



Element distribution in the corrosion layer and cytotoxicity of alloy Mg–10Dy during in vitro biodegradation[☆]



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ABSTRACT

The present work investigates the corrosion behaviour, the element distribution in the corrosion layer and the cytocompatibility of alloy Mg–10Dy. The corrosion experiments were performed in a cell culture medium (CCM) under cell culture conditions close to the in vivo environment. The element distribution on the surface as well as in cross-sections of the corrosion layer was investigated using scanning electron microscopy, energy-dispersive X-ray analysis, X-ray photoelectron spectroscopy and X-ray diffraction. The cytocompatibility of alloy Mg–10Dy with primary human osteoblasts was evaluated by MTT, cell adhesion and live/dead staining tests. The results show that the corrosion layer was enriched in Dy, while the P and Ca content gradually decreased from the surface to the bottom of the corrosion layer. In addition, large amounts of MgCO₃·3H₂O formed in the corrosion layer after 28 days immersion. Both extracts and the Dy-enriched corrosion layer of alloy Mg–10Dy showed no cytotoxicity to primary human osteoblasts.

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1. Introduction

A number of recent studies have emphasized the possibilities of using Mg alloys as a new class of degradable biomaterials for orthopaedic applications [1–4]. As Mg–RE (RE, rare earth) alloys have shown a good combination of mechanical and corrosion properties extensive investigations have been performed on RE-containing Mg alloys, such as WE43 [5,6] (4 wt.% yttrium, 3 wt.% RE), LAE442 (4 wt.% lithium, 4 wt.% aluminum, 2 wt.% RE) [7,8], Mg–Gd [9], Mg–Dy [10,11], Mg–Y–Zn [12,13] and Mg–Nd–Zn–Zr [14,15], for medical applications. Alloy WE43 has already been used in clinical studies [16].

It is essential to investigate corrosion layers, especially the distribution of alloying elements in the layer and their influence on the biocompatibility of Mg–RE alloys used in biomedical applications. The corrosion layer acts as the interface between Mg alloys and body fluids, as well as body tissues. It plays a very important role in determining both the corrosion behaviour and the biocompatibility of Mg alloys. Investigations have already been conducted on the corrosion layers of Mg alloys such as Mg–Mn–Zn [17], AZ31 [18] and WE43 [19]. These investigations focus on the microstructure and composition of the surface of the corrosion layer. In order

to further understand the biodegradation process and the distribution of different elements across the corrosion layer it is also necessary to investigate cross-sections of the corrosion layer. Previous investigations on cross-sections of the corrosion layer of pure Mg samples has shown that the distribution of elements is heterogeneous [20].

Dy is one of the best tolerated RE elements based on an in vitro study [21] and has a very high solubility in Mg (maximum 25.3 wt.%) [22]. A previous work on Mg–Dy alloys showed that solution heat treated alloy Mg–10Dy has a good combination of mechanical and corrosion properties [10]. Hence, solution heat treated alloy Mg–10Dy, in which all the second phase is dissolved in the Mg matrix, was selected in the present work for further investigation. The composition and element distribution on the surface and in a cross-section of the corrosion layer were investigated. Furthermore, the cytocompatibility of extracts and the corrosion layer was evaluated. As one of the potential applications of alloy Mg–10Dy is as bone fixtures, osteoblasts were used in the cytocompatibility tests.

2. Experimental procedures

2.1. Materials preparation

High purity Mg (99.94%) (MEL, UK) was melted in a mild steel crucible under a protective gas atmosphere (Ar + 2% SF₆). 10 wt.% (1.6 at.%) of pure Dy (99.5%) (Griem, Beijing, China) was added

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Table 1

Detailed composition and impurity levels of the Mg–10Dy alloy and pure Mg used in this work (wt.%).

	Dy	Fe	Ni	Cu	Mg
Mg–10Dy	9.2	0.005	<0.004	0.007	Balance
Pure Mg		0.005	<0.002	0.001	Balance

at a melt temperature of 720 °C. The melt was then mechanically stirred with a stirrer rotating at 200 rpm for 30 min. The permanent mould direct chill casting technique [23] was used to prepare ingots (20 × 12 × 6 cm). Pure Mg was prepared using the same melting process as a reference material for corrosion rate and cytocompatibility tests. Solution treatment was performed on the Mg–10Dy alloy at 520 °C for 24 h. The chemical compositions of pure Mg and the Mg–10Dy alloy were analysed using an X-ray fluorescence (XRF) analyser (Bruker AXS S4 Explorer, Germany) and spark optical emission spectroscopy (Spectrolab M9, Kleve, Germany) and are listed in Table 1.

2.2. Corrosion tests

The specimens for the corrosion tests were prepared by grinding each side of the sample with 2400 grit emery paper, followed by degreasing with ethanol and drying in air. Three samples were used for each corrosion condition.

