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“Petal effect”-inspired superhydrophobic and highly adhesive coating on magnesium with enhanced corrosion resistance and biocompatibility

Feng Peng^{1,2}, Donghui Wang^{1,2}, Xiaohan Ma¹, Hongqin Zhu¹, Yuqin Qiao¹ and Xuanyong Liu^{1*}

ABSTRACT With properties of complete degradation and favorable mechanical behavior, Mg and its alloys are regarded as the next generation medical metal materials. However, fast degradation and poor surface biocompatibility hinder their clinical applications. Inspired by the “petal effect”, we successfully constructed a superhydrophobic and highly adhesive coating on pure Mg *via* a simple hydrothermal treatment in a solution containing sodium oleate. The superhydrophobicity of the fabricated coating results from its flake-like micro-nanostructure and the low-surface-energy oleate group. Water droplet on the superhydrophobic coating cannot roll off even when the sample is turned upside down, owing to the sealed air-pockets and the van der Waals’ attraction at the solid-liquid interface, indicating a highly adhesive force. The chemical and mechanical stability of the superhydrophobic coating were measured. Potentiodynamic polarization and electrochemical impedance spectroscopy measurements suggest enhanced corrosion resistance of the as-prepared sample. Furthermore, cell cytotoxicity, migration and adhesion data of human umbilical vein endothelial cells (HUVECs) reveal an improved cytocompatibility of the modified surface. Finally, hemolysis assay and platelet adhesion assay suggest an improved hemocompatibility. It is believed that the facile and low-cost method can expand the new application of superhydrophobic surface with highly adhesive on Mg in biomedical fields.

Keywords: magnesium, petal-effect, superhydrophobic, corrosion resistance, biocompatibility

INTRODUCTION

Magnesium (Mg) based materials are attracting much

attention and are regarded as the next generation biomedical metal materials [1,2]. Mg is a necessary and important trace metal element within human body, playing important roles in protein and nucleic acid synthesis, as well as many other cellular functions. Originating from its low standard electrode potential (-2.37 V vs. normal hydrogen electrode), Mg can totally degrade *via* corrosion and the intermediate corrosion product can be either absorbed or metabolized in the form of Mg^{2+} . More importantly, Mg possesses appropriate mechanical strength that is close to cortical bone, which may reduce stress shielding. However, fast degradation and poor surface biocompatibility hinder its clinical application. Many efforts have been devoted to improving Mg alloys’ corrosion resistance and their biological performance *via* surface modifications, such as plasma electrolytic oxidation coating [3], apatite coating [4] and organic polymer coating [5]. However, none of the coatings mentioned above can separate the corrosive aqueous from the material. Once Mg or the degradable coating contacts with aqueous environments, the corrosion begins. Bearing this situation in mind, fabricating a superhydrophobic surface on Mg alloys could effectively reduce the contact area between the material and corrosive aqueous medium, leading to an improved corrosion resistance.

However, a superhydrophobic surface with water-repellent property is not suitable for cell adhesion. Nature is an excellent school for academic research. In 2008, inspired by the petal of red rose, Feng *et al.* [6], for the first time, defined the superhydrophobic surface with highly adhesive as “petal effect”. A water droplet on such surface

¹ State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China

² University of Chinese Academy of Sciences, Beijing 100049, China

* Corresponding author (email: xyliu@mail.sic.ac.cn)

cannot roll off even when the surface is turned upside down. Up to now, the study of superhydrophobic and highly adhesive surface is limited in the potential application of no-loss liquid transfer [7,8]. Water on such a surface is not totally repellent, but half is contact with the surface and the other is sealed with air. The contact area between solution and surface is favorable for cell adhesion. Bearing these situations in mind, constructing a superhydrophobic surface with highly adhesive on Mg alloy might achieve a balance between the corrosion resistance and cell adhesion, providing a new surface modification direction on Mg alloy for clinical application.

A rough surface and low surface energy are the two key elements for the design of superhydrophobic surface [9]. The water adhesive force of superhydrophobic surface varies with its structural characteristics [10]. A close array of micropapillae endows the surface of the petal of red rose a superhydrophobic ability with highly adhesive force. Hu *et al.* [11] regulated the adhesive force from 4.4 to 89.6 μN continuously on 1H,1H,2H,2H-perfluorooctyltriethoxysilane-modified TiO_2 nanotube arrays by changing the structural characteristics, such as tubular diameter, length and density. On the other hand, our previous study reported that nanoflake-like structure consisting of $\text{Mg}(\text{OH})_2$ and Mg-Al layered double hydroxide (LDH) could effectively enhance the corrosion resistance and biocompatibility of Mg alloy [12]. Inspired by the petal effect, if we further fabricate the surface with a low surface energy composition, the micro-flake-like structure may act as the micropapillae, resulting in the superhydrophobic surface with highly adhesive.

