Acta Biomaterialia xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Effect of the addition of low rare earth elements (lanthanum, neodymium, cerium) on the biodegradation and biocompatibility of magnesium

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ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 15 September 2014
Accepted 22 September 2014
Available online xxxx

Keywords:
Magnesium alloy
Rare earth elements
Biocompatibility
Biodegradable implants
In vitro

ABSTRACT

Rare earth elements are promising alloying element candidates for magnesium alloys used as biodegradable devices in biomedical applications. Rare earth elements have significant effects on the high temperature strength as well as the creep resistance of alloys and they improve magnesium corrosion resistance. We focused on lanthanum, neodymium and cerium to produce magnesium alloys with commonly used rare earth element concentrations. We showed that low concentrations of rare earth elements do not promote bone growth inside a 750 μm broad area around the implant. However, increased bone growth was observed at a greater distance from the degrading alloys. Clinically and histologically, the alloys and their corrosion products caused no systematic or local cytotoxicological effects. Using microtomography and in vitro experiments, we could show that the magnesium–rare earth element alloys showed low corrosion rates, both in in vitro and in vivo. The lanthanum- and cerium-containing alloys degraded at comparable rates, whereas the neodymium-containing alloy showed the lowest corrosion rates.

1. Introduction

One of the actual and much-needed demands in orthopaedics is the clinical availability of biodegradable implants [1–5]. In some clinical applications, such as fracture treatment, permanent metal implants are not necessary or even disadvantageous and a temporary implant concept would be much more suitable. Temporary implants made of biodegradable materials are destined to corrode and dissolve postoperatively and hence a second surgery for implant removal is not necessary, eliminating surgery and accompanied additional costs and unnecessary health risks for the patients. The requirements for such temporary implants are multi-fold and depend upon the site of their utilization. However, some properties are essential: (i) the material should provide a controlled and adequate degradation profile, whilst allowing (ii) necessary spatial and temporal mechanical stability. (iii) The biodegradable material or its components should not only be biocompatible [6,7], but moreover, (iv) it should at best assist specific and desired biological effects such as stimulating regeneration, healing processes or supporting anti-inflammatory mechanisms. (v) Finally and optimally, the biodegradable material should be completely replaced by host tissue. Amongst other candidates, magnesium-based alloys are very promising materials for such temporary implants [4,8,9]. These alloys offer some remarkable physicochemical properties [10–14] while they disintegrate gradually up to complete dissolution in physiological environments. Magnesium and its alloys show high degrees of biocompatibility [15–19] and very interestingly, an increased bone growth has been repeatedly reported in the vicinity of corroding magnesium implants or its

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http://dx.doi.org/10.1016/j.actbio.2014.09.041
1742-7061/

corrosion products [20–23]. Actually, a drug-eluting magnesium stent [24] and a magnesium screw consisting of MgYREZr (a material similar to WE43) that contains >90 wt.% magnesium [25] are already in clinical use.

Despite the remarkable progress which was achieved in the development of Mg-based alloys, the important issues of corrosion resistance, gas formation, biocompatibility and mechanical stability need more research effort. In our search of suitable alloying elements for biomedically usable magnesium alloys, we concentrated on cerium (Ce), lanthanum (La) and neodymium (Nd), three rare earth elements (REEs). REE are widely used in different commercially available alloys such as AE, QE, WE or ZE series alloys [26–28]. The addition of REEs has significant effects on the high temperature strength and creep resistance [29] and they improve magnesium corrosion resistance [30]. For engineering applications, REEs are usually added as a mischmetal of various compositions commonly rich in Ce, La and Nd; however, this mischmetal can vary in its composition depending on its source. For biomedical applications a more defined approach is desirable, since reproducibility is a major requirement for medical devices. In a first approach, we focused on the collectivity of eligible REEs on these commonly occurring three elements and we prepared Mg–Ce, Mg–La and Mg–Nd alloys to observe the in vitro and in vivo effects. The alloy compositions were used at a concentration which was determined from an average of the most common representatives of Mg–REE alloys, e.g. 2.0–2.5% neodymium was used for WE43A alloy, according to ASTM B275-04 [31,32]. We expected these concentrations to be toxicologically noncritical but sufficient enough to influence positively the mechanical properties of the alloy matrix architecture.

This study investigated the microstructures, the in vitro corrosion behaviour and the cytotoxicity of low concentrations of REE in Mg alloys. To observe the effects of these different Mg–REE alloys on the reactions of bone tissue, they were implanted, within the limits of a pilot study, into rabbit femur condyles. General biocompatibility as well as changes in the surrounding tissues using histomorphological analysis was investigated.