The corrosion experiments were conducted under sterile conditions. The samples were sterilized in a solution of 70% ethanol for 15 min. After drying each sample was immersed in a cell culture medium (CCM) consisting of Dulbecco's modified Eagle's medium (DMEM) Glutamax-I (Life Technologies, Darmstadt, Germany) and 10% fetal bovine serum (FBS) (PAA Laboratories, Linz, Austria). The samples were then incubated in an incubator (Heraeus BBD 6620, Thermo Fisher Scientific, Schwerte, Germany) under cell culture conditions (37 °C, 21% O₂, 5% CO₂, 95% relative humidity). The ratio of corrosion medium to surface area of sample was 1.5 ml cm⁻². The composition of DMEM is shown in Table 2. After immersion for up to 28 days without changing the medium the samples were cleaned with distilled water, dried and kept in a vacuum drying chamber. For weight loss measurement the corrosion products of specimens after immersion for 3, 7 and 14 days were removed by immersing the corroded specimens in chromic acid solution (180 g l⁻¹) for 20 min at room temperature. The corrosion rate was calculated as millimeters per year (mm year⁻¹) using the equation:

$$CR = \frac{8.76 \times 10^4 \times \Delta g}{A \cdot t \cdot \rho} \quad (1)$$

Table 2

Composition of DMEM (mg l⁻¹).

	Concentration
<i>Inorganic salts</i>	
Calcium chloride (CaCl ₂ ·2H ₂ O)	264
Ferric nitrate (Fe(NO ₃) ₃ ·9H ₂ O)	0.1
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	200
Potassium chloride (KCl)	400
Sodium bicarbonate (NaHCO ₃)	3700
Sodium chloride (NaCl)	6400
Sodium phosphate monobasic (NaH ₂ PO ₄ ·2H ₂ O)	141
<i>Organic salts</i>	
Vitamins	35.6
Amino acids	1852
D-Glucose (dextrose)	4500
Phenol Red	15
Sodium pyruvate	110

where Δg is the weight change (g), A is the surface area (cm²), t is the immersion time (h) and ρ is the density of the alloy (g cm⁻³).

2.3. Characterization of the corrosion layer

The macro-corrosion morphology was examined using optical microscopy. Observation of the micro-corrosion morphology and analysis of the corrosion layer composition were performed using a Zeiss Ultra 55 (Carl Zeiss GmbH, Oberkochen, Germany) scanning electron microscope equipped for energy dispersive X-ray (EDX) analysis at an operating voltage of 15 keV. The thickness of the corrosion layer was measured using a coating thickness gauge (Mini-Test 600, ElectroPhysik, Germany).

A cross-section of the corroded sample (after 3 days immersion) was prepared by cutting the sample with a 30 keV gallium focused ion beam (FIB) attached to a scanning electron microscope (Auriga, Carl Zeiss GmbH, Oberkochen, Germany). To prevent damage to the corrosion layer and obtain precise cutting along the corrosion layer a layer of platinum was deposited on the surface of the corrosion layer. Observation of the morphology, line scanning and mapping of cross-sections of the corrosion layer were performed directly after cutting, operating at 15 keV.

X-ray induced photoelectron spectroscopy (XPS) measurements were carried out on a Kratos Axis Ultra DLD (Kratos Analytical Ltd., Manchester, UK) with an attached 15 keV X-ray gun using monochromatic Al K_α radiation. Samples of Mg–10Dy alloy and pure Dy metal immersed in the CCM under cell culture conditions for 24 h were used for XPS studies. Samples were cleaned with distilled water and dried under vacuum. The XPS measurements were conducted on an area of 700 × 300 μm at a pass energy of 40 eV in the regions of measurement and 160 eV for survey scans. As a result of the non-conductive nature of the corrosion products a charge neutralizer was used to correct the chemical shifts caused by charging. Argon ions (4000 eV) with an etching rate of about 40 nm min⁻¹ were used to etch the specimens in order to investigate the composition of the corrosion layer at different depths. Curve fitting of the spectra was conducted with Casa 2.3.15 software (Casa Software Ltd., Teignmouth, UK). The NIST Standard Reference Database 20, Version 3.5, database was used to obtain element binding energies.

X-ray diffraction (XRD) was used to identify the phases in the corrosion layer. The XRD measurements were carried out using a diffractometer (Siemens D5000, Germany) with Cu K_{α1} radiation (wavelength $k = 0.15406$ nm). The operating voltage and current were 40 kV and 40 mA, respectively. The step size was 0.02° with a dwell time of 3 s.

2.4. Isolation and culture of cells

2.4.1. SaoS-2 cells

To save time in the isolation and culture of primary human osteoblasts the human osteosarcoma cell line SaoS-2 (human osteoblast-like cells) was used to evaluate the cytotoxicity of Dy³⁺ ions. The human osteosarcoma cell line SaoS-2 was obtained from the European Collection of Cell Cultures (Salisbury, UK). The cells were cultured under standard cell culture conditions in McCoy's 5A medium (Life Technologies, Darmstadt, Germany) with 15% FBS. Cells were passaged at subconfluency (70–80%) and reseeded at a density of 2 × 10⁴ cells cm⁻². For cell culture experiments cells after passage 5 were used.

2.4.2. Primary human osteoblasts

Osteoblasts were grown out of bone chips from a patient undergoing total hip arthroplasty following the protocol of Gallagher [24]. The isolation protocol was approved by the local ethics committee. In brief, cancellous bone was removed from the femoral

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