Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Influence of trace impurities on the *in vitro* and *in vivo* degradation of biodegradable Mg–5Zn–0.3Ca alloys

J. Hofstetter [a], E. Martinelli [b], S. Pogatscher [a], P. Schmutz [c], E. Povoden-Karadeniz [d], A.M. Weinberg [b], P.J. Uggowitzer [a], J.F. Löffler [a],*

[a] *Laboratory of Metal Physics and Technology, Department of Materials, ETH Zurich, 8093 Zurich, Switzerland*
[b] *Department of Orthopaedics and Orthopaedic Surgery, Medical University Graz, 8036 Graz, Austria*
[c] *Laboratory for Joining Technologies and Corrosion, EMPA, Swiss Federal Laboratories for Materials Science and Technology, 8600 Dübendorf, Switzerland*
[d] *Christian Doppler Laboratory for Early Stages of Precipitation, Vienna University of Technology, 1040 Vienna, Austria*

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 5 May 2015
Accepted 9 May 2015
Available online xxxx

Keywords:
Magnesium
Mg–Zn–Ca alloys
Biodegradation
Intermetallic phases
Corrosion

ABSTRACT

The hydrogen evolution method and animal experiments were deployed to investigate the effect of trace impurity elements on the degradation behavior of high-strength Mg alloys of type ZX50 (Mg–5Zn–0.3Ca). It is shown that trace impurity elements increase the degradation rate, predominantly in the initial period of the tests, and also increase the material's susceptibility to localized corrosion attack. These effects are explained on the basis of the corrosion potential of the intermetallic phases present in the alloys. The Zn-rich phases present in ZX50 are nobler than the Mg matrix, and thus act as cathodic sites. The impurity elements Fe and Mn in the alloy of conventional purity are incorporated in these Zn-rich intermetallic phases and therefore increase their cathodic efficiency. A design rule for circumventing the formation of noble intermetallic particles and thus avoiding galvanically accelerated dissolution of the Mg matrix is proposed.

1. Introduction

Magnesium alloys are of special interest in the context of structural lightweight applications in the transport and aerospace industries, and for temporary implants in medicine because of their biocompatibility and biodegradability [1,2]. One of the major obstacles to widespread application of magnesium alloys, however, is their high corrosion rate [3,4], which is often attributed to the presence of specific impurity elements. Elements such as Fe, Ni, Cu and Co can significantly accelerate Mg corrosion when their concentrations are above their tolerance limits [5–9]. Fe, for example, is the most common impurity element; its tolerance limit is reported as 170 ppm in unalloyed as-cast Mg [3] and roughly 5 ppm in wrought Mg [6,9]. Below this limit, when no Fe-rich particles are formed and thus no electrochemically active cathodic sites exist to accelerate corrosive attack, the corrosion rate decreases dramatically. Recent studies have added silicon (Si) to the above-mentioned reactive impurity elements detrimental to corrosion. Si plays a critical role in promoting the formation and growth of Fe-rich particles and should thus also be considered as corrosion-provoking element [9,10].

For osteosynthesis implants and stent applications, not only electrochemical and biocompatible requirements are crucial, but also mechanical properties [1]. Recent studies have demonstrated that the alloying system Mg–Zn–Ca offers simultaneous high strength and high ductility, with values of $R_{p0.2} > 250$ MPa, $R_m > 300$ MPa and $A_f > 20\%$. These attractive large values were achieved within a composition window of 5–6 wt.% Zn and 0.2–0.4 wt.% Ca [11,12]. Recently the *in vivo* behavior of an alloy with 5 wt.% Zn and 0.25 wt.% Ca (ZX50) of conventional purity (CP) was investigated, and undesired rapid degradation was observed [13]. In the present study, the alloy ZX50 was chosen again, but this time raw materials of two different purities were used. The first alloy was made of CP Mg identical to that used in [13], but the second was synthesized using ultrahigh-purity (XHP) Mg [14]. The research aim of this study was to compare the *in vitro* and *in vivo* behavior of the two alloys CP ZX50 and XHP ZX50, and to clarify the influence of trace elements on degradation characteristics and mechanisms. The *in vitro* corrosion was determined via the hydrogen evolution method [15], deploying a newly designed testing device [9]. For the *in vivo* examination pins of each alloy were implanted in the femoral shafts of rats and their degradation was studied over a period of 12 weeks (see also Ref. [13]). A few pins were also explanted after two weeks to investigate their surface topography and elucidate the degradation mechanisms.