2. Materials and methods

2.1. Preparation of Mg–REE alloys and microstructure observation

Three binary Mg–REE alloys (Mg–Ce: 1.27 wt.% cerium; Mg–La: 0.69 wt.% lanthanum; Mg–Nd: 2.13 wt.% neodymium) were melted and cast in pure Mg (99.95%) and commercially pure REE under a mixed gas atmosphere of SF_6 and CO_2 using a mild steel crucible. The chemical compositions of Mg–REE alloys were measured by inductively coupled plasma atomic emission spectrometry (Profile ICP-AES, Leeman Labs, Husdon, USA). The cylindrical samples (3 mm diameter and 5 mm height) were cut from casting ingots and further machined and polished using a computer numerical controlled lathe machine. The chemical polishing was then performed using a solution of 20 ml glycerol, 2 ml hydrochloride acid, 3 ml nitric acid and 5 ml acetic acid to remove the remaining fine scratches from the surface. The implants final size was 2.99 ± 0.01 mm in diameter and 5.00 ± 0.02 mm in height. All samples were ultrasonically cleaned in acetone, absolute ethanol and distilled water. To observe the microstructure, samples were polished and etched with a mixture of 1 g oxalic acid, 1 ml acetic acid, 1 ml nitric acid and 98 ml water and characterized by an environmental scanning electron microscopy (ESEM; Quanta 200FEG, FEI Company, Eindhoven, Netherlands). X-ray phase analysis was conducted via an X-ray diffractometer (XRD; DMAX 2400, Rigaku, Tokyo, Japan) using CuKα radiation. Before implantation, the implants were gamma-sterilized with 25–29 kGy of cobalt-60 radiation (BBF Sterilisations service, Kernen, Germany). All chemical reagents were analytical reagent grade and were purchased from Beijing Chemical Reagent Co., Beijing, China.

2.2. Electrochemical corrosion test

The electrochemical tests were carried out in simulated body fluid (SBF; [29]) at 37 °C using an electro-chemical workstation (CHI660C, Chenhua, Shanghai, China). A three-electrode cell was used with the counter electrode being made of platinum and the reference electrode as saturated calomel. Three 10 × 10 × 2 mm square plate samples were used for electrochemical testing. The exposed area of the Mg–REE alloys to the solution was 1 cm^2. An average of three measurements was taken for each group. The corrosion rate was calculated using the equation described in ASTM-G102-89:

$$CR = K\frac{i_{corr}W}{n\rho}$$

where CR is the corrosion rate in mm $year^{-1}$, $K = 3.27 \times 10^{-3}$ mm in g μA^{-1} cm^{-1} $year^{-1}$, i_{corr} is the corrosion current density, ρ is the density in g cm^{-3}, W is the atomic weight of the magnesium and n is the number of electrons involved in the corrosion reaction.

2.3. Immersion test

The immersion test was carried out according to ASTM-G31-72 in SBF at 37 °C. The hydrogen evolution volume was measured as a function of the immersion time. After different immersion time intervals, the samples were removed from solution, gently rinsed with distilled water and dried at room temperature. Changes to the surface morphologies of the samples before and after immersion were characterized by ESEM, equipped with an energy-dispersive spectrometer (EDS) attachment and XRD. An average of three measurements were taken for each group. The molar volume of hydrogen is 22.4 l at standard temperature and pressure and thus 1 ml of hydrogen evolved corresponds to 1 mg of Mg dissolved. The corrosion rate was calculated following the equation described in ASTM-G31-72:

$$CR = \frac{K \cdot W}{A \cdot T \cdot D}$$

where CR is the corrosion rate in mm $year^{-1}$, $K = 8.76 \times 10^4$, T is the exposure time, A is the exposure area, W is the mass loss and D is the density in g cm^{-3}.

2.4. Cytotoxicity test

Murine calvarial preosteoblasts (MC3T3-E1; purchased from Peking Union Medical College, Beijing, China) were cultured in Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum (FBS), 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. All samples were sterilized using ultraviolet radiation for at least 2 h before the cell experiment. The cytotoxicity test was evaluated using the Mg–REE alloys extracts, prepared with a surface area to extraction medium ratio of 1 ml cm^{-2} in a humidified atmosphere with 5% CO_2 at 37 °C for 72 h. The supernatant fluid was withdrawn, centrifuged, serially diluted (100%, 50% and 10%) and then refrigerated at 4 °C before the cytotoxicity test. The control groups involved the use of DMEM medium as negative control. Cells were incubated in 96-well culture plates at 3×10^3 cells/100 μl medium in each well and incubated for 24 h to allow attachment. The medium was then replaced with 100 μl of pure and diluted extracts. After 1, 3 and 5 days the extracts were replaced by media and 10 μl MTT was added to each well for 4 h and 100 μl formazan solubilization

Please cite this article in press as: Willbold E et al. Effect of the addition of low rare earth elements (lanthanum, neodymium, cerium) on the biodegradation and biocompatibility of magnesium. Acta Biomater (2014), http://dx.doi.org/10.1016/j.actbio.2014.09.041