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Effects of grain refinement on the biocorrosion and in vitro bioactivity of magnesium

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ABSTRACT

Magnesium is a new class of biodegradable metals potentially suitable for bone fracture fixation due to its suitable mechanical properties, high degradability and biocompatibility. However, rapid corrosion and loss in mechanical strength under physiological conditions render it unsuitable for load-bearing applications. In the present study, grain refinement was implemented to control bio-corrosion demonstrating improved in vitro bioactivity of magnesium. Pure commercial magnesium was grain refined using different amounts of zirconium (0.25 and 1.0 wt.%). Corrosion behavior was studied by potentiodynamic polarization (PDP) and mass loss immersion tests demonstrating corrosion rate decrease with grain size reduction. In vitro biocompatibility tests conducted by MC3T3-E1 pre-osteoblast cells and measured by DNA quantification demonstrate significant increase in cell proliferation for Mg–1 wt.% Zr at day 5. Similarly, alkaline phosphatase (ALP) activity was higher for grain refined Mg. Alloys were also tested for ability to support osteoclast differentiation using RAW264.7 monocytes with receptor activator of nuclear factor kappa-β ligand (RANKL) supplemented cell culture. Osteoclast differentiation process was observed to be severely restricted for smaller grained Mg. Overall, the results indicate grain refinement to be useful not only for improving corrosion resistance of Mg implants for bone fixation devices but also potentially modulate bone regeneration around the implant.

1. Introduction

Synthetic resorbable biomaterials are in ever increasing demand for repairing or substituting fractured bones, which can exhibit controlled resorption characteristics with time and invariably bypass the complicated invasive surgical procedures that is very much common in the case of permanent inert fixtures [1]. The ideal material for this purpose should exhibit osteoconductive/osteoinductive characteristics coupled with mechanical and resorbable properties similar to that of bone. In this regard, magnesium (Mg) based metallic biomaterials appear promising as next generation implants suitable for fracture fixation devices such as screw and fixation plates due to their resorption characteristics and similar mechanical properties to those of cortical bone [2–4]. Besides, the elastic modulus of Mg is also much closer to the natural bone compared to other commonly used metallic implants which considerably reduce the risk of stress shielding [3,5].

Earlier scientific studies have revealed that pure Mg has poor corrosion resistance in physiological environments and rapidly loses its mechanical strength rendering them not suitable for load-bearing implants for long-term use [2,3]. This rapid increase in corrosion results in loss of mechanical strength of the implant with concomitant formation of gas pockets around the implanted sites and leads to patient discomfort and morbidity [2,3,6]. Efforts have been made to improve the corrosion characteristics and mechanical strength of pure magnesium by adopting various routes which include alloy development, surface treatments, extrusion by means of severe plastic deformation, formation of a protective coating, and microstructural refinement etc. [1,2,7,8]. So far, the most widespread technique employed to improve the corrosion resistance and mechanical strength of pure magnesium has been alloy design with the development of magnesium based systems using aluminum, zinc, yttrium, other rare earths, silver, calcium, manganese etc. as common alloying elements owing to their thermodynamic preference and observed in vitro biocompatibility. However, the magnesium alloys developed so far have raised long term compatibility issues [9–11]

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requiring further investigation to prove their safety, efficacy and biocompatibility [12,13].

Applicability of magnesium based degradable implant is not only related to its strength and corrosion properties in physiological conditions, but is also related to its biocompatibility. Bioactivity, specifically enhancement of osteoblast bone forming activity of cells, of bone replacement materials has been correlated to the material's grain size [14–16]. All classes of bone replacement materials such as ceramics (hydroxyapatite, alumina, titania), metals (Ti, Ti6Al4V, and CoCrMo), polymers (PLA and polyurethane) and carbon nanotube/nanofiber based composites have shown to enhance osteoblast cell proliferation and differentiation when the grain size is reduced to the submicron size range. The enhanced osteoblast performance has primarily been associated with higher protein adhesion on these submicron sized materials [17]. With regard to inert metallic implants (Ti, Ti6Al4V), selective protein adhesion has been noticed at grain boundaries [15]. Since the submicron sized material has higher grain boundary area per unit area, it shows higher specific osteoblast activity.

Due to the several positive attributes of smaller grain size from improved mechanical strength, and bioactivity, the present study is aimed to investigate the effects of grain refinement of magnesium on its biodegradation behavior in Hank's buffered salt solution (HBSS). Zirconium (Zr) is a biologically inert element and is a well-known grain refiner used for pure magnesium and its alloys [8,18]. The excellent grain refining ability of Zr is due to its similar crystal structure and small lattice disregistry with the Mg phase (Zr: a = 0.323 nm, c = 0.514 nm and Mg: a = 0.320 nm, c = 0.520 nm), as well as the highest growth restriction factor among all the elements that promotes grain refinement in magnesium [8]. In this regard, pure magnesium (used as the baseline) and grain refined magnesium with two different levels of zirconium–0.25 wt.% (Mg–0.25Zr), and 1 wt.% (Mg–1Zr) were cast under identical conditions and studied for their biodegradation behavior and loss of mechanical strength with time. Effects of grain size on the biocompatibility of magnesium were studied using MC3T3 and osteoclast precursor RAW264.7 cells. Cell–material interactions were analyzed by alkaline phosphate activity (ALP), DNA proliferation assay, and TRAP assay for their intended use as biodegradable implant materials. The results of the above experimental studies conducted on Mg–0.25Zr and Mg–1Zr samples generated by melting/casting and subsequent processing are compared with pure magnesium. Correspondingly, the results of the materials and the biological characterization to analyze the efficacy of microstructural refinement outlined above are discussed in the present manuscript.