---

* Corresponding author. Tel.: +41 44 632 2565; fax: +41 44 633 1421.
E-mail address: joerg.loeffler@mat.ethz.ch (J.F. Löffler).

http://dx.doi.org/10.1016/j.actbio.2015.05.004
1742-7061/© 2015 Published by Elsevier Ltd.

Please cite this article in press as: J. Hofstetter et al., Influence of trace impurities on the *in vitro* and *in vivo* degradation of biodegradable Mg–5Zn–0.3Ca alloys, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.05.004

2. Experimental methods

2.1. Materials and methods

CP and XHP ZX50 were each processed to extruded rods, from which specimens for the *in vivo* and *in vitro* tests were machined. Conventional pure Mg (99.95%), Zn (99.5%), and Ca (99.5%) were used as raw materials for CP ZX50. Mg and the alloying elements were melted in an electric furnace at a temperature of approximately 690 °C, treated with $MnCl_2$ to reduce the Fe-content [16], and then cast by vertical direct chill casting on an industrial scale (billet diameter: 185 mm) at a speed of approximately 1.6 mm/s with continuous water cooling. The alloy XHP ZX50 was produced using XHP Mg (99.999%), Zn (99.999%), and Ca (99.99%). The ultrahigh-purity (XHP) Mg was produced using an in-house vacuum distillation setup to reduce the impurity content of conventional-pure (CP) Mg. High-purity graphite crucibles were used to avoid any contamination [9,14]. The raw materials of XHP ZX50 were synthesized using a protective gas mixture (pure Ar with 1 vol.% of SF_6) at 750 °C. The melt was poured into a graphite crucible of approx. 150 mm in height and 55 mm in diameter. Directional solidification was achieved by cooling the bottom of the mold, which ensures cavity-free shrinkage. The CP and XHP ZX50 cast billets were both homogenized at 330 °C for 12 h, cooled with pressurized air and machined to extrusion billets. The sizes of the extrusion billets were 120 mm in diameter and 250 mm in length for CP ZX50, and 50 mm in diameter and 120 mm in length for XHP ZX50. Direct extrusion was performed at 325 °C with a ram speed of ≈0.5 mm/s for both alloys. The extruded rods had diameters of 20 mm for CP ZX50 and 8 mm for XHP ZX50, which corresponds to extrusion ratios of 36:1 and 39:1, respectively. Table 1 shows the chemical compositions of the alloys, determined by glow discharge mass spectrometry in the as-cast state.

Samples for *in vitro* tests were prepared from the extruded rods; the sample surfaces were $857 \pm 2.8\ mm^2$ for CP ZX50 and $459 \pm 12.8\ mm^2$ for XHP ZX50. These surface areas result from the different rod diameters used to ensure similar extrusion ratios. Pins for the *in vivo* tests were machined to 1.6 mm in diameter and 8 mm in length, corresponding to a total surface area of approx. 44 mm^2 and an initial volume of ~16 mm^3. The *in vitro* samples were ground using abrasive SiC paper of granularity 4000 and then polished with a 1 μm polishing cloth. The samples were cleaned in an ultrasonic bath using isopropyl alcohol and dried in hot air just before immersion. The specimens for the *in vivo* tests were cleaned in a cascade of pure ethanol in an ultrasonic bath and dried with hot air.

The microstructure analysis of the as-extruded material was performed by optical microscopy (OM) after etching with picric acid. The average grain size was determined using the linear intercept method. Elemental distribution maps of alloy microstructure and the surfaces of explanted pins were recorded using a Hitachi SU-70 scanning electron microscope (SEM, Schottky-type field emission gun), equipped with an X-Max energy dispersive X-ray (EDX) detector from Oxford instruments. The SEM was operated at 15 kV in the secondary electron (SE) and back-scattered electron (BSE) imaging modes. For SEM investigations, the pins were embedded longitudinally in Bakelite with some bony material at its surface. The embedded samples were coated with an 8-nm-thick carbon layer using a Balzers SCD 050 sputter coater.

2.2. In vitro testing

The *in vitro* degradation of each alloy was analyzed using the hydrogen evolution method [15]. Three samples per batch were tested in CO_2-buffered simulated body fluid (SBF) [17] over two weeks of immersion. The immersion tests were performed using a special setup, designed in-house, for slow-corroding material and investigated under the conditions given in Ref. [9]. This setup has the advantages of very stable pH and temperature control, and the possibility to incorporate atmospheric pressure fluctuations and gas dissolution [9]. It has already been shown to adequately simulate the *in vivo* situation of pure Mg, Y-containing Mg alloys, and other Mg–Zn–Ca-alloying systems [9,18,19]. The pH was set to 7.44 ± 0.043 with a temperature of 36.8 ± 0.34 °C, and the atmospheric pressure was 1008.72 ± 6.86 mbar during the two weeks of immersion. All data points were corrected for pressure fluctuations and gas dissolution, and the mean hydrogen amount was evaluated to ml/cm^2 [9].

2.3. In vivo testing

The animal experiments were conducted according to established principles of ethical respect for animals, and authorized by the Austrian Ministry of Science and Research (BMWF-66.010/0087-II/3b/2011). Eight male Sprague–Dawley® rats with body weights of 140–160 g and 5 weeks of age were used. Under general anesthesia each rat had two identical pins implanted in its femur shafts. An accurate description of the surgical procedure and postoperative treatment is given in Ref. [13]. Micro-computer tomography (μCT) scans were performed at four prearranged times after pin implantation: 1 week, 4 weeks, 8 weeks and 12 weeks. During the μCT examinations the animals were anesthetized using volatile isoflurane (Forane®, Abbot AG, Baar, Switzerland). The rats were scanned using a Siemens Inveon Acquisition Workplace (version 1.2.2.2). Scans were performed at 70 kV voltage, 500 μA current, and with 1000 ms exposure time. The volume of each implant was measured by 3-D morphometric analysis and quantified using the software program 'Mimics®' (Version 15.0, materialise, Leuven, Belgium); for more details see Ref. [13]. Two rats were euthanized after 2 weeks to inspect the surface topography and elemental distribution in the near-surface regions of the pins. Note that for the present study only the alloy XHP ZX50 was used for the *in vivo* testing. μCT data and explanted pins for surface topography investigations of the alloy CP ZX50 were taken from Kraus et al. [13]. Both the CP ZX50 [13] and XHP ZX50 studies were conducted according to Standard Operation Protocols (SOPs), which were established in the same working group using the same infrastructure, software and animal model. They are thus comparable.

3. Results

3.1. Microstructure

Fig. 1 shows the microstructure of the alloys tested. The grain size D of CP ZX50 is slightly smaller ($D \approx 6$ μm) than that of XHP ZX50 ($D \approx 7.5$ μm). Both alloys contain second-phase particles,

Table 1
Chemical compositions of CP ZX50 (conventional purity) and XHP ZX50 (ultrahigh-purity).

Alloy	Zn (wt.%)	Ca (wt.%)	Mn (ppm)	Si (ppm)	Fe (ppm)	Cu (ppm)	Ni (ppm)	Co (ppm)
CP ZX50	5.24	0.27	1630	440	42	9	8	8
XHP ZX50	5.26	0.31	0.8	2.9	0.5	0.09	0.05	<0.05

Please cite this article in press as: J. Hofstetter et al., Influence of trace impurities on the *in vitro* and *in vivo* degradation of biodegradable Mg–5Zn–0.3Ca alloys, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.05.004