also have enhanced cytocompatibility and antibacterial characteristics. Common elements are silver [16], zinc [17] and copper [18], all of which have good antibacterial activity, though an excess amount may cause deleterious effects. Zirconium has good cytocompatibility and antibacterial properties [19–21], and zirconium oxide has been reported to exhibit low toxicity while having the ability to inhibit bacterial colonization on the surface [22–25]. In this work, zirconium and oxygen ion implantation is conducted sequentially to modify Mg–Ca and Mg–Sr alloys and their corrosion resistance, cytocompatibility and antimicrobial characteristics are determined systematically.

2. Experimental details

2.1. Sample preparation

A Mg–Ca (1 wt.% Ca content) alloy ingot was cast from commercial pure magnesium (99.98%) and Ca (99.95%) in a crucible under a mixed atmosphere of SF_6 and CO_2, then extruded into a rod at 210 °C at a reduction ratio of 17 [8]. Mg–Sr (0.5 wt.% Sr content) alloys were prepared from pure magnesium (99.98%) and a Mg–10 wt.% Sr master alloy in a crucible under mixed SF_6 and CO_2. After holding at 740–760 °C for 30 min, the melt was poured into a steel mold preheated to 300 °C [6]. The Mg–Ca and Mg–Sr alloy samples, $10 \times 10 \times 2$ mm^3 in size, were machined from the extruded Mg–Ca rod and as-cast Mg–Sr ingot, mechanically polished up to 1200 grit and ultrasonically cleaned in acetone and ethanol. The resulting magnesium alloy samples were subjected to zirconium ion implantation on an HEMII-80 ion implanter (Plasma Technology Ltd., Hong Kong, China) equipped with a zirconium cathodic arc source. The samples were implanted for 0.5 h at a terminal voltage of 25 kV and a base pressure of 1.5×10^{-3} Pa. Afterwards, oxygen PIII was conducted on the GPI-100 ion implanter (Plasma Technology Ltd., Hong Kong, China). Oxygen gas was introduced at a flow rate of 30 sccm and the plasma was triggered by a 1000 W radio frequency. Using a pulsed voltage of −20 kV, a pulse width of 100 μs and a pulsing frequency of 50 Hz, oxygen PIII was conducted for 3 h.

2.2. Surface characterization

X-ray photoelectron spectroscopy (XPS; Physical Electronics PHI 5802) with Al K_α irradiation was used to determine the chemical states and acquire the elemental depth profiles before and after PIII&D. The sputtering rate was estimated to be about 5.8 nm min^{-1}, based on similar sputtering experiments conducted on a SiO_2 reference. Static water contact angle measurements were performed by the sessile drop method on a Ramé-Hart (USA) instrument at ambient humidity and temperature to determine the surface hydrophilicity. Five-microliter water droplets were laid on the sample surface and the image of the droplet was immediately captured to calculate the contact angle. Each data point represents the average and standard deviation of five measurements conducted on different areas of each specimen for statistical analyses. Atomic force microscopy (AFM) using a Park Scientific Instruments/Auto Probe CP was employed to evaluate the surface morphology before and after ion implantation. The AFM images were obtained using the contact mode, and the root-mean-square roughness was determined by averaging the results obtained from three different areas.

2.3. Corrosion behavior

Weight loss measurements are often used to evaluate the corrosion rate of magnesium alloys. The samples with a surface area of 10×10 mm^2 were exposed to simulated body fluid (SBF) at pH 7.40 (ion concentrations (mM) of Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, $HPO_4^{2}-$ 1.0 and SO_4^{2-} 0.5) [26] at 37 °C for 1 and 3 days. The corrosion products formed on the sample surfaces during corrosion were removed by immersing the samples in chromic acid (200 g l^{-1} CrO_3 +10 g l^{-1} $AgNO_3$) for 5 min. Afterwards, the samples were rinsed in distilled water and alcohol, and dried overnight prior to the measurement.

The electrochemical tests are used to determine corrosion mechanism of magnesium alloy. The measurement was performed on a Zahner Zennium electrochemical workstation using the three-electrode technique. The potential was referenced to a saturated calomel electrode (SCE) and a platinum sheet served as the counter electrode. The test milieu was SBF, tryptic soy broth (TSB; Bacto) and complete cell culture medium consisting of a mixture of Dulbecco's modified Eagle's medium (DMEM; Gibco) and 10% fetal bovine serum (FBS; Gibco). The samples, with a surface area of 10×10 mm^2, were exposed to the solution at 37 °C. Electrochemical impedance spectroscopy (EIS) measurement was carried out after stabilization in the solution for 5 min. The data were recorded from 100 kHz to 100 mHz, with a 5 mV sinusoidal perturbing signal at the open-circuit potential. The polarization curves were acquired by scanning the potential at a rate of 1 mV s^{-1} from −300 to +600 mV following the EIS measurement.

Among the SBF, TSB and cell culture medium (DMEM+10% FBS), the cell culture medium is the closest to the physiological environment and was therefore used as the immersion solution to further evaluate the corrosion resistance of the ion implanted magnesium alloys. After immersion for 3 days in the cell culture medium at 37 °C, the samples were removed from the solution, rinsed with distilled water and dried in air. The surface morphology and microstructure were characterized by scanning electron microscopy (JEOL JSM-820 with an energy-dispersive spectroscopy (EDS) attachment).

2.4. Indirect cell viability and cell proliferation evaluation

Mouse MC3T3-E1 pre-osteoblasts were used in the in vitro cell culture experiments. They were cultured in DMEM supplemented with 10% FBS, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Prior to the experiments, all the samples were sterilized by 70 vol.% ethanol for 30 min and rinsed three times with sterile phosphate-buffered saline (PBS).

The cell viability was evaluated by extract assay. The extracts were prepared with a sample surface area to extraction medium (DMEM) ratio of 1 ml cm^{-2} in a humidified atmosphere with 5% CO_2 at 37 °C for 3 days. The supernatant fluid was withdrawn and stored at 4 °C prior to the cytotoxicity test. The cells were cultured on a 96-well tissue culture plate at a density of 5000 cells