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In vitro study of nanostructured diopside coating on Mg alloy orthopedic implants

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ABSTRACT

The high corrosion rate of Mg alloys has hindered their application in various areas, particularly for orthopedic applications. In order to decrease the corrosion rate and to improve the bioactivity, mechanical stability and cytocompatibility of the Mg alloy, nanostructured diopside ($CaMgSi_2O_6$) has been coated on AZ91 Mg alloy using a combined micro arc oxidation (MAO) and electrophoretic deposition (EPD) method. The crystalline structure, the morphology and the composition of the samples were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Electrochemical corrosion test, immersion test, and compression test were used to evaluate the corrosion resistance, the in vitro bioactivity and the mechanical stability of the samples, respectively. The cytocompatibility of the samples was tested by the cell viability and the cell attachment of L-929 cells. The results confirmed that the diopside coating not only slows down the corrosion rate, but also enhances the in vitro bioactivity, mechanical stability and cytocompatibility of AZ91 Mg alloy. Therefore, Mg alloy coated with nanostructured diopside offers a promising approach for biodegradable bone implants.

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1. Introduction

Due to their reliable mechanical properties, the use of permanent metallic bone implants in load bearing applications is one of the most popular approaches for orthopedic treatment [1,2]. However, second surgeries may be required for many patients in whom the metals are rejected by the body and cause allergic responses, bursitis or tendonitis [1,3]. Such second surgeries are highly unfavorable as the implant removal can be inconvenient and expensive after healing the bone [4,5]. To avoid the second surgery issue, it is preferable that the implants be biodegradable [6–10]. However, most of the biodegradable materials, usually polymers, do not have good mechanical properties to be reliable for bearing the load of the body [1,4,11,12]. Mg alloys, by incorporating the two characteristics of 1 — good mechanical strength and 2 — biodegradability, can be an excellent candidate for this purpose [13–15]. However, the serious disadvantage of Mg alloys is their poor corrosion resistance, which needs to be addressed before they can be clinically employed as bone implants [16,17]. A practical solution, which is general for many metal implants, is a suitable surface modification and coating on the surface of the implants [18–20]. One popular surface treatment for metal implants is coating by bioactive ceramics [21–24], specifically silicate bioceramics [25,26].

Recently, Chang et al. reported the superiority of silicate bioceramics in terms of material degradation and inducing in vivo bone formation, compared to the β-tricalcium phosphate (β-TCP) ceramic scaffolds, suggesting that the silicate ceramics have potential application in bone regeneration [27]. Among silicate biomaterials, diopside ceramics have the ability to induce in vitro bone-like apatite formation in the simulated body fluid (SBF) and in vivo bone formation [28]. Moreover, detailed in vitro and in vivo studies by Nonami and Tsutsumi and Miake et al. confirmed that dense diopside bulks possessed improved bioactivity compared to other types of bioceramics such as akermanite and bredigite [28,29]. Due to these advantages, we chose to employ the diopside coating on AZ91 Mg substrate, which is a biomedical grade Mg alloy with the highest corrosion resistance in this paper [15,30].

Since the in vitro bioactivity and osteoconductivity of biomaterials could be further improved if the coating materials were made in nano-size [3,11,19], we made a nanostructured configuration of

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diopside coating. More specifically, we used cost effective EPD technique which has been extensively employed to deposit bioactive ceramics on a variety of biomedical metallic substrates, including stainless steel and titanium alloys [31,32]. As an intermediate layer between the AZ91 Mg substrate and diopside coating, micro arc oxidation (MAO) was used to produce a rough structure on the surfaces of the AZ91 Mg alloy to strengthen the interfacial bonding and to provide more favorable sites for EPD of nanostructured diopside powders [33]. The MAO layer itself can further assist in enhancing the corrosion resistance and in vitro bioactivity of the substrates [34,35].

It is important to consider that the surface modification of substrates can significantly influence the viability, attachment and proliferation of the cells [36], which is the subject of our investigation in this paper. The formation of the corrosion products, as well as their departure due to the high hydrogen release of Mg implants, causes serious difficulties for cell attachment and proliferation [37,38]. Thus, we expect that the coating of Mg implants is able to positively influence the cytocompatibility.

The main aim of our study in this paper was improving the cytocompatibility, mechanical stability, corrosion resistance and in vitro bioactivity of biodegradable AZ91 Mg alloy using a nanostructured diopside coating that was prepared on the AZ91 Mg alloy through combined MAO and EPD methods.

2. Materials and methods

2.1. Samples preparation

AZ91 Mg alloy (Al 9%, Zn 1%, all in wt.%) was machined to obtain the substrates of 20 mm × 15 mm × 5 mm, which were ground with SiC papers progressively to 600 grit.

