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Degradation and cytotoxicity of lotus-type porous pure magnesium as potential tissue engineering scaffold material

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ABSTRACT

The lotus-type porous pure magnesium was prepared using a metal/gas eutectic unidirectional solidification method (GASAR process). The corrosion behavior, decay of mechanical property and the cytocompatibility were evaluated with the compact pure Mg as control. The porous pure Mg indicates better corrosion resistance than that of compact pure Mg in SBF at 37 °C. The compressive yield strength of compact and porous pure Mg is (110.3 ± 8.5) MPa and (23.9 ± 4.9) MPa before immersion test, and porous pure Mg exhibits slower decay in compressive yield strength with the extension of immersion period than that of compact pure Mg. With larger exposed surface area, porous pure Mg shows higher Mg concentration in the extract than that of compact pure Mg, which leads to a higher osmotic pressure to cells and might affect its indirect cytotoxicity assay result, but is still within the Grade I RGR value (no toxicity), implying the feasibility as potential tissue engineering scaffold.

1. Introduction

Magnesium alloys have been a new class of degradable biomaterials with low densities and unique combination of good mechanical properties, biocompatibilities and biodegradation properties [1]. Porous Mg alloys maybe promising for bone tissue engineering application, especially at the load bearing area compared with the polymer or ceramic scaffolds [2], due to their good mechanical property and porous structure similar to natural bone. The porous structure also offers opportunities for the invasion of cells, the formation of blood vessels and eventually replacement by newly formed bone after gradual degradation and absorption of the magnesium ion. Recently Witte et al. [3] reported that the degrading AZ91D scaffold induced extended peri-implant bone remodeling with a good biocompatibility.

Many methods have been developed to prepare porous magnesium, such as infiltration process [4], powder metallurgy [5] and negative salt-pattern molding process [3]. For biomedical purpose, the residual spacer holder or salt inside the porous materials may impair their biocompatibility. In this study, the lotus-type porous Mg, with the average pore size of 170 μm was designed to allow well ingrowth of cells and easy transportation of the body fluid, was fabricated by metal/gas unidirectional solidification under pressurized hydrogen without other operative additions by the present authors [6]. The biodegradation behavior was systematically investigated, with the associated mechanical property decay and the influence of corrosion products on cell models being characterized to explore its feasibility as bone tissue engineering scaffold material.

2. Material and methods

99.9 wt.% magnesium was melted in the crucible in vacuum, and high pressure hydrogen was introduced into the chamber with the hydrogen pressure 1 MPa. The melt was superheated until the dissolved hydrogen reached its saturation, then it was poured into the water-cooled copper mold below. Thus the melt was solidified unidirectionally upwards, straight pores were formed by supersaturated hydrogen and precipitated during solidification. Finally the lotus-type porous pure Mg with long cylindrical pores ordered and aligned in one direction was fabricated. The average porosity and mean pore diameter were about 28 ± 1.3% and 170 ± 19 μm respectively, as measured according to [7].

The 10 × 10 × 10 mm^3 cuboid porous Mg and the same master compact pure Mg samples, with the surfaces being mechanically polished up to 2000 grit, were immersed in 150 ml SBF [8] at 37 °C. At different immersion time points, samples were taken out, cleaned with boiled CrO_3 solution and observed by environmental scanning electron microscopy (ESEM, Quanta 200FEG). The change of pH value of immersion solution and H_2 evolution during the corrosion process were monitored, with the same method described in our previous work [1]. The uniaxial compression testing of experimental samples

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after different immersion times was conducted with an Instron 8562 testing machine at a constant nominal strain rate of $2\times10^{-4}s^{-1}$ at room temperature to measure the loss of mechanical integrity during corrosion procedure. An average of three measurements was taken for each test.

The indirect cell experiments were carried out using murine fibroblast cells (L-929 cell). L-929 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The extract was prepared using DMEM with the mass of solution ratio 0.2 g/ml for 72 h. The supernatant fluid was withdrawn, centrifuged and refrigerated at 4 °C before cytotoxicity test. Cells were seeded in 96-well flat-bottomed cell culture plates at 5×10^4 cells/ml and incubated for 24 h to allow attachment. The medium was then replaced with the extract and incubated for 1, 3 and 5 days. 10 μl MTT was added to each well and incubated at 37 °C for 4 h. After that, 100 μl formazan solubilization solution was added overnight in the incubator. The spectrophotometrical absorbance was measured by microplate reader (Bio-RAD680) at 570 nm with a reference wavelength of 630 nm. The control groups involved the use of DMEM medium as negative control and 10% DMSO DMEM medium as positive controls. The pH value and Mg concentrations in extracts were also measured.

3. Results and discussion

Fig. 1(a) shows the change of hydrogen evolution rate during the immersion period. It can be seen that the hydrogen evolved quickly and exhibited a much slower evolution rate after 60 h of immersion. After 250 h of immersion period, the compact pure Mg samples lost nearly 60% weight whereas the porous pure Mg samples lost about 10% weight, as shown in Fig. 1(b). That is to say, the porous pure Mg exhibits lower corrosion rate than that of the compact pure Mg. Fig. 1(c) indicates that the pH value rapidly increases in the first 72 h and stabilizes afterwards, for both compact and porous pure Mg groups.

