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Tailoring the degradation and biological response of a magnesium–strontium alloy for potential bone substitute application

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ABSTRACT

Bone defects are very challenging in orthopedic practice. There are many practical and clinical shortcomings in the repair of the defect by using autografts, allografts or xenografts, which continue to motivate the search for better alternatives. The ideal bone grafts should provide mechanical support, fill osseous voids and enhance the bone healing. Biodegradable magnesium–strontium (Mg–Sr) alloys demonstrate good biocompatibility and osteoconductive properties, which are promising biomaterials for bone substitutes. The aim of this study was to evaluate and pair the degradation of Mg–Sr alloys for grafting with their clinical demands. The microstructure and performance of Mg–Sr alloys, *in vitro* degradation and biological properties including *in vitro* cytocompatibility and *in vivo* implantation were investigated. The results showed that the as-cast Mg–Sr alloy exhibited a rapid degradation rate compared with the as-extruded alloy due to the intergranular distribution of the second phase and micro-galvanic corrosion. However, the initial degradation could be tailored by the coating protection, which was proved to be cytocompatible and also suitable for bone repair observed by *in vivo* implantation. The integrated fracture calluses were formed and bridged the fracture gap without gas bubble accumulation, meanwhile the substitutes simultaneously degraded. In conclusion, the as-cast Mg–Sr alloy with coating is potential to be used for bone substitute alternative.

1. Introduction

Bone grafting is a frequently performed procedure to enhance bone regeneration in a variety of conditions in orthopedic; it has been estimated that more than 2.2 million grafting procedures are performed worldwide each year [1,2]. Bone grafting is usually required to stimulate bone healing, which is carried out in spinal fusions, filling defects following removal of bone tumor and several congenital diseases [3]. From the viewpoint of the clinical demands, autologous bone grafting is currently considered as the gold standard to restore bone defects due to its osteoinductivity and excellent outcomes [4]. However, the morbidity from its harvesting and its restricted availability generated the need for the development of alternative bone substitutes.

Ideally, bone substitute should provide necessary mechanical support for the specific location; promote rapid formation of bone and bridging/filling of the defects at 95% of the time, and remodel over time [5]. Moreover, it should be preferably bioresorbable. There is a wide spectrum of materials used today for the purpose of grafting, such as calcium phosphate based in the form of hydroxyapatite (HA), Beta tricalcium phosphate (β-TCP); calcium sulfate ($CaSO_4$), bioactive glasses and biological/synthetic composites [6,7]. They vary in composition, mechanism of action and special characteristics; however, their ultimate goal remains same — to form functionally viable bone that meets the needs of the site. It is important to note that they all are osteoconductive, but offer various levels of structural support, and have little, if any, ability for osteoinduction [8]. Furthermore, there is a range of performance for these materials. Some of those are quickly resorbed before they can perform their scaffolding function, some remain at the surgical site for many months beyond the healing period and others are fairly inert with only minimal resorption in the lifetime of the patients [5]. Thus how to pair new materials for grafting with these surgical options is always a large problem for clinical use.

Magnesium based metals have been increasingly researched in the past decade; most studies are involved in their potential to be used as biodegradable implants due to their biocompatibility combined with outstanding physical and mechanical properties [9,10]. *In vitro* studies found that magnesium increased cell proliferation and expression of osteogenic markers [11]. Furthermore, magnesium promoted new bone formation, thus allowing bone healing and regeneration in the implant site and it has also been shown to increase osteoconductivity *in vivo* [12]. In comparison with commercial synthetic substitute materials, magnesium provides the merits of osteoinductive, osteoconductive effects, tailored degradation and excellent mechanical property, which

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inspire the utilization of biodegradable magnesium alloys as the potential bone substitute alternatives.

In our previous study [13], magnesium strontium (Mg–Sr) alloys were designed for the bioresorbable bone substitute application. Strontium (Sr) is a bioactive element which could stimulate bone formation and improve bone density [14]. It is known that Sr can activate osteoblastic cell replication through the calcium sensing receptor (CaSR) and signal-regulated kinase (ERK) 1/2 phosphorylation, and inhibit bone resorption by increasing osteoprotegerin (OPG) and decreasing receptor activator of nuclear factor kappa B ligand (RANKL) expression by osteoblasts [15]. Additionally, the addition of Sr as an alloying element contributed to precipitation strengthening and galvanic corrosion due to intermetallic phases. The mechanical strength, degradation rate and cytocompatibility of Mg–Sr alloys were evaluated and showed predominant advantages compared with commercial bone substitutes (HA, TCP and $CaSO_4$).

Regarding established indication and relevant *in vivo* application, such as intraosseous bone defects or large segmental defects, what would be desirable is a biodegradable device that has the compatible degradation/resorption with the bone healing and reconstruction. As mentioned before, substitutes with excessive or slow degradation time cannot ultimately be incorporated and replaced by the bone, and may inhibit full bone regeneration. Moreover the degradation of magnesium *in vivo* would produce high alkalinity and evolving hydrogen gas, which would further influence the bone and wound healing [16]. In the prior study, the Mg–1.5 wt.% Sr alloy showed better combined properties for potential application as bone substitute material. However, what kind of degradation rate especially *in vivo* could satisfy the clinical demand is still unknown. Thus it is important to evaluate the degradation and relevant biological response of magnesium based bone substitutes for further optimization and application.