2.2. Diopside preparation

0.125 mol of $Ca(NO_3)_2 \cdot 4H_2O$ and 0.125 mol of $MgCl_2 \cdot 6H_2O$ were dissolved in 150 mL of ethanol. The solution was stirred with a stirrer at 80 °C for 30 min. 0.25 mol $Si(OC_2H_5)_4$ was added to this solution. The homogeneous solution was slowly stirred for a few hours to yield a precursor wet gel. The wet gel was dried in an oven at 100 °C for 24 h. The dried gel powder was calcined at 700 °C for 2 h in the air [39]. Finally, the prepared diopside powder was ball milled with ball/powder ratio of 10/1 and rotational speed of 250 rpm for 10 h.

2.3. MAO process

To perform the MAO process, a power supply was employed in which the AZ91 sample and a stainless steel plate were used as the anode and cathode, respectively. The electrolyte in the MAO process was an aqueous solution of NaOH (200 g/L) and Na_2SiO_3 (200 g/L). During the MAO, the voltage and the time were fixed at 60 V and 30 min, respectively.

2.4. EPD process

Suspensions of nanostructured diopside powders at a total concentration of 100 g/L were prepared in methanol and treated in an ultrasonic bath for 1 h and then the suspensions were stirred with a magnetic stirrer for 30 min to ensure a good dispersion of the particles. EPD was performed under constant voltage of 100 V for 3 min. AZ91 sample was used as the cathode and a graphite electrode was used as the anode.

2.5. Coatings characterization

Phase structure analysis was performed using an X-ray diffractometer (XRD, Philips Xpert). The grain size of the prepared diopside powders was estimated by Williamson–Hall equation [40]:

$$\beta \cos\theta = 0.89\lambda/D + 2\varepsilon \sin\theta. \quad (1)$$

In which β (rad) is the full width half maximum of peaks (FWHM), θ (°) is the Bragg's angle, λ (nm) is the wavelength of the X-ray, D (nm) is the grain size and ε is the structural strain.

The morphology and elemental composition of samples were investigated by a scanning electron microscope (SEM: Philips XL 30: Eindhoven) equipped with energy dispersive spectroscopy (EDS).

Transmission electron microscope (TEM: JEOL JEM-2100) was utilized to study the morphology and to determine the size of diopside nanoparticles after ball milling.

2.6. Electrochemical test

The electrochemical corrosion behavior of AZ91, MAO and diopside coated samples was investigated in the simulated body fluid (SBF) using an Ametek potentiostat (model PARSTAT 2273). A three-electrode cell with the sample as the working electrode, calomel electrode as the reference electrode, and platinum electrode as the counter electrode was employed. The experiment started after the sample was immersed in the test solution for 1 h. A scanning rate of 1 mVs^{-1} was applied during the polarization experiment and the scan range of polarization test was in the range of −250 to +1000 mV (vs. OCP). The impedance data were recorded from the frequency between 100 kHz to 10 mHz and the amplitude of applied AC signal was 10 mV.

2.7. Immersion test

The immersion test was carried out in the SBF according to ASTM-G31-72 standard [41] at different time points to monitor the corrosion resistance and bioactivity of the AZ91, MAO and diopside coated samples. The corrosion products on the sample surface during the corrosion were removed by immersing the samples into the chromic acid (200 g/L) for 5 min [3].

The difference in weight before and after chromic acid immersion indicated the amount of weight loss. The corrosion rate of samples was calculated by weight loss as a function of immersion time, according to Eq. (2) [9]:

$$CR = W/At \quad (2)$$

where CR ($mg/cm^2/h$) is the corrosion rate, W (mg) is the weight loss, A (cm^2) is the initial surface area exposed to the SBF, and t (h) is the immersion time.

The release of Mg ions from the samples was measured using inductively coupled plasma (ICP: PERKIN-ELMER 2380). The pH values of samples were also monitored with a pH-Meter (pH & ION meter GLP 22, Crison, Spain).

The in vitro bioactivity was evaluated using SEM imaging and the functional groups of precipitated layer on the surface of samples in the SBF solution were analyzed by Fourier transform infrared spectroscopy (FTIR, Agilent 680 IR). For FTIR analysis, the precipitated white particles were collected from the formed corrosion products on the surface of the diopside coated sample.

2.8. Compression test

The samples for the compression test were machined according to ASTM E9 standard and immersed in the SBF for 4 weeks. The uniaxial compression tests were carried out using INSTRON 8562 universal tensile testing machine at cross head displacement rate of 0.5 mm/min to measure the residual compressive properties for each sample.

2.9. Cytocompatibility evaluation

L-929 fibroblast cell line was employed for cytocompatibility examination. The cells were first cultured in 89% Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum