In vitro degradation of ZnO flowered coated Zn-Mg alloys in simulated physiological conditions

Marta M. Alves [a,*], Tomas Prosek [b], Catarina F. Santos [a,c], Maria F. Montemor [a]

[a] *CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*
[b] *Technopark Kralupy, University of Chemistry and Technology in Prague, Zizkova 7, 278 01 Kralupy nad Vltavou, Czech Republic*
[c] *EST Setúbal, DEM, Instituto Politécnico de Setúbal, Campus IPS, 2910 Setúbal, Portugal*

ARTICLE INFO

ABSTRACT

Flowered coatings composed by ZnO crystals were successfully electrodeposited on Zn-Mg alloys. The distinct coatings morphologies were found to be dependent upon the solid interfaces distribution, with the smaller number of bigger flowers (ø 46 μm) obtained on Zn-Mg alloy containing 1 wt.% Mg (Zn-1Mg) contrasting with the higher number of smaller flowers (ø 38 μm) achieved on Zn-Mg alloy with 2 wt.% Mg (Zn-2Mg). To assess the *in vitro* behaviour of these novel resorbable materials, a detailed evaluation of the degradation behaviour, in simulated physiological conditions, was performed by electrochemical impedance spectroscopy (EIS). The opposite behaviours observed in the corrosion resistances resulted in the build-up of distinct corrosion layers. The products forming these layers, preferentially detected at the flowers, were identified and their spatial distribution disclosed by EDS and Raman spectroscopy techniques. The presence of smithsonite, simonkolleite, hydrozincite, skorpionite and hydroxyapatite were assigned to both corrosion layers. However the distinct spatial distributions depicted may impact the biocompatibility of these resorbable materials, with the bone analogue compounds (hydroxyapatite and skorpionite) depicted in-between the ZnO crystals and on the top corrosion layer of Zn-1Mg flowers clearly contrasting with the hindered layer formed at the interface of the substrate with the flowers on Zn-2Mg.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Metallic biomaterials are of utmost relevance because their resistance to mechanical stress and tension make them especially suitable for load bearing implants. Inside this class of biomaterials, bioresorbable metals and alloys are growing in interest [1]. In transient wound-healing pathologies, a biomaterial would ideally be absorbed by the organism, eliminating the need of a second surgery for implant removal. Amongst the available resorbable metallic materials, Zn has up surged as a promising candidate [2]. As a micronutrient, Zn has a tolerable upper intake of 40 mg/day, with beneficial effects in wound-healing, namely in skeletal, muscle remodelling and associated inflammatory responses [3]. In elderly, Zn supplementation was described to decrease severity and extent of common colds, blindness and mortality due to cardiovascular diseases [3,4]. Under physiological conditions, metallic Zn has been described to have adequate corrosion rates for tissue healing [2,5]. However, when considering load-bearing implants the poor mechanical strength of Zn limits its use. This drawback can be solved by alloying Zn with physiological compatible relevant elements, a strategy that results in superior strength and castability [6]. Mg, in particular, is physiologically non-toxic and supports important biological processes, as bones growth and development [7] and is well known as bioresorbable element [2,8]. Whatsoever, the addition of Mg to Zn yielded alloys with a maximum strength and elongation for Mg contents only around 1 wt.% [6,9] with *in vitro* tests confirming Zn-Mg alloys biocompatibility [9–13]. To further modulate the corrosion behaviour of Zn-Mg alloys the addition of functional coatings can be considered. The addition of ZnO flowered coatings has been reported to favoured the formation of biomimetic compounds, osteoblasts proliferation, collagen secretion and extracellular matrix mineralization [14–16]. Besides the increased biocompatibility envisaged by these coatings, the reported antimicrobial properties of ZnO flowers [14,17] could additionally present an effective strategy to overcome the world-wide increasing implant-associated infections.

Therefore, the aim of this work was to functionalize Zn-Mg alloys with ZnO flowered coatings. A detailed evaluation of the *in vitro* corrosion behaviour, using simulated physiological conditions, was performed by combining electrochemical impedance spectroscopy (EIS), Raman Spectroscopy, scanning electron microscopy (SEM) and Energy dispersive X-Ray (EDS) analyses.

2. Materials and methods

2.1. Preparation of the materials

The preparation of the alloys was previously described by Prosek et al. [18]. Briefly, Zn was melted and $ZnCl_2$ flux was added at the temperature of 450 °C. Magnesium was set in at the temperature by 50 K higher than the temperature of liquidus of each alloy. The melt was strongly homogenized by a graphite bar and poured into a mould with a quatrefoil section. After solidification, it was machined to a bar with diameter of 20 mm and cut to coin-like samples. The ZnO flowered coatings were obtained by electrodeposition, as previously described by the authors for pure Zn [19]. Briefly, Zn-1Mg and Zn-2Mg samples were mounted in cold curing epoxy resin, polished with SiC paper up to 2500 grit, washed with absolute ethanol and dried in air prior to electrodeposition. The electrodeposition was carried out in a three-electrode electrochemical cell with platinum as counter electrode, saturated calomel electrode (SCE) as reference electrode and the alloys as working electrode. The electrolyte was composed of 50 mM $Zn(NO_3)_2$ and 50 mM H_3BO_3 at pH 6. The electrodeposition was carried out at the constant cathodic potential of −1.9 V for 20 s in a Voltalab PGZ 100 potentiostat (Radiometer Analytical). The electrodeposited coupons were washed with absolute ethanol and dried at room temperature.

2.2. Electrochemical impedance spectroscopy (EIS)

The corrosion behaviour of coated alloys, mounted in the epoxy resin, was studied by submitting the samples to simulated physiological simulated conditions, using a 3-electrodes cell arrangement. A simulated body fluid (SBF) solution (137.5 mM NaCl, 4.2 mM $NaHCO_3$, 3.0 mM KCl, 1.0 mM $K_2HPO_4 \cdot 3H_2O$, 1.5 mM $MgCl_2 \cdot 6H_2O$, 2.6 mM $CaCl_2$, 0.5 mM Na_2SO_4, 50.5 mM $(HOCH_2)_3CNH_2$ at pH 7.4) [20] was used and the immersed samples were kept at 37 °C for 24 h. The EIS measurements were carried out with an AUTOLAB PGSTAT 302N by applying 10 mV perturbation. The measuring frequency ranged from 10^5 Hz down to 10^{-2} Hz. The EIS spectra were periodically recorded at open circuit potential (OCP). A three-electrode electrochemical cell, with a ZnO flowered coated sample as working electrode, SCE as reference electrode and a platinum coil as counter electrode was used. The EIS data analyses, the equivalent circuit modelling and corresponding elements values were calculated with Zview software.

2.3. Physico-chemical characterization

The morphologies of the prepared surfaces and corrosion products formed during immersion in SBF were analysed by scanning electron microscopy (SEM), using Hitachi S2400 apparatus, or by FEG-SEM, using a JEOL-JSM7001F apparatus, and the elemental chemical composition by the respective energy dispersive X-ray (EDS). Raman spectra and maps were collected (Horiba LabRAM HR800 Evolution) using the radiation source with a solid-state laser operating at 532 nm with an output power of 20 mW. A spectrograph with a 600 lines/mm grating was used. A 50 or 100 times objective lens focused the laser beam on the samples surface. Spectra were obtained with acquisition time of 10 s and 10 accumulations.

3. Results and discussion

3.1. Electrodeposition of flower-like ZnO coatings

Coating surfaces with ZnO flowers has been proposed as an expedite route to functionalise metallic materials [14,19]. In this work flowered coatings were successfully prepared by electrodepositing ZnO on Zn-Mg alloys.

As depicted in Fig. 1, distinct ZnO flowered coatings were obtained on Zn-1Mg and Zn-2Mg. A low density of larger flowers grew on Zn-1Mg whereas a higher density of smaller flowers was observed on Zn-2Mg. These results revealed that the flowers density was inversely related to their diameter. These flowers, composed by several branches from where diverse ramifications emerge, are the result of the assembly of several small ZnO lamina-like subunits. Depending on the alloys, distinct flower morphologies were obtained: on Zn-1Mg the ZnO flowers presented thicker branches and average diameters of 45.9 ± 0.8 μm whereas on Zn-2Mg the thinner branched flowers had average diameters of 38.2 ± 0.3 μm. The distinct flowers' distributions and morphologies on the alloys' surfaces were investigated by the current-transient plots obtained along the electrodeposition time.

As depicted in Fig. 2, the current-transient curves show that a lower current density is required to produce the flowered coatings on Zn-1Mg than on Zn-2Mg. In the early beginning of electrodeposition an increased current density can be related with double layer discharge events. On Zn-1Mg a slight current density increase was clearly noticed whereas for Zn-2Mg this effect was much attenuated. After that stage, a decrease in current density can be associated to the nucleation step. On Zn-1Mg a decreased current density occurred until reaching another plateau after *c.a.* 5 s while for Zn-2Mg a sharper decrease occurred with the stable plateau starting at around 2 s. During this flower seeding process the slight decrease in current density observed for Zn-1Mg suggested a low nucleation density, when compared with that of Zn-2Mg, where a higher nucleation density is related with the sharp current density decrease. These findings are associated with the number of flowers present on the alloys surfaces (Fig. 1). Thereafter a less pronounced decreasing trend in the current density was associated to flowers growth. This trend, more pronounced on Zn-2Mg than on Zn-1Mg, is in agreement with the previously reported formation of nanostructured ZnO flowers on pure Zn [19]. Although having small differences in the Mg composition, the impact on the current density distribution on Zn-1Mg and Zn-2Mg was enough to yield distinct ZnO flowered coatings.

To confirm the presence of ZnO as part of the flowers composition, EDS maps and Raman spectra of the flowered coating were carried out. As depicted in Fig. 3, the EDS maps revealed that the presence of Zn and oxygen could be assigned to the electrodeposited flowers (Fig. 3a), with the higher intense spots observed in the oxygen maps being speculated to be part of a $Zn(OH)_2$ veil as suggested by Wadowska et al. [21].

Magnesium EDS maps revealed the phase distribution typical of these alloys: $Zn + Mg_2Zn_{11}$ eutectic interdendritic areas and Zn dendrites, as reported elsewhere [18]. The comparison of the magnesium EDS map with the corresponding SEM images depicted the localization of the nucleation points. As outlined in Fig. 3a the nucleation points occurred preferentially in the solid interfaces with the presence of fewer interfaces in Zn-1Mg being consistent with the lower number of flowers formed on this alloy (Fig. 1). Conversely, the higher number of interfaces is in agreement with the high number of flowers formed on Zn-2Mg surface (Fig. 1). As depicted in Fig. 3b, the Raman spectra collected from flowers revealed the presence of E_{2H}–E_{2L} (318 cm^{-1}), E_{2H} (431 cm^{-1}), 2LA (~507 and 533 cm^{-1}), $2B_{1L}$ (533 cm^{-1}) and A_{1LO} (571 cm^{-1}) vibrational modes, typical assigned to ZnO [22]. The strong peak corresponding to E_{2H} photon mode, and related weaker multi-phonon mode (E_{2H}–E_{2L}), were assigned to wurtzite ZnO crystal [19, 23]. The presence of A_{1LO} phonon mode, with weak intensity, suggested the presence of well-ordered ZnO crystals. These results evidenced that flower units were formed by wurtzite and well-organized ZnO crystals on both alloys. The additional peak detected at 550 cm^{-1} in the substrate has been previously ascribed to the surface optical (SO) phonon vibrations. This may result from disorderly arranged areas or boundaries between alloys surface and deposited and/or disordered regions of ZnO crystals [22]. Despite the solid phase distribution in the Zn alloys being a crucial factor for flowers distribution and morphology, as previously discussed, the presence of small amounts of Mg alloying Zn did not to modify the chemical nature of the ZnO crystals units.