LDHs are made up of positively charged brucite-like layers and an interlayer region containing various anions and solvation molecules [13,14]. Thus, it is possible to bond inorganic anions with low surface energy on the surface of positively charged layer. In addition, sodium oleate (SO), usually working as a surfactant, is a low surface energy material with excellent biocompatibility [15]. Chen *et al.* [16] successfully modified ultralong hydroxyapatite nanowires with SO and obtained a superhydrophobic surface with self-cleaning and oil/water separation abilities. Considering these context, it is promising to fabricate a superhydrophobic surface with highly adhesion on Mg alloy for biomedical applications.

In this work, a superhydrophobic surface with highly adhesion was prepared on pure Mg *via* facile, low-cost, one-step hydrothermal treatment. The formation mechanism of the superhydrophobic surface was investigated. Moreover, the chemical and mechanical

stability, corrosion resistance, cytocompatibility and hemocompatibility of the superhydrophobic surface were measured.

EXPERIMENTAL SECTION

Sample preparation

The dimension of pure Mg specimens was $10\text{ mm} \times 10\text{ mm} \times 2\text{ mm}$. Before the hydrothermal treatment process, the specimens were polished with a series of SiC abrasive paper (up to 1000 grit), ultrasonically cleaned in ethyl alcohol for 10 min, and dried in the air.

The hydrothermal treatment process was performed according to our previous work [12]. In a typical process, the Mg specimens were placed in Teflon liners with different reaction solutions at 120°C . The reaction solutions contained 50 mL aluminum nitrate solution (0.02 mol L^{-1}), adding 600 μL NaOH (10 mol L^{-1}) to adjust the pH value, and different SO concentrations (0, 200, 400, 800, and 1200 $\mu\text{mol L}^{-1}$, respectively). The reaction time varied from 3 to 24 h.

Sample surface characterization

The surface and cross-sectional morphology of the samples were observed by scanning electron microscopy (SEM, Hitachi-S3400N, Hitachi, Japan), and surface elemental compositions of the samples were evaluated by energy dispersive spectrometry (EDS, IXRF-550i, IXRF SYSTEMS, USA). Meanwhile, scanning maps (Mg, O) of cross-section of LDH and LDH/SO samples were also measured by EDS.

The phase compositions of Mg, LDH and LDH/SO samples were analyzed by X-ray diffraction (XRD, D/Max, RIGAKU, Tokyo, Japan). The chemical states of surface elements were measured by X-ray photoelectron spectroscopy (XPS, PHI-5000C ESCA system PerkinElmer, USA). Fourier transform infrared (FTIR) spectra were recorded using a FTIR spectrometer (FTIR-7600, Lambda Scientific, Australia).

The water contact angles (CAs) were measured on an optical contact angle system (Model SL200A/B/D) at ambient temperature using a 3 μL deionized water droplet.

Chemical and mechanical stability

For the chemical stability evaluation, deionized water with a series of pH value (1–13) was used in the test. The pH value of deionized water was adjusted by 0.1 mol L^{-1} NaOH or HCl. The CAs of the water with different pH values on LDH/SO samples were measured as mentioned

above.

For the mechanical stability evaluation, the LDH/SO samples were dragged to move on 1000 grit SiC abrasive paper in one direction under a pressure of 2.45 kPa. The changes of the CAs with sliding distance were measured.

Electrochemical tests

The corrosion resistance of Mg, LDH and LDH/SO was evaluated by potentiodynamic polarization tests and electrochemical impedance spectroscopy (EIS) *via* a CHI760C electrochemical analyzer (Shanghai, China) in phosphate buffer saline (PBS) at 37°C. Both processes were conducted in a three-electrode electrochemical cell with a saturated calomel electrode (SCE) as the reference electrode, a graphite rod as the counter electrode and the sample with exposing area of 0.255 cm² as the working electrode. Ahead of the test, the samples were stabilized in PBS for 400 seconds. The test of potentiodynamic polarization was conducted at a scanning rate of 10 mV s⁻¹. The corrosion potential (E_{corr}), current density (i_{corr}) and corrosion resistance (R_p) were calculated according to Tafel extrapolation. For the test of EIS, the amplitude of sinusoidal perturbing was 5 mV, and the frequency varied from 100 kHz to 10 mHz. The DC voltage applied for the EIS tests was open circuit potential. The EIS results were analyzed with ZViewTM software. Both potentiodynamic polarization test and EIS tests were performed under air condition with steady air pressure and temperature.

Immersion test

The Mg, LDH and LDH/SO samples were immersed in PBS at 37°C for one month to evaluate their long-term corrosion resistance. Each sample was placed inside a tube containing 40 mL PBS. After one month, all the samples were cleaned by chromic acid solution (200 g of CrO₃ and 10 g of AgNO₃ per liter of water). The mass loss of each sample was measured by electronic balance (AL204-IC Mettler Toledo) and the surface morphology was taken by a digital camera.

Cell migration

Human umbilical vein endothelial cells (HUVECs, ScienceCell, USA) were used to evaluate the cytocompatibility (cytotoxicity, cell proliferation and adhesion) of the Mg, LDH and LDH/SO samples. The cells were cultured with endothelial cell medium (ECM) at 37°C in a humidified atmosphere of 5% CO₂ in air. Cells mentioned in this work are referred to HUVECs, if not specified. All the samples were sealed by water-proof silica gel, leaving one side for the following cell tests, and sterilized *via* ultra-

violet irradiation over 12 h.

The samples were incubated in ECM for 24 h with a sample-area/extraction-medium ratio of 0.25 cm² mL⁻¹. At the same time, 1 mL of cell suspensions with a cell density of 2×10⁵ cell/mL were added to each well of a 24-well culture plate. After 1 day, lines crossing the cell well were drawn by a 200 μL pipet tip, and the culture medium was replaced with 1 mL extraction. After cells migrating 9 h, the actin were stained with fluorescein isothiocyanate (FITC)-phalloidin (Sigma, USA) and nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Chemical International). Subsequently, the cells were observed *via* fluorescence microscopy (Olympus, Japan) and the numbers of cells migrating into the crossing line were counted.

Cytotoxicity evaluation

The extracted solution, obtained as mentioned in the previous section, was designated as 100%, and diluted to 60% and 30% with ECM. 100 μL cell suspensions with a cell density of 5×10⁴ cell/mL were added to each well of a 96-well culture plate. After 24 h, the culture medium was replaced by 100 μL extracted solution with different concentrations and cells were incubated for another one day. ECM without extract served as the control group. Cell number was tested by the alamarBlue assay (AbD Serotec Ltd, UK) according to the manufacturer's instruction. The cell viability was calculated by the following equation:

$$\text{Viability} = \frac{F_s}{F_c} \times 100\%,$$

where F_s is the fluorescence intensity of the sample group and F_c is the fluorescence intensity of the control group.

Cell adhesion with direct incubation

The cells were seeded on the samples with a density of 5 × 10⁴ cells/mL. After culturing 1, 4 and 24 h, the samples were rinsed with PBS. Then the cells were fixed, permeabilized and blocked successively by 4% paraformaldehyde (PFA) diluent, 0.1% (v/v) Triton X-100 (Amresco, USA) and 1 wt.% bovine serum albumin (BSA, Sigma, USA) respectively. Shortly afterwards, FITC-phalloidin was added to stain F-actin, and DAPI to stain nucleus. The samples were rinsed with PBS after each step. Finally, the samples were observed by confocal laser scanning microscopy (CLSM, Leica SP8, Germany).

Platelet adhesion

Platelet-rich plasma (PRP) was prepared by centrifuging (1500 rpm, 15 min) human whole blood. 50 μL PRP was

Figure 1 SEM images and (inset) water angles of the as-prepared superhydrophobic surfaces reacted for different reaction time with a SO concentration of 400 μmol L⁻¹: (a) 3, (b) 6, (c) 12, and (d) 24 h. SEM images and (inset) water angles of the as-prepared superhydrophobic surfaces reacted for 12 h with different SO concentrations: (e) 200, (f) 400, (g) 800, and (h) 1200 μmol L⁻¹. CA varies with reaction time (i) and SO concentration (j) (mean ± SD, *N* = 3).

added to the surface of the sample and incubated for 2 h at 37°C. After washing with 0.9% (*w/v*) sodium chloride solution (NaCl), the samples were fixed with 2.5% glutaraldehyde. The samples were then dehydrated in a gradual ethanol series (30, 50, 75, 90, 95, and 100% *v/v*) for 10 min, respectively. The platelets adhering on the surface of the sample were detected by SEM.

Hemolysis assay

Human venous blood was collected from healthy young volunteers. Blood (4 mL) was diluted with 0.9% (*w/v*) NaCl (5 mL). The samples were immersed in 1.5 mL 0.9% (*w/v*) NaCl for 30 min at 37°C. Untreated 0.9% (*w/v*) NaCl and distilled water were served as negative and positive controls, respectively. After that, 30 μL diluted blood was added to the samples. After incubated for another 60 min at 37°C, the solutions were centrifuged for 5 min at 3000 rpm. The optical density of the supernatant was measured at 545 nm to determine the released hemoglobin. The hemolysis rate (HR) was calculated *via* the following equation:

$$\text{HR} = \frac{\text{AS}_{545} - \text{AN}_{545}}{\text{AP}_{545} - \text{AN}_{545}} \times 100\%,$$

where AS₅₄₅ is the absorption value of the samples, AN₅₄₅ is the absorption value of the negative control, and AP₅₄₅

is the absorption value of the positive control.

Statistical analysis

Statistically significant differences (*p*) between various groups were measured using one-way analysis of variance and Tukey's multiple comparison tests on a GraphPad Prism 5 statistical software package. All the data are expressed as the mean ± standard deviation (SD). A value of *p* < 0.05 was considered to be statistically significant and denoted as "*", *p* < 0.01 was "**", and *p* < 0.001 was "***".

RESULTS AND DISCUSSION

Effect of hydrothermal treatment time and SO concentration on the morphology and wettability

Fig. 1a–d display the SEM images of the as-prepared surfaces in 400 μmol L⁻¹ SO for different time. Though reaction time varies, all the surfaces show compact nanoflake-like structure, which is in agreement with the traditional alkali heat treated surface structure [17–19]. The only difference is that with the increase of reaction time, the nanoflake-like structure grows up. The corresponding surface elemental compositions are shown in Table 1. The contents of C are almost the same, while the content of Al increases with the extension of reaction

Table 1 Surface elemental compositions of coated samples prepared in different reaction conditions measured by EDS (corresponding with figure, mean $\pm$ SD, $N = 3$)

	C (wt.%)	O (wt.%)	Mg (wt.%)	Al (wt.%)
3 h	25.95 $\pm$ 0.75	23.07 $\pm$ 0.68	49.74 $\pm$ 0.03	1.25 $\pm$ 0.06
6 h	27.08 $\pm$ 0.33	41.60 $\pm$ 0.03	29.34 $\pm$ 0.37	1.99 $\pm$ 0.07
12 h	23.43 $\pm$ 0.86	48.53 $\pm$ 0.53	25.73 $\pm$ 0.23	2.32 $\pm$ 0.11
24 h	26.43 $\pm$ 1.00	47.67 $\pm$ 0.32	23.14 $\pm$ 0.83	2.761 $\pm$ 0.15
200 $\mu\text{mol L}^{-1}$	17.59 $\pm$ 2.54	53.01 $\pm$ 1.60	27.17 $\pm$ 0.76	2.15 $\pm$ 0.19
400 $\mu\text{mol L}^{-1}$	23.43 $\pm$ 0.86	48.53 $\pm$ 0.53	25.73 $\pm$ 0.23	2.32 $\pm$ 0.11
800 $\mu\text{mol L}^{-1}$	36.500.37	39.56 $\pm$ 0.27	21.65 $\pm$ 0.29	2.30 $\pm$ 0.05
1200 $\mu\text{mol L}^{-1}$	43.46 $\pm$ 0.67	34.69 $\pm$ 0.26	19.19 $\pm$ 0.56	2.65 $\pm$ 0.15

time. It indicates that the concentration of SO group on the sample surface does not change, while the coating thickness increases with the extension of reaction time. Fig. 1e–h display the surface morphology of the as-prepared surfaces with different concentrations of SO for 12 h. The basic morphology of the sample surfaces is also compact nanoflake-like structure. With increased concentration of SO, the nanoflake-like structure becomes more intensive and smaller, implying that high SO concentration will hinder the growth of crystal. In contrast to the trend that C and Al content vary with reaction time, the C content increases with the increase of SO concentration, while Al content almost unchanged. It means that, more SO in the reaction solution will result in more SO group binding on the sample surface. However, the coating thickness does not change with SO concentration.

As shown in Fig. 1i, the CAs of all the samples reached 150°, and change slightly with the reaction time. The CA was about 140° for the reaction solution only containing 200 $\mu\text{mol L}^{-1}$ SO (Fig. 1j). Once the SO concentration reached or over 400 $\mu\text{mol L}^{-1}$, the CA was stable above 150°. It is easy to conclude that the CAs varies with the SO concentration, but not with the reaction time. In addition, the water droplet does not slide even when the LDH/SO sample is tilted vertically (Fig. 2a) or turned upside down (Fig. 2b), indicating a highly adhesive force.

Coating characteristics, formation mechanism and mechanism of the superhydrophobicity

On the basis of the analysis of the effects of reaction time and SO concentration on the morphology and wettability, an reaction time of 12 h and SO concentration of 400 $\mu\text{mol L}^{-1}$ (denoted as LDH/SO) were chosen as the optimal parameters to fabricate the superhydrophobic surface, and the control group without SO was denoted as LDH.

Fig. 2c displays the XRD patterns of the samples. In the pattern of Mg, only feature peaks of Mg are observed.

Both $\text{Mg}(\text{OH})_2$ (feature peak at 18°) and Mg-Al LDH (feature peak at 12°) are detected in the pattern of LDH sample [20,21], which is consistent with our previous study [12]. Compared with the LDH sample, there is no obvious change of the crystallinity of LDH/SO sample. According to the result of EDS (Table 1), it is definite that oleate groups exist on the surface of LDH/SO sample. Since SO is an amorphous organic matter and oleate groups only exist on the outermost of the coating, it does not show any peak in the XRD pattern of LDH/SO sample. The results indicate that the addition of SO into the reaction solution does not change the crystalline of the coating. High-resolution spectra of O 1s and Al 2p of the LDH and LDH/SO samples are shown in Fig. 3a–d. O 1s peak of both samples can be divided into two peaks centered at 530.9 and 531.9 eV corresponding to oxygen peak in hydroxyl bonding with Mg and Al. Al 2p peak of the LDH and LDH/SO samples centered at 74.2 eV is ascribed to aluminum peak bonding with hydroxyl. The results of XPS further confirm the existence of Mg-Al LDH on the treated samples. Fig. 3e shows the FTIR spectra. For the LDH and LDH/SO samples, the broad band at 3442 cm^{-1} is ascribed to the vibrations of OH^- of either the water molecules or hydroxyl groups in the brucite-like layers [22,23], while the peak at 1635 cm^{-1} is associated with the absorbed water in the interlayer of Mg-Al LDH structure [24]. The peaks at 2922 cm^{-1} and 2852 cm^{-1} are only detected in the spectra of SO powder and LDH/SO sample, attributed to the asymmetric and symmetric C–H stretching vibration of the alkyl group of oleate groups, further indicating that oleate groups are successfully anchored on the surface of LDH/SO sample. The cross-sectional morphology of LDH and LDH/SO are presented in Fig. 4. The thickness of LDH and LDH/SO samples are $3.67 \pm 0.37 \mu\text{m}$ and $1.93 \pm 0.12 \mu\text{m}$, respectively, and the results are confirmed by the corresponding scanning maps. It is worth noting that no C element was detected on the cross sectional surface of LDH/SO sam-

Figure 2 Shapes of water droplets on the surface of the LDH/SO sample with different tilt angles: 90° (a) and 180° (b). (c) XRD patterns of the Mg, LDH and LDH/SO samples. (d) SEM image and (inset) water angle of the LDH sample.

Figure 3 High-resolution spectra of O 1s of LDH (a) and LDH/SO (b), Al 2p of LDH (c) and LDH/SO (d). (e) FTIR spectra of SO, LDH and LDH/SO samples.

Figure 4 Cross-sectional morphology and scanning maps of Mg and O of LDH (a) and LDH/SO (b).

ple, demonstrating that oleate groups only exist on the outermost surface.

Without adding SO into the reaction solution, the as-obtained LDH sample shows intrinsic hydrophilicity (Fig. 2d). After adding SO, the as-obtained LDH/SO sample shows superhydrophobicity with highly adhesive force (Figs 1c and 2a, b). It is widely accepted that a rough surface morphology and a low surface energy would lead to a superhydrophobic surface [25,26]. Considering that surface morphology (Figs 1c and 2d) and crystalline phase compositions (Fig. 2c) of LDH and LDO/SO samples are similar, it can be concluded that it is the oleate groups on the surface of LDH/SO sample result in its superhydrophobicity. During the process of hydrothermal treatment, oleate groups adhere to the surface of the LDH/SO coating *via* two ways as depicted in Fig. 5a. A spot of oleate groups bind on the crystal of $\text{Mg}(\text{OH})_2$ *via* the van der Waals force and hydrogen bond [27]. At the same time, the electrostatic attraction between the positive metal hydroxide layer of Mg-Al LDH and the negative oleate groups would result in a large number of oleate groups binding on the surface of LDH/SO sample [16,28]. In order to verify this deduction, pure $\text{Mg}(\text{OH})_2$ coating and $\text{Mg}(\text{OH})_2/\text{SO}$ coating were fabricated on pure Mg (Fig. S1). In the absence of Mg-Al LDH phase, the C content of $\text{Mg}(\text{OH})_2/\text{SO}$ sample ($6.22 \pm 0.51\%$) was much less than LDH/SO ($23.43 \pm 0.86\%$) (Table S1 and Table 1), indicating less oleate groups binding on $\text{Mg}(\text{OH})_2/\text{SO}$. As a consequence, the CA of $\text{Mg}(\text{OH})_2/\text{SO}$ was only $70.3 \pm 9.5^\circ$, far lower than that of LDH/SO ($151.2 \pm 2.4^\circ$).

Fig. 5b, c illustrate the status of the drop water in contact with the LDH or LDH/SO samples. For LDH, though its surface is rough, hydroxyls on its surface leads

Figure 5 (a) Schematic illustration of oleate groups bound on $\text{Mg}(\text{OH})_2$ and Mg-Al LDH. (b, c) Schematic illustration of water in contact with the surface of LDH and LDH/SO samples.

to its hydrophilicity (Fig. 5a). Different with Cassie's state (a classic model for lotus effect, Fig. S2), LDH/SO sample shows highly adhesive force, and thus a petal effect model (Cassie impregnating wetting state) is used (Fig. 5b) [6]. The nanoflake-like surface combines with hydrophobic long-chain aliphatic groups of oleate are responsible for its superhydrophobicity [16]. The highly adhesive property results from the cooperation effect between the negative pressures induced by the volume change of sealed air pockets and the van der Waals' attraction at the solid-liquid interface [7,11].

Chemical stability and mechanical stability

As shown in Fig. 6a, though the pH value varies from 4 to 12, the CAs of water on the LDH/SO sample are stable (around 150°). When the pH value is lower than 4, the CA sharply decreases, which was resulted from the collapse of $\text{Mg}(\text{OH})_2$ and Mg-Al LDH phases in a strongly acidic environment [29]. The CA also slightly decreases at pH 13, owing to that the bond between oleate groups and Mg-Al LDH layer would be destroyed in an environment rich with Na^+ , and then some oleate groups would bond with Na^+ . The reaction equations are described as follows [30,31]:

These results indicate that the LDH/SO sample can bear a moderate environment. Since the pH value of human body is around 7.4 [32], the as-prepared surface should show superhydrophobic in human body.

Implant, especially orthopedic implant, is unavoidable to rub with the surrounding tissue. Considering that, an abrasion test was used to characterize the samples' mechanical stability, as displayed in Fig. 6b. After moving 500 mm on a 1000# SiC abrasive paper in one direction under a pressure of 2.45 kPa, the CA of the sample was still higher than 140°. During the moving process, the oleate groups in the connected section between the sample and the abrasive paper were destructed, while profiting from its nanoflake like structure, most regions were not connected with the abrasive paper. On the other hand, the SiC powder adhering on the LDH/SO sample surface was also disadvantage for its superhydrophobicity. As a consequence, the CA was slightly decreased after abrasion. Nevertheless, the LDH/SO sample shows good mechanical durability to some extent.

Corrosion resistance performance

For the clinical application of Mg, the first problem needs to be solved is its poor corrosion resistance. The polarization curves are presented in Fig. 7a and corresponding corrosion parameters are shown in Table 2. Compared with Mg, both the LDH and LDH/SO samples show significant lower free current density i_{corr} and higher corrosion potential E_{corr} , especially for the LDH/SO sample. In addition, the polarization resistance R_p of LDH sample is slightly higher than that of Mg, while R_p of the LDH/SO sample is about twenty times higher than that of Mg and LDH sample. It can be easily concluded that LDH/SO coating shows the best corrosion resistance. This result comes from the joint effect of the sealed air, which would

Figure 6 (a) CA of the LDH/SO sample as a function of pH. (b) Schematic illustration of the abrasion test and CA of the LDH/SO sample as a function of abrasion length (mean $\pm$ SD, $N = 3$).

reduce the corrosion area, and the oleate groups on its surface, which would inhibit the transportation of electrons.

EIS was also used to evaluate the protective ability of LDH and LDH/SO coating, and the results are shown in Fig. 7b, c. The LDH/SO sample exhibits remarkably larger overall impedance in the entire scanned frequency range than the other two samples. The equivalent electrical circuit model of these samples can be described as $R_s(Q_f(R_f(Q_{dl}R_{ct})))$, as drawn in Fig. 7d [5,33]. R_s represents the solution resistance. Q_f and R_f are the capacitance and resistance of the corrosion production or coating on Mg, respectively. R_{ct} and Q_{dl} are the charge transfer resistance and the constant phase element of the electrical double layer, respectively. The fitting results are listed in Table 2. It is noteworthy that R_{ct} directly refers to the impedance of the corrosion reaction. The R_{ct} value of LDH/SO is two times and five times larger than that of LDH and Mg, respectively, indicating the favorable corrosion resistance of LDH/SO coating, which is consistent with the result of polarization curves.

The results of long-term corrosion resistance are dis-

Figure 7 (a) Potentiodynamic polarization curves of Mg, LDH and LDH/SO samples in PBS at 37°C. (b) EIS results of Mg, LDH and LDH/SO samples in PBS at 37°C; the magnification of the block in (b) shown in (c). (d) Equivalent circuit of EIS fitting for Mg, LDH and LDH/SO samples.

Table 2 Free corrosion potential (E_{corr}), corrosion current densities (i_{corr}), polarization resistance (R_p) calculated according to the polarization curves and fitted results of EIS spectra of Mg, LDH and LDH/SO samples

Samples	Polarization curves			Nyquist EIS spectra				
	j_{corr} ($\mu\text{A cm}^{-2}$)	E_{corr} (V)	R_p ($\text{k}\Omega \text{ cm}^{-2}$)	R_s ($\Omega \text{ cm}^2$)	Q_f ($\mu\text{F cm}^{-2}$)	R_f ($\Omega \text{ cm}^2$)	Q_{dl} ($\mu\text{F cm}^{-2}$)	R_{ct} ($\Omega \text{ cm}^2$)
Mg	22.2	-1.54	58	23.8	0.094	51.5	76.8	415.8
LDH	14.4	-1.47	79.8	18.36	0.026	541.7	4.05	1021
LDH/SO	0.98	-1.45	1160	12.1	0.034	338.3	9.47	2004

played in Fig. 8. The average mass loss of LDH/SO sample is 21.4 mg (Fig. 8b), which is substantially lower than that of the LDH sample (26 mg) and Mg (123.3 mg), suggesting its best long-term corrosion resistance. The result is further confirmed by the corrosion morphology of the samples (Fig. 8a).

The above results demonstrate that, whether from the point of electrochemical test or directly observation of the degradation behavior, the LDH/SO sample with a super-hydrophobic surface, shows superior protective effect.

Cytocompatibility

Faster migration of endothelial cells (ECs) would stimulate faster wound healing after injury or stent implantation [34]. Scarification method is a conventional means to evaluate cell migration [35]. Here, the extraction was used

to evaluate the migration of HUVECs. As shown in Fig. 9a, only a handful of cells in Mg extraction migrated into the line. After coated with LDH, cell migration was significantly enhanced. It should be noted that cells in the LDH/SO extraction almost migrated across the line, indicating the fastest migration rate. The quantitative results of cell migration are expressed in Fig. 9b, which further confirms the qualitative results (Fig. 9a). Compared with the Mg and LDH samples, the LDH/SO sample shows the best corrosion resistance, and thus pH value and Mg^{2+} of its extraction are changed least, leading to the fastest migration rate.

For cytotoxicity evaluation, the extraction was used to evaluate the cell viability, and the results are displayed in Fig. 9c. Before diluted, both extraction of the Mg and LDH samples show obviously cytotoxicity, whose cell

Figure 8 (a) Corrosion morphology and (b) mass loss of Mg, LDH and LDH/SO samples after immersion in PBS for 30 days (mean $\pm$ SD, $N = 3$).

viability are 19.6% and 64.3%, respectively. Compared with the Mg and LDH extractions, the cell viability of LDH/SO extraction was significantly higher (84.7%). After diluted to 60%, the extraction of LDH and LDH/SO samples showed no cytotoxicity. Further diluted to 30%, the cell viability in Mg extraction sharply increased and cell proliferation was promoted. With the dilution of extractions, the pH value and Mg^{2+} concentration decreased, leading to a more suitable environment for cell proliferation. Whether diluted or not, the LDH/SO extraction showed little cytotoxicity, suggesting its excellent cytocompatibility.

Cell adhesion and spreading are critical steps for cell survival and then proliferation on substrates. Fig. 10 shows the initial cell adhesion process of HUVECs seeded on the samples. After cultured for 1 h, the expression of filamentous F-actin for cells on the LDH and LDH/SO samples was better than that on the Mg sample. After cultured for 4 h, the cells on the Mg sample remained their round morphology, while cells on the LDH and LDH/SO samples showed polygon shape. SEM morphology confirms the phenomenon (Fig. S3). After 24 h of incubation, few cells were detected on the Mg sample. Cells on the LDH/SO sample displayed the same spreading degree with that on the LDH sample, and there were more cells adhered on the surface of LDH/SO sample. These results indicate that superhydrophobic

Figure 9 (a) Migration of HUVECs after incubation 9 h in extractions of Mg, LDH and LDH/SO samples (the scratch area is between the two yellow dotted lines) and (b) numbers of cells migrating into the blank area (mean $\pm$ SD, $N = 3$). (c) Viability of HUVECs after incubation for 1 day in different concentrations of extractions of Mg, LDH and LDH/SO samples.

Figure 10 Cell adhesion of HUVECs after cultured on Mg, LDH and LDH/SO samples for 1, 4 and 24 h, actin stained with FITC (green) and nucleus stained with DAPI.

surface of the LDH/SO sample would not influence the cells' adhesion and spreading process. The superhydrophobic surface based on lotus effect (Fig. S2) has a small solid-liquid contact area, resulting in the inhibition of cell adhesion. In contrast, the as-prepared superhydrophobic in this work is based on petal effect (Fig. 5c) with an impregnating wetting state. As a consequence, the solid-liquid contact area is greatly increased and cells on such superhydrophobic surface (the LDH/SO sample) can adhere and spread as on hydrophilic surface (the LDH sample). Furthermore, the better corrosion resistance of the LDH/SO sample leads to a milder environment for cell proliferation (Fig. S4).

Hemocompatibility

Since hemocompatibility is a vital evaluation for blood-contacting materials, platelet adhesion assay and HR assay were conducted in this work. The adherent platelets on the Mg, LDH and LDH/SO samples are shown in Fig. 11a–f. As the Mg sample is severely corroded (Fig. 11a, d), it is hard to distinguish platelets from the corrosion

Figure 11 (a–f) SEM images of platelets adhered on the surface of Mg (a, d), LDH (b, e) and LDH/SO (c, f) samples. (g) Hemolysis rate of Mg, LDH and LDH/SO samples (mean $\pm$ SD, $N = 4$).

products. After modified with LDH coating, the corrosion resistance of the sample was greatly enhanced (Fig. 7), and there are many platelets on its surface (Fig. 11b, e). The shape of the platelets is not round, indicating that the adhered platelets are damaged. Further fabricating a superhydrophobic surface, the amount of adherent platelets was significantly decreased (Fig. 11c, f). The results reveal that the LDH/SO sample with a superhydrophobic surface could effectively inhibit the adhesion of platelets, indicating a promising anticoagulation effect. The different sizes of HUVECs and platelets might play roles in their different adhesion activities. The HR value represents the degree of erythrocyte destruction. As shown in Fig. 11g, pure Mg shows a high HR value (13.9%) caused by its rapid degradation. The LDH sample presents a much lower HR value (1.2%), which is within the safe value of 5%. Compared with the LDH sample, the corrosion resistance of LDH/SO sample is further enhanced, resulting in an even lower HR value. The results of platelet adhesion and HR value suggest that LDH/SO sample coating is suitable for blood-contacting biomaterials.

CONCLUSIONS

Inspired by the petal effect, a superhydrophobic and highly adhesive coating was successfully fabricated on the surface of Mg *via* a simple one-step method. The superhydrophobic property of the coating was determined by the SO concentration of the reaction solution, and was independent of the reaction time. The oleate groups on the surface of LDH/SO sample were mainly bound with the positive layer of Mg-Al LDH *via* electrostatic attraction. The as-prepared LDH/SO coating could effectively improve the corrosion resistance of the substrate. With better corrosion resistance, cell migration and viability in the extraction of LDH/SO sample were greatly enhanced. Furthermore, owing to its impregnating wetting state, cells directly cultured on its surface showed the same degree of adhesion and spreading with hydrophilic surface. Finally, the platelet adhesion was effectively inhibited and the hemolysis ratio was reduced to a level for clinical application. With favorable corrosion resistance, cytocompatibility and hemocompatibility, the LDH/SO coating in the present study shows a promising application in clinic.

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Author contributions Liu X conceived and designed the experiments; Peng F performed the experiments and analyzed the data. Peng F and Liu X co-wrote the manuscript. Wang D, Ma X, Zhu H and Qiao Y provided technical guidance and participated in the data analysis.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Supplementary data are available in the online version of the paper.

Peng Feng was born in 1992. He received his bachelor degree in materials science from the School of Materials Science and Engineering, Central South University, in 2014. He is now a doctoral candidate at Shanghai Institute of Ceramics Chinese Academy of Sciences, and his current research interest is focused on biodegradable magnesium alloys for biomedical application.

Liu Xuanyong was born in 1974. He received his BSc and MSc degrees in materials science and engineering from Hunan University in 1996 and 1999, respectively, and PhD in materials science and engineering from Shanghai Institute of Ceramics Chinese Academy of Sciences, in 2002. His research fields include nanosized and functionalized surface of biomedical Ti alloys, PEEK and biodegradable magnesium alloys, surface modification of biomaterials using plasma immersion ion implantation & deposition (PIII&D) technology, plasma sprayed bioactive ceramic coatings.

仿花瓣效应超疏水涂层提高纯镁抗腐蚀性和生物相容性

彭峰^{1,2}, 王东辉^{1,2}, 马小涵¹, 朱红芹¹, 乔玉琴¹, 刘宣勇^{1*}

摘要 由于具有完全可降解性和良好的机械性能, 镁及其合金被誉为“下一代生物医用金属材料”。然而, 过快的降解速率和较差的生物相容性制约着它们在临床上的应用。受“花瓣效应”启发, 本文利用含有油酸钠的溶液, 通过水热法在纯镁表面制备了超疏水且具有高粘附力的涂层。涂层表面的片状微纳结构和低表面自由能的油酸根赋予涂层超疏水性。水滴与涂层表面之间封存的空气以及固液界面间的范德华力, 使水滴在涂层表面显示出高粘附性(即使倒置180°, 水滴也不会从材料表面脱落)。本工作评价了所制备超疏水涂层的化学稳定性和机械稳定性。动电位极化曲线和阻抗谱测试都表明所制备涂层具有良好的抗腐蚀性。此外, 细胞(人脐静脉内皮细胞)毒性、细胞迁移以及细胞粘附等结果都表明超疏水涂层具有良好的细胞相容性。最后, 溶血率和血小板粘附测试表明超疏水涂层的血液相容性也有明显提升。利用超疏水高粘附力涂层改善纯镁抗腐蚀性和生物相容性的思路有望拓展镁在生物医用领域的应用。