The SEM morphologies of compact pure Mg, cross section and longitudinal section of lotus-type porous pure Mg before and after different immersion periods are shown in Fig. 2. The compact pure Mg exhibits a flat surface with some micro-pores enveloped in the corrosion product film after 1 day of immersion and indicates serious local corrosion after 4 and 7 days of immersion. The porous pure Mg sample maintains its porous structure during 7 days of immersion and local corrosion phenomena were observed for the porous samples with some pores merged into bigger ones, marked in Fig. 2(h,k).

Fig. 3(a) demonstrates the influence of biocorrosion on the compressive yield strength of compact and porous pure Mg after different immersion periods. According to Gibson–Ashby model, the relationship between compressive strength and the relative density is given as $\sigma_{p1}/\sigma_{ys}=C(\rho/\rho_s)^{3/2}$, here σ_{p1} and σ_{ys} are the compressive yield strength of porous and compact pure Mg; C is a constant of 0.3 from the data of cellular metals and polymers [5]. The compressive yield strength of porous pure Mg (23.9 ± 4.9 MPa) before the immersion test is close to the calculated data (20.3 ± 1.6 MPa), indicating higher strength than that of natural cancellous bone (4.5 ± 1.9 MPa) [9]. Compact pure Mg exhibits a large decrease of compressive strength after 1 day of immersion and slower reduction afterwards. It is due to compact pure Mg that suffers severe local corrosion (Fig. 2(d,g,j)) and results in stress concentration in the surface defects. Similarly, Zhang et al. [10] reported a rapid decrease of bending strength of Mg-6Zn alloy in the early corrosion stage. However, porous pure Mg, with the porous surface feature, maybe not as sensitive to the surface defects as compact pure Mg. On the other hand, its relative lower corrosion rate and even corrosion morphology may also contribute to the slower reduction of compressive strength for porous pure Mg.

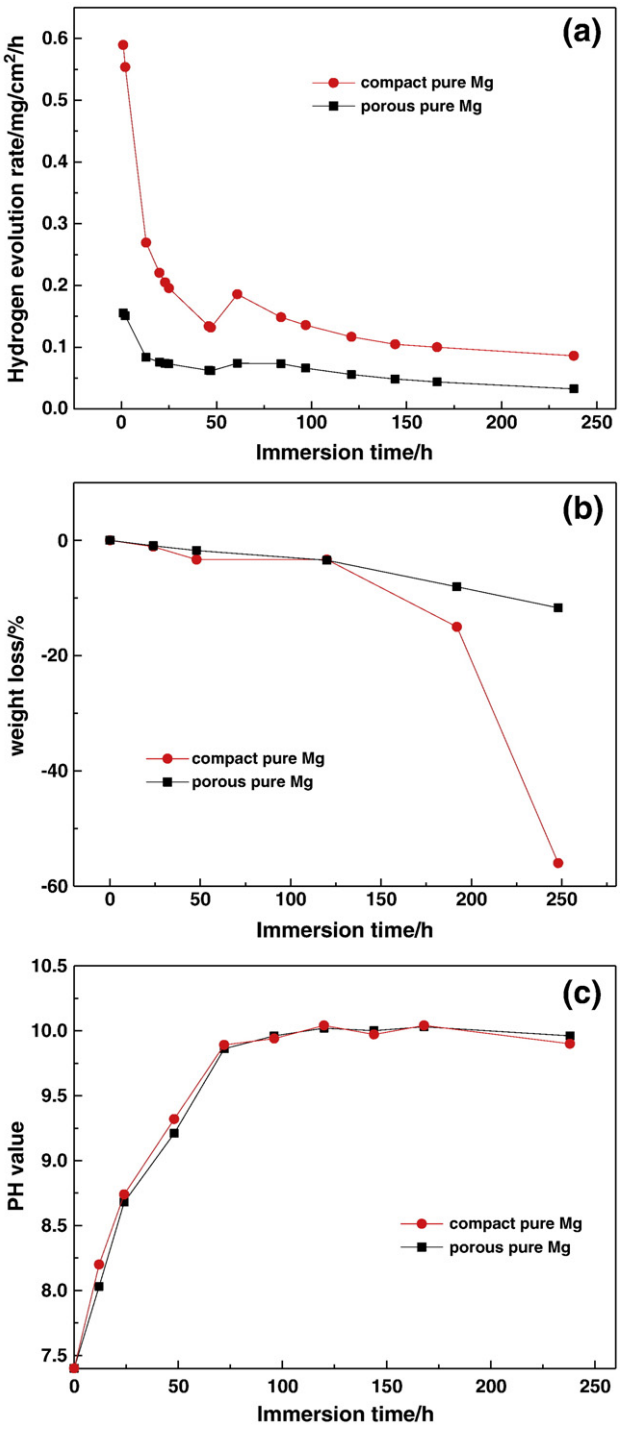

Fig. 1. (a) The hydrogen evolution rate, (b) the weight loss and (c) the change of pH values of SBF incubating compact pure Mg and porous pure Mg as a function of immersion time.

Fig. 3(b) shows the results of the indirect cytotoxicity test for compact and porous pure Mg after 1, 3 and 5 days of culture. The pH value of compact and porous pure Mg extract is (8.70 ± 0.62) and (8.74 ± 0.85). The Mg concentration in compact and porous pure Mg extract is measured to be (203.6 ± 29.2) μg/ml and (256 ± 31.8) μg/ml. The higher Mg concentration in porous pure Mg extract is attributed to the much larger exposed area to extract. The higher Mg concentration may lead to a higher osmotic pressure to cells and thus influence the viability of cells [11]. Though the porous pure Mg