In this work a range of magnesium strontium alloys in status of as-cast, as-extruded and as-cast with coating were explored to investigate the microstructure and performance of the alloys, *in vitro* degradation and biological properties including *in vitro* cytocompatibility and *in vivo* implantation. The overall goal was to develop an optimized magnesium based bone substitutes by tailoring the degradation and relevant biological response.

2. Materials and methods

2.1. Materials and preparation of MAO coatings

Mg–Sr alloys with the actual composition of 1.5 wt.% Sr (confirmed by ICP) [13] were prepared by melting down pure Mg (99.9 wt.%) and pure Sr (99.99 wt.%) in a high purity graphite crucible. SF_6 (1 vol.%) and CO_2 (balance) were used at a flow rate of 1.1 L/min as a protective gas to prevent burning during melting and casting. After being held at 780 °C for 30 min the melt was poured into a steel mold preheated to 300 °C. The as-cast ingots were hot extruded into bars with extrusion ratio of 64:1 at 350 °C. The samples were cut into pieces with dimensions of $\Phi 10 \times 2$ mm^3. All samples were grounded with SiC paper up to 2000 grit, followed by ultrasonic cleaning in acetone, absolute ethanol, and distilled water for 10 min each and then sterilization with ethylene oxide.

A pulsed bipolar electrical source (WHD-20) with power of 2 kW was used to prepare the micro-arc oxidation (MAO) coatings. The following electrolyte was chosen for the MAO treatment: 8 g/L $KF{\cdot}2H_2O$, 4 g/L $(NaPO_3)_6$, 0.8 g/L $Ca(OH)_2$ and 0.8 g/L $Sr(OH)_2$. A voltage static mode was adopted to prepare the MAO coatings. The work voltage, work frequency, work duty cycle and preparation time were 360 V, 1000 Hz, 40% and 5 min, respectively according to previous study [17]. The temperature of the electrolyte was kept at 20–25 °C by a water cooling system.

2.2. Morphology and phase composition

Optical microscopy (OM) was carried out to observe the microstructure of the Mg–Sr alloys. The cross-sections of specimens were ground, polished and etched with the solution of 1.5 g/L picric acid, 5 mL/L glacial acetic acid and 10 mL/L alcohol. An X-ray diffractometer (XRD, Rigaku D/MAX 2500 Diffractometer) using Cu K_α radiation was employed to identify the crystal structure of the phases. Diffraction patterns were generated between 2θ values of 10–85°, with a step increment of 0.04° and a scanning speed of 4° min^{-1}. Scanning electron microscopy (SEM, S-3400N, Hitachi, Japan) equipped with energy-dispersive spectrometry (EDS) was employed to study the microstructure of the Mg–Sr alloys.

2.3. Physicochemical characterization of MAO coating

The surface and cross-section morphologies of the MAO coatings were studied on the SEM and EDS. The phase composition was analyzed using XRD. The chemical composition of the coatings was characterized by X-ray photoelectron spectroscopy (XPS, ESCALAB 250, Thermo, VG, USA) using an X-ray source of Al K_α (1486.6 eV) with a spot size of 500 μm diameter. The energy resolution was 0.1 and 1 eV for the high resolution and survey scan, respectively. To prevent charging, spectra were collected using a flood gun. Samples were etched with Ar ions *in situ*, in the XPS analysis chamber, to remove surface contamination. XPS peak 4.1 software was used to analyze the data.

2.4. Electrochemical test

The electrochemical tests were carried out using an electrochemical workstation (Reference 60, Gamry, USA). A three electrode cell, using platinum (10 mm × 10 mm × 1 mm) as the counter-electrode and a saturated calomel electrode (SCE) as the reference electrode, was used for electrochemical measurements. A wire lead was attached to one section of each sample and closely sealed with epoxy resin, leaving one end surface (with a cross-sectional area of 1 cm^2) exposed to solution. All the tests were carried out in at a temperature of 37 ± 0.5 °C in Hank's solution (8 g/L NaCl, 0.4 g/L KCl, 0.06 g/L KH_2PO_4, 0.12 g/L Na_2HPO_4, 0.14 g/L $CaCl_2$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.35 g/L $NaHCO_3$, 1 g/L Glucose, pH = 7.4). The samples were stabilized for 10 min in the solution and the test was conducted with a scan rate of 0.5 mV/s for all the measurements. Three duplicates were taken for each group to control the experimental scatter for statistics.

2.5. Immersion test

The samples were immersed in the Hank's solution at 37.5 ± 0.5 °C for 14 days. The ratio of surface area/solution volume was 1.25 cm^2/mL. The immersion solutions were refreshed every day to simulate the real *in vivo* condition. The pH value of the solution was measured every 24 h during the immersion test. Samples were removed after 14 days, rinsed in distilled water and air dried overnight. The changes in surface morphology and composition of samples after degradation in the solution were analyzed by SEM and EDS.

The weight loss was measured at three time points (3, 7, 14 days) to calculate the corrosion rate. Corrosion products were cleaned using chromic acid solution (200 g/L CrO_3 and 10 g/L $AgNO_3$) for 15 min in an ultrasonic bath at room temperature until all signs of corrosion products were removed. The morphology of the samples after cleaned was observed by SEM. An average of three measurements was taken for each group. The *in vitro* corrosion rate was calculated according to ASTM G31-72 using the following equation:

$$CR = KW/ATD$$

where CR is the corrosion rate (mm/year), K is a constant, 8.76×10^4, W is the mass loss (g), A is the surface area (cm^2), T is the time of exposure