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Biofunctional composite coating architectures based on polycaprolactone and nanohydroxyapatite for controlled corrosion activity and enhanced biocompatibility of magnesium AZ31 alloy

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ABSTRACT

In this work a biofunctional composite coating architecture for controlled corrosion activity and enhanced cellular adhesion of AZ31 Mg alloys is proposed. The composite coating consists of a polycaprolactone (PCL) matrix modified with nanohydroxyapatite (HA) applied over a nanometric layer of polyetherimide (PEI).
The protective properties of the coating were studied by electrochemical impedance spectroscopy (EIS), a non-disturbing technique, and the coating morphology was investigated by field emission scanning electron microscopy (FE-SEM).
The results show that the composite coating protects the AZ31 substrate. The barrier properties of the coating can be optimized by changing the PCL concentration. The presence of nanohydroxyapatite particles influences the coating morphology and decreases the corrosion resistance.
The biocompatibility was assessed by studying the response of osteoblastic cells on coated samples through resazurin assay, confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The results show that the polycaprolactone to hydroxyapatite ratio affects the cell behavior and that the presence of hydroxyapatite induces high osteoblastic differentiation.

1. Introduction

Metallic materials currently used as orthopedic implants include stainless steel (biomedical grades), titanium and its alloys, and cobalt and chromium-based alloys [1–3]. Recently, bioresorbable Mg alloys have been extensively discussed as alternative metallic materials for applications that require temporary implants. Slow dissolution of Mg implants may eliminate the need of a second surgery for implant removal. The Mg ions that are released in the process are used in the regular metabolism and, to date, no critical toxic limits have been reported for magnesium ions, which are the fourth most abundant ions in the human body [1,4–6]. On the other hand, magnesium alloys have elastic modulus and density values similar to those of the bone. These properties create better load bearing mechanical properties, without stress shielding effects that are commonly observed in some conventional metallic materials such as stainless steel [7]. Thus, Mg alloys have been classified as bioresorbable implants.

The major drawback related to the use of Mg alloys is the high reactivity of Mg in the presence of moisture. In fact, in aqueous solutions, Mg alloys corrode very fast, releasing hydrogen (H_2) gas and creating local alkalization [8,9]. These events affect cellular adhesion and cell growth. The fast degradation rate can also result in early failure of the implant before it can complete the required temporary function [7,10,11]. In order to overcome these drawbacks, one possible solution is the application of protective coatings with a twofold objective: (i) controlling the corrosion rate and (ii) enhancing biocompatibility of the healing tissues [12,13].

Various surface modification strategies have been proposed for corrosion protection of bioresorbable Mg implants. These include conversion coatings [14,15], sol–gel coatings [15], chemical deposition [16,17], micro-arc oxidation [18–20], hydroxyapatite coatings [21,22] and organic coatings [23]. For example, fluoride-based surface treatments of Mg alloys enhance bone regeneration [24,25] and delay the corrosion process as a result of MgF_2 formation but do not provide long term corrosion protection, so early failure of the implant may occur [26]. On the other hand, some coatings can increase the corrosion resistance in such an extent that the degradation process becomes too slow for a temporary implant [15,27]. Another problem has been identified for thick ceramic coatings that can develop early cracks and mechanical failures [28].

More complex biocompatible coating architectures, like multilayered coatings, can be designed to control the corrosion activity, in parallel with introduction of other functionalities, such as drug

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delivery ability, increased biocompatibility and anti-bacterial effects [29–31]. Thus, this work reports the fabrication and testing of an advanced biointerface for controlled corrosion rate of the Mg alloy AZ31. The work focuses on the evaluation of the corrosion resistance of coated AZ31 alloys and on the assessment of the coating biocompatibility. The composite coating consists of a polycaprolactone (PCL) matrix modified with nanohydroxyapatite, applied over a very thin layer of polyetherimide (PEI), which in turn, was applied over hydrofluoric acid (HF) pre-treated AZ31 alloy. PCL is used in research and clinical applications and is approved by the Food and Drug Administration (FDA) as a biodegradable polymer [32]. Polycaprolactone is characterized by a slow degradation rate under physiological media [33], being used for example to store nanoparticles or for drug delivery. However, its use as coating for corrosion protection of bioresorbable magnesium alloys has been little explored because it lacks adhesion to Mg substrates. In this work this problem was overcame by applying an inner polyetherimide (PEI) layer with a nanometric thickness that acts as an adhesion promoter between the AZ31 and the PCL coating. On previous studies, PEI was shown to exhibit an excellent osteoblastic cytocompatibility [23].

The corrosion protection of the composite multilayered coating was studied by electrochemical impedance spectroscopy, a non-disturbing technique, which allows long-term monitoring of the coating performance. The morphological features were investigated by scanning electron microscopy. The osteoblastic cell response of the composite coating was assessed by using the MG63 cell line.

The results show that the PCL composite coating architecture can be tailored for controlling the corrosion rate of the substrate. The corrosion protective performance depends upon the content of PCL and hydroxyapatite particles. The results also demonstrate that the presence of hydroxyapatite particles induces higher osteoblastic differentiation. Moreover, the cell differentiation process was sensitive to the corrosion activity of the coated alloy.

2. Materials and methods

2.1. Substrate preparation

AZ31 magnesium alloy was supplied by Goodfellow, Inc. The nominal composition of the alloy is depicted in Table 1.

The samples consisted of flat coupons having dimensions of 30 × 25 × 2 mm that were progressively polished up to 2100 grit SiC paper. The coupons were degreased in alcohol and pre-treated by immersion in a 12% HF solution for 1 h.

The coating system was composed of two layers applied in separated steps. The first polymeric layer was synthesized by mixing PEI in N,N-dimethylacetamide (DMAc), as solvent, in concentration of 2.5 wt.%. The coating was applied by dip coating and the coated samples were cured in an oven at 150 °C for 2 h.

The second polymeric layer was prepared by dissolving PCL granules (Mn 70,000–90,000 Da) in chloroform, under magnetic stirring for 2 h. Nanohydroxyapatite particles were added to the obtained solution. Details on the coating composition are depicted in Table 2.

The PCL coatings were applied over the PEI-coated metallic coupons by dip coating.

2.2. Corrosion tests

Electrochemical impedance spectroscopy (EIS) measurements were carried out for different immersion times in Hank's solution with an initial of pH 7.4; the chemical composition of this electrolyte being found elsewhere [15], at room temperature (25 °C), using an AUTOLAB PGSTAT 302N. A three-electrode electrochemical cell was placed inside a Faraday cage. The cell consisted of the coated AZ31 working electrode (3.1 cm^2 of exposed area), a saturated calomel electrode as reference and a platinum coil as counter electrode. The spectra were taken by sweeping the frequency from 10^5 Hz down to 10^{-2} Hz with 10 mV (RMS) amplitude. All the experiments were performed at the open circuit potential using at least two replicates.

The pH of the Hank's solution was controlled during the experiment and the solution was regularly replaced with fresh Hank's solution.

The morphological features of the coated AZ31 coupons were studied by a field emission gun scanning electron microscope (FEG-SEM) using a JEOL-JSM7001F apparatus.

Table 1
Nominal chemical composition of AZ31 magnesium alloys.

Elements	Aluminum (Al)	Zinc (Zn)	Manganese (Mn)	Magnesium (Mg)
Contents	2.5–3.5%	0.7–1.3%	0.2–1%	Balanced

Table 2
Chemical composition of the coating applied on AZ31, including the composition of polycaprolactone (PCL) and hydroxyapatite (HA).

PEI (wt.%)	PCL (wt.%)	HA (wt.%)
2.5	–	–
2.5	5	–
2.5	10	–
2.5	5	5
2.5	10	5

2.3. Osteoblastic cytocompatibility studies

MG63 osteoblastic-like cells (ATCC) were cultured (37 °C, 5% CO_2/air, humidified atmosphere) in α-MEM containing 10% fetal bovine serum, 50 μg/ml ascorbic acid, 100 IU/ml penicillin, 2.5 μg/ml streptomycin and 2.5 μg/ml amphotericin B. At about 70–80% confluence, cells were enzymatically detached (0.05% trypsin and 0.5 mM EDTA) and re-suspended in the culture medium. The cell suspension was used to test the cell response of the coated specimens. The following coating compositions were tested: 5% PCL, 5% PCL–5% HA and 10% PCL–5% HA (see Table 2). Two sets of experiments were performed. In the first one, the cells were seeded ($5 \times 10^4/cm^2$) onto the coatings applied over glass coverslips, in order to evaluate cell behavior without interference of the bare magnesium alloy. In the second set of experiments, cells were seeded ($5 \times 10^4/cm^2$) on the same coating applied over the AZ31 Mg alloy. Cultures were maintained for 1 and 5 days.

Coated samples (glass coverslips and AZ31 alloy) were characterized for cell viability/proliferation (resazurin assay) at days 1 and 5, and for alkaline phosphatase (ALP) activity at day 5. In these assays, material samples without seeded cells were incubated in the same experimental conditions as the seeded materials, to be used as blank controls, in order to account for the possibility of interference of the released Mg ions in this analysis [6]. Coated glass samples were observed by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

2.4. Resazurin assay

In the resazurin assay, a non-fluorescent blue component is reduced by the living cells to a pink fluorescent component. At each time-point (day 1 and day 5), the culture medium was removed, and fresh medium with 10% (v/v) of resazurin was added to the cells. Cultures were incubated at 37 °C in humidified atmosphere of 95% air and 5% CO_2 for 3 h. Then, 100 μl was transferred to a 96-well plate and the fluorescence intensity was measured in a microplate reader (Synergy HT, BioTek, USA) at 535 nm excitation wavelength and 590 nm emission wavelength. The results were expressed in relative fluorescence units.

2.5. Alkaline phosphatase (ALP) activity

ALP activity was evaluated in cell lysates (0.1% Triton X-100, 5 min) by the hydrolysis of *p*-nitrophenyl phosphate in alkaline buffer solution