Supporting Information *for*

Formation and *In vitro* evaluation of a deep eutectic solvent conversion film on biodegradable magnesium alloy

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1. Experimental

1.1. Materials and chemicals

The LiCl–urea (LU) DES system was prepared by stirring a mixture of 18.55 g lithium chloride (LiCl, AR, ⩾98.0%) and 131.45 g urea ([$CO(NH_2)_2$], AR, ⩾99.0%) in a beaker. Then the mixture was heated to 80 °C for about 60 minutes until a clear, colorless liquid was acquired. The substrates were pure Mg (~99.8% Mg) and AZ31B Mg alloy (~95.7% Mg, ~2.9% Al, ~0.8% Zn, ~0.6% Mn) sheets with a size of 30 mm×17 mm×1 mm.

1.2. Preparation of conversion coatings

The conversion coating was prepared by a heat-treatment process in LU-DES. Firstly, substrates were polished by using SiC papers, and then samples were placed in autoclaves (100 mL) with DES (45 ml) at 200 °C for 30 min. The surfaces of samples were rinsed and dried entirely. Finally, the DES-treated Mg and AZ31B samples were named as LU-Mg and LU-AZ, respectively.

1.3. Surface characterization

A field emission scanning electron microscope (FE-SEM, Hitachi SU-70) was adopted to characterize the surface morphology and chemical composition of the conversion films. Crystalline structure of conversion films was analyzed by high-resolution transmission electron microscope (HRTEM, Tecnai-F200) with energy dispersive X-ray spectrometer (EDS) attachment and X-ray diffraction (XRD, XPert Pro-MPD with CuKα radiation). The cross section of samples for TEM observations was prepared by focused ion beam (FIB, QUANTA 3D FEG). A protection layer of Pt with 1–2 μm was deposited on the target area before preparation. Surface chemical valence state was characterized by X-ray photoelectron

spectroscopy (XPS, AXIS UTLTRADLD) with monochromatic AlK_{α} radiation. XPS spectra were referred to the C1s peak at 284.6 eV. The thermogravimetric differential scanning calorimeter-Mass spectrometry (TG-DSC-MS) experiment was performed on Netzsch 209 F1 in nitrogen. The sample weights about 4 mg, and is heated to 350 °C at a heating rate of 1 °C/min. Mass spectrometers were used to analyze the gas products produced by the reaction, and then analyze the reaction mechanism.

1.4. Corrosion resistance tests

1.4.1 Potentiodynamic polarization measurement

The corrosion resistance of the conversion films was evaluated by potentiodynamic polarization measurement in a Hank's solution at 37±0.5°C. The electrochemical measurement was conducted by a three-electrode cell (Chenhua Instruments Inc., China) with Pt as counter electrode and Ag/AgCl (saturated KCl) as reference electrode at a scan rate of 1 mV s^{-1}. At least 15 min is needed for each sample (area of ~1 cm^2) to be exposed in the solution for to stabilize the OCP before test. From the polarization curves, corrosion potential (E_{corr}) and corrosion current density (i_{corr}) were derived.

1.4.2 pH measurement

Firstly, the sample weight was measured and recorded with an electronic microbalance. Then the sample (area of 6 cm^2) was placed into 50 mL centrifuge tubes with 4.8 mL Hank's solution at 37±0.5°C. The solution is changed once a day. The pH value of the solution in each tube was tested and recorded every other day for 25 days. After 25 days, samples were cleaned with chromic acid solution (200 g/L),[1] distilled water, and alcohol for 10 minutes to compute the mass loss, which was calculated according to the following relation:

$$\text{Mass loss} = \frac{(m_0 - m_1)}{S} \quad \text{(S1)},$$

where m_0 and m_1 mean the masses of samples before and after treatment. S is the surface area of samples.[2]

1.5 In vitro biocompatibility evaluation

1.5.1 Hemolysis tests

Hemolysis tests of the Mg and AZ31B alloy substrates with and without conversion films were carried out according to ISO 10993-4: 2002.[3] The hemolysis procedures were approved by the Ethics Committee of Zhejiang University beforehand and were performed in strict accordance with institutional guidelines.[4] Samples were placed into sterile centrifuge tubes with PBS solution at 37±0.5 °C incubator for 30 min. Samples were placed into distilled water as the positive control. In contrast, samples were placed into PBS solution as negative control. The sample surface area/extraction medium volume equals 1.25:1 $cm^2\ mL^{-1}$. 10 mL venous blood from healthy volunteers was collected with heparin-containing blood collection tubes. Anticoagulant and PBS solution were mixed after autoclaving at a volume ratio of 4:5. Then centrifuge tubes were filled by 4.8 mL diluted blood and continually cultured at 37 °C for 60 min and 24 h. After this, the samples were centrifuged at 3000 r/min for 5 min. Then a 96-well plate was pipetted by 150 μL of the supernatant. A microplate photometer (Multiskan MK3, Thermo Scientific, USA) was adpoted to measure absorbance values at a wavelength of 545 nm. The hemolysis percentage was calculated according to the following formula:[4]

$$\text{Hemolysis percentages} = \frac{A_S - A_N}{A_P - A_N} \quad \text{(S2)},$$

where A_S is the absorbency of the samples; A_P and A_N are the absorbency of the positive

control and the negative control, respectively.

1.5.2 *In vitro* cytotoxicity evaluation

In vitro evaluation was performed by an indirect method according to description of the paper.[5] The main investigation is the effect of extract on cell growth. Cell viability assay was carried out using the CCK-8 kit and MC3T3-E1 pre-osteoblast cells (mouse cell lines, MC3T3-E1). Firstly, the MC3T3-E1 was disposed with 0.25% EDTA trypsin and centrifuged. Then the cell was inoculated in a 96-well plate at a cell density of 5×10^3 cells/mL, and cultured for 24 h. According to ISO 10993-5: 2009,[6] the preparation of extract is immersing the substrates with and without conversion films in the 50 mL centrifuge tubes for 72 h at a ratio of 1.25 cm^2 mL^{-1}. The diluted extracts were added into the original medium of cells, and then incubated for 1, 3, and 5 d. After incubation for the corresponding time, 10 μL CCK-8 solution was added to each well, wobbled carefully and sequentially incubated for 2 h in a 37 ^oC incubator. In the experiment, a microplate reader (Thermo, Multiskan go) was used to detect optical density of samples. Relative cell growth rate of the MC3T3-E1 of samples was calculated according to the following formula:[4]

$$\text{Relative cell growth rate}(\%) = \frac{A_t}{A_c} \times 100\% \quad \text{(S3)},$$

where the absorbance of samples and control groups is represented by A_t and A_c, respectively.

1.5.3 Antibacterial assays

Antibacterial properties of samples were evaluated using *Staphylococcus aureus* (*S. aureus*) as model bacteria. Firstly, S. aureus activated colonies was acquired by using the burned bacteria inoculation ring, and then inoculated into medium. Afterwards, the bacterial

solution was diluted to a concentration of 5.0×10^5 cfu/mL with PBS solution.[4] The sterilized samples were immersed in bacterial suspension and cultured for 6 h, 24 h, and 72 h, respectively. After reaching the prescribed incubation time, each group of bacterial suspension was diluted with physiological saline to 5.0×10^2 cfu/mL, and 50 μL diluted bacterial suspension was coated on a blood plate overnight. The number of colonies on each blood plate was counted after taking pictures to evaluate the sterilizing ability of the material.

1.5.4 Cytoskeleton staining experiment

MC3T3-E1 cells in logarithmic growth phase were inoculated into a 24-well plate at a cell density of 1×10^4 cells/mL, and cultured in a cell incubator at 37 °C for 24 h at 5% CO_2. The extract solution of the sample was obtained by the same procedure with the cytotoxicity evaluation. Then extracts and 500 μL of 10% fetal bovine serum were added into each well. An equal amount of 10% fetal bovine and Alpha-MEM medium were added into the control group. After cultured in the incubator for 12 hours, the plate was washed with PBS solution. Then 500 μL of 4% paraformaldehyde, Triton X-100 and rhodamine-labeled phalloidin stain, 4,6-diamidino-2-phenylindole (DAPI) and 300 μL anti-fluorescence attenuation mount were added into each well one after another. Finally, cytoskeleton staining was observed and taken pictures under an inverted fluorescence microscope.

1.5.5 Statistical analysis

In this study, statistical graphs were made with GraphPad Prism6 and data were represented with means ± standard deviations. In addition, statistical significance was tested with the Student t test. $P < 0.05$ was considered to meet the standard.

References

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