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In vitro degradation, cytocompatibility and hemolysis tests of CaF_2 doped TiO_2-SiO_2 composite coating on AZ31 alloy

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ABSTRACT

In this study, a CaF_2 doped TiO_2-SiO_2 composite coating was successfully coated onto AZ31 alloy by sol-gel method. Electrochemical tests, in vitro degradation, direct cellular experiment and hemolysis tests were conducted and the results showed that the CaF_2 doped TiO_2-SiO_2 composite coating can not only improve the corrosion resistance, but also enhance the biocompatibility of AZ31 alloy. XRD, SEM and EDX were also performed to characterize the crystalline structures, morphologies and chemical compositions of the coatings.

1. Introduction

Magnesium and its alloys as biodegradable implant materials have attracted a lot of research interest due to their desirable mechanical properties, biodegradability and biocompatibility [1–11]. Biodegradable magnesium material without secondary operations is a better choice in orthopaedic operations compared with nondegradable implant materials and the released Mg^{2+} can be absorbed by human body and involved in metabolism and bone self-healing [4,5]. However, the rapid corrosion of magnesium and its alloys inhibits their clinical applications since the magnesium implants cannot maintain integrity before the injured bone is healed well enough [12].

The applications of coatings have been proved to be the most direct and effective way to protect magnesium from corrosion [13–40]. In various studies of metallic biomaterials, too much attention has been paid to Ca-P coatings [13–26], while studies of other coatings are not enough. In recent years, researches of titanium dioxide [27–29] and silicon dioxide [30,31] coatings have also emerged. TiO_2- and SiO_2- containing coatings are widely used in the protection of Ti-based orthopaedic implants. And recently, research interests have been attracted in TiO_2- and SiO_2- containing coatings for the protection of magnesium-based biomaterials [33,34]. Cao et al. studied TiO_2 coating on OH-terminated $Mg_3(PO_3)_2$ surface of Mg-Zn-Ca alloy and proved its influence on the improvement of corrosion resistance [34]. Khalajabadi et al. investigated the corrosion behaviours of nano-Si and nano-ZnO/Si coatings on Mg/HA/TiO_2/MgO nanocomposites. And the improvement of corrosion resistance by nano-Si and nano-ZnO/Si coatings was also confirmed [35].

To further improve the corrosion resistance and biocompatibility of SiO_2 and TiO_2 coated magnesium alloys, Ca- and CaF_2-containing TiO_2-SiO_2 composite coatings were prepared onto AZ31 alloy in this paper. Fluorine is one of the necessary trace elements for human body. The right amount of fluoride can play a positive role in human metabolism, particularly in the defence against teeth decay. The biocompatibility of F-containing coatings has been proved by many studies, such as well known FHA and MgF_2 coatings [36–40]. The corrosion behavior and biocompatibility were studied by in vitro degradation and cellular compatibility tests, respectively. Then the coating and degradation processes were also discussed.

2. Experimental procedure

2.1. Materials and sample preparation

The AZ31 alloy (2.84%Al, 0.81%Zn, 0.37%Mn, 0.002%Cu and balance Mg) used in this study was purchased from Qingdao De Xing Sheng Metal Materials Co., Ltd and cut into discs with the dimension of 20mm × 20mm × 2 mm. Before coating, all the AZ31 discs were gradually ground by 500/800/1200/1500 grits emery papers

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and then ultrasonically cleaned for 10 min in absolute ethanol and acetone, respectively.

2.2. Synthesis of sols

All the studied coatings (TiO_2, TiO_2-SiO_2 (denoted TS), Ca doped TiO-SiO_2 (denoted Ca-TS) and CaF_2 doped TiO_2-SiO_2 (denoted CaF_2-TS) coatings) were prepared by sol-gel method. And the chemicals used in this study are all analytical grade and used without any more purification.

The TiO_2 sol was prepared according to our previous work which was in volume ratio of tetrabutyl titanate (TTB, $C_{16}H_{36}O_4Ti$): deionized water: acetylacetone ($C_5H_8O_2$): nitric acid = 20:80:3:3:0.1 [41].

The SiO_2 sol was prepared in the ratio of tetraethoxysilane (TEOS, $C_8H_{20}O_4Si$): absolute ethanol: deionized water: polyethylene glycol (PEG, $HO(CH_2CH_2O)_nH$): nitric acid = 20 mL:80 mL:4 mL:1 g:0.1 mL. First, 20 mL TEOS was titrated into 40 mL absolute ethanol under continuous stirring to obtain TEOS/ethanol solution. Next, 4 mL deionized water, 1 g PEG and 0.1 mL nitric acid were added into another 40 mL absolute ethanol. Then the PEG/deionized water/nitric acid/ethanol solution was ultrasonically treated for 10 min until the PEG was completely dissolved and titrated into TEOS/ethanol solution under continuous stirring. At last, the mixed solution was stirred vigorously for another 4 h at 35 °C to obtain SiO_2 sol.

The TiO_2-SiO_2 mixed sol was prepared by titrating the newly-prepared SiO_2 sol into newly-prepared TiO_2 sol at the volume ratio of TiO_2:SiO_2 = 1:1 under continuous stirring at room temperature.