2. Experimental

2.1. Material preparation

Pure Mg (US Magnesium Inc., Salt Lake City, UT, 99.97%) ingot was melted at 780 °C in a mild steel crucible using an electrical resistance furnace (Wenesco Inc., Chicago, IL) under the Ar + 0.5% SF_6 protection atmosphere. Once the desired pouring temperature reached 780 °C, an equivalent amount of Zr (0.25 and 1.0 wt.%) was added using Zirmax® (Mg–33.3 wt.% Zr) master alloy (Magnesium Elektron Ltd., Manchester, UK). After Zr addition, the melt was stirred for 10 s at 1 and 5 min intervals to dissolve and disperse the Zr particles uniformly into the melt. The melt was further held for 30 min at 780 °C and poured into a cylindrical mild steel mold (44.5 mm diameter × 82.5 mm length) preheated to 500 °C. The required holding time of 30 min at the pouring temperature is essential to release Zr particles from the Zirmax® master alloy uniformly in the Mg melt in order to achieve optimal grain refinement as well as to settle the undissolved large Zr particles (particles >5 μm) to the bottom of the crucible due to the density difference of pure Zr and the liquid Mg (ρ_{Zr}: 6.52 g/cm^3, ρ_{Mg}: 1.58 g/cm^3) [18–20]. Pure Mg was also prepared following the same metallurgical route and used as the control baseline sample.

2.2. Microstructural characterization

For microstructural characterization, square samples (10 mm × 10 mm × 1 mm) from the center of the as-cast specimens (pure Mg, Mg–0.25Zr, and Mg–1.0Zr) were epoxy mounted, mechanically polished (Tegramin-20, Struers, Ballerup, Denmark), and chemically etched in acetic-picral solution. Optical micrographs were captured using an Axiovert 40 MAT (Carl Zeiss, Jena, Germany) optical microscope at different magnifications. The average grain size was measured using the mean lineal intercept method.

2.3. Electrochemical and immersion corrosion

Electrochemical corrosion of pure Mg, Mg–0.25Zr, and Mg–1Zr were also measured by the potentiodynamic polarization (PDP) technique. Samples were connected to a copper wire using silver epoxy and mounted in epoxy resin. The mounted samples of dimensions Ø 8 mm × 2 mm were mechanically polished, sonicated in isopropanol, and dried in air. The potentiodynamic polarization study was carried out using an electrochemical workstation (CH-604A, CH Instruments, Inc., Austin, TX) at a scanning rate of 1 mV/s and potential window of 500 mV above and below the open circuit potential. A three electrode cell was employed utilizing platinum as the counter electrode, Ag/AgCl as the reference electrode, and the sample mounted in epoxy resin used as the working electrode. The test was performed in HBSS at pH 7.2 ± 0.2 and held at 37.4 °C. Before each measurement, the sample was immersed in the corrosion media for 5 min to provide voltage stability. The cathodic and anodic portions of the generated Tafel plots were accordingly extrapolated to calculate the corrosion potential, E_{corr}, and corrosion current density, i_{corr}. The corrosion product after the potentiodynamic polarization test was removed using 200 g/L of chromic acid and 10 g/L of $AgNO_3$ solution for 10 min [21] and the surface was characterized using SEM to determine the corroded morphology.

Immersion corrosion measurements were performed in conformation with ASTM G31-72 standards [22]. Cylindrical samples from pure Mg, Mg–0.25Zr, and Mg–1Zr were prepared in a dimension of Ø 5 mm × 10 mm and polished up to 1200 grit using a SiC paper. Surface area and the weight of each sample were carefully recorded before the immersion test. The samples were thoroughly cleaned in acetone using a sonicated bath followed by UV sterilization for 30 min each side. After sterilization, the samples were immersed in HBSS at 37.4 °C. HBSS media volume to surface area ratio was 20 ml/cm^2 according to the ASTM G31-72 standard. Samples were removed after 16 and 32 days of immersion and rinsed with ethanol and dried at 60 °C for 24 h. The corrosion product on the sample surfaces was removed using 200 g/L of chromic acid and 10 g/L of $AgNO_3$ solution for 10 min [21]. The corrosion rate was calculated according to ASTM G31-72 using the following equation:

$$C = (K \times W)/(A \times T \times D)$$

where C is the corrosion rate in mm/year (mmpy), the constant K is 8.76×10^4, W is the mass difference before and after immersion in grams, A is the sample area exposed to solution, measured in cm^2, T is the time of exposure in h, and D is the density of the material in g/cm^{-3}. An average and standard deviation of three measurements were taken for each group. Data are presented as mean ± standard deviation. Statistical analysis was performed using Student's t test, and $p < 0.05$ was considered statistically significant. During immersion corrosion study, solution pH was also recorded at a regular interval using a digital pH meter. 500 μl of the HBSS solution was also collected at regular intervals and the Mg and Zr ionic concentrations were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCAP duo 6500 Thermo Fisher, Waltham, MA).