To prepare Ca-containing TiO_2-SiO_2 mixed sol, 13.89 g $Ca(NO_3)_2$ was added into 60 mL TTB/ethanol solution first and the Ca-containing TTB/ethanol solution was subsequently ultrasonically treated until the $Ca(NO_3)_2$ was completely dissolved. Then the Ca-containing solution was used to prepare Ca-containing TiO_2 in the same procedure of TiO_2 sol preparation process and mixed with SiO_2 sol in the volume ratio of 1:1 to obtain Ca-containing TiO_2-SiO_2 mixed sol.

To prepare CaF_2-TiO_2-SiO_2 mixed sol, 4 mL trifluoroacetic acid (TFA, CF_3COOH) and 13.89 g $Ca(NO_3)_2$ were added into another 60 mL TTB/ethanol solution, respectively, and the Ca/TFA-containing TTB/ethanol solution was also ultrasonically treated to let $Ca(NO_3)_2$ and TFA dissolve completely. After that, its pH would be adjusted to the initial pH of TTB/ethanol solution (pH = 7.0) by NH_4OH and HNO_3 to eliminate the influence of H^+ from TFA on the hydrolysis speed of TTB. Then Ca/TFA-containing TTB/ethanol solution was added into deionized water/acetylacetone/nitric acid/ethanol solution to prepare Ca/TFA-containing TiO_2 sol and subsequently mixed with newly-prepared SiO_2 sol in under continuous stirring in the volume ratio of 1:1 to obtain Ca/TFA-containing TiO_2-SiO_2 mixed sol.

Prior to the dip-coating, all the newly prepared mixed sols were stirred vigorously at 35 °C for another 4 h and aged for 24 h to get the sols stabilized.

2.3. Dip-coating process

AZ31 discs were labeled and respectively immersed into corresponding sols for 5 min to allow wetting and subsequently withdrawn at the speed of 3 mm/min. The dip-coating processes of all coatings were repeated for three times. After dip-coating, all the coated discs were dried in a vacuum chamber at 50 °C for 1 h and annealed at 500 °C for 2 h in air.

2.4. Characterization

The phase analysis of the coated samples was identified by X-ray diffraction (XRD) which uses Cu Kα line at 0.15405 nm as source. The XRD peaks were assigned by a Jade 6.0 software and the peaks were compared with TiO_2 (JCPDS No. 21-1272), $CaTiO_3$ (JCPDS No. 42-0423) and CaF_2 (JCPDS No. 65-0535). Scanning electron microscope (SEM, equipped with energy dispersive X-ray spectroscopy (EDX)) was used to investigate the morphologies of the samples before and after immersion.

2.5. Electrochemical tests

Hank's solution (8.010 g/L NaCl, 0.400 g/L KCl, 0.475 g/L $NaHCO_3$, 0.060 g/L KH_2PO_4, 1.000 g/L Glucose and 0.126 g/L $Na_2HPO_4{\cdot}12H_2O$) with initial pH value of 7.4 was used as the medium of the electrochemical tests. The coated samples were molded into epoxy resin carefully with a surface of 4 cm^2 exposed to Hank's solution. The electrochemical tests were conducted on an CHI660E electrochemical workstation with a three-electrode cell in which the platinum served as the counter electrode and AZ31 disc and a saturated calomel electrode (SCE) were used as the working electrode and reference electrode, respectively.

Before electrochemical tests, all the samples were cleaned with deionized water and dried in air. Each sample was immersed in Hank's solution at 37 °C for 1 h to stabilize the potential. Then the potentiodynamic polarization curves were recorded at the scanning rate of 1 mV/s from the initial potential of 300 mV below the stabilized potential.

2.6. Immersion corrosion tests

All the samples used in immersion corrosion tests were also molded into epoxy resin with a surface of 4 cm^2 exposed. The uncoated, TiO_2, TS, Ca-TS and CaF_2-TS coated samples were immersed in Hank's solution at 37 °C for 21 days to study their corrosion behavior.

The pH value of the Hank's solution and the volume of collected H_2 were recorded every 24 h during the immersion. After being immersed for certain time, the immersed samples were cleaned in a chromic acid solution (180 g/L CrO_3 + 10 g/L $AgNO_3$) for 10 min and subsequently washed with distilled water to remove corrosion products. The morphologies and weight loss of immersed samples after cleaning were studied. The corrosion rates were calculated by Eq. (1):

$$C_R = (K \times W)/(A \times T \times D) \quad (1)$$

C_R represents the corrosion rate in mm/year. K is conversion coefficient ($K = 8.76 \times 10^4$). W is the weight loss in g. A is surface area in cm^2. T stands for the immersion time in hour. D is the density of AZ31 alloy which is 1.738 g/cm^3 [42].

2.7. Biocompatibility tests

2.7.1. Cell culture

L-929 mouse fibroblast cells (provided by Donghua University, China) were used for the biocompatibility evaluation of the studied samples due to the good proliferation in vitro, wide distribution and large number of fibroblast cells in human body. It was also found that the collagenous fiber and cellular matrix excreted from fibroblast cells were favorable in the bone healing process [32,43,44]. The L-929 mouse fibroblast cells were cultured in a Dulbecco's modified Eagle's medium (DMEM, supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin)