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In vitro degradation behavior and cytocompatibility of a bioceramic anodization films on the biodegradable magnesium alloy

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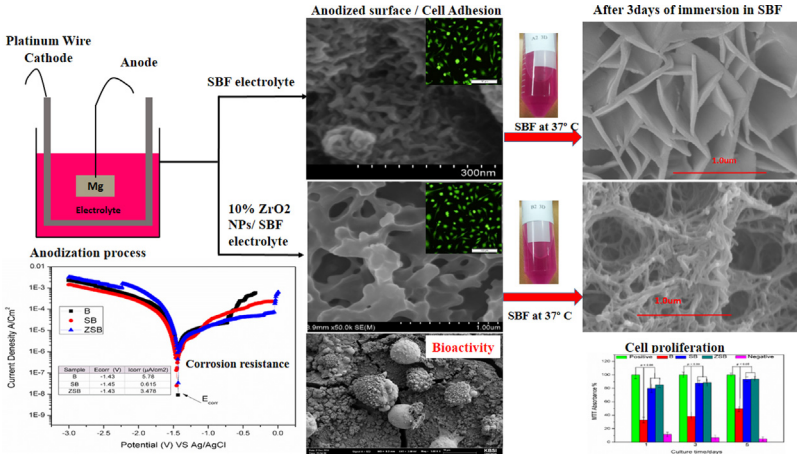
HIGHLIGHTS

- AZ31 Mg alloy was anodized using SBF with and without ZrO_2 NPs as electrolyte.
- ZrO_2 NPs containing SBF improve magnesium chemical and biological properties.
- Electrochemical and immersion tests enhanced the corrosion resistance of Mg alloy.
- Nanostructure resulted on the anodized surface before and after immersion test.
- EA.hy926 endothelial cells show good cell proliferation and attachment.

GRAPHICAL ABSTRACT



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ABSTRACT

Magnesium (Mg), and its alloys have good potential for using in biomedical applications due to its good mechanical properties and biodegradability. However, the poor corrosion resistance and insufficient mechanical stability of Mg in biological fluids have limited its clinical applicability. As a result, surface modification techniques are being investigated to improve corrosion resistance for biomedical applications. In this study, a bioactive CaP film were deposited on the surface of the AZ31B magnesium alloy *via* anodization with SBF solution used as the electrolyte with and without ZrO_2 Nanoparticles (NPs). The samples were evaluated and characterized *in vitro* to assess their degradation and cytocompatability behaviour *via* FE-SEM, electrochemical corrosion test, immersion test, and cell culture tests. The results indicate that the AZ31B Mg alloy surface anodized in SBF with and without ZrO_2 NPs consists of CaP nanoplate leaf-like apatite structures and porous 3-D structures, respectively. The nanomorphology showed uniform growth on the anodized surface that had created the porous layer on the surface of the samples three days after immersion in the SBF solution. The formation of such nanostructures on the anodized sample could provide the implant materials with extra biocompatibility. Moreover, this unique

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deposited layer can improve the corrosion resistance and cytocompatability of the Mg alloys, which make it a promising material for use in biomedical applications.

1. Introduction

Magnesium (Mg) and its alloys are biodegradable materials that have attracted an increasing attentions nowadays due to their potential ability to address the challenges of using permanent implants, including the foreign body response, painful, and expensive post-healing removal surgery [1]. Magnesium is considered the fourth abundant cations in the human body[2], where the adult body contains approximately 24 g of Mg per 70 kg, and about 50–65 wt% of total magnesium is located in the bone [3]. Moreover, magnesium is very important for the human metabolism [4]. However, some of the disadvantages of using Mg alloy are that it has a high rate of corrosion accompanied with hydrogen gas release and an increase in the alkalinity of body fluids, all of which limit its clinical applicability [5]. Once, Mg implant is used to repair damaged tissue *in vivo*, the implant can lose its mechanical properties before healing has been fully completed due to its fast corrosion and low bioactivity [6]. Therefore, introducing a surface coating can be a suitable technique to protect the magnesium surface subsequently improve its stability and corrosion control as well as its bioactivity at the tissue interface [4]. Since the natural bone tissue consists of a hydroxyapatite nanostructure, it is better to employ a bioceramic coating that mimics the bone structure as a coating layer to improve the bioactivity of the Mg alloy. *In vivo* tests have shown that the CaP coating is degradable in a physiological environment and improves the osseo-integration ability of the implant [7]. Calcium phosphate based biomaterials have also recently received an increasing attention as a result of their bioactivity and ability to develop bone tissue when in contact with the physiological environment [8,9]. Apatite formation on the biomaterial surface through *in vitro* immersion and bioactivity depend on the chemical composition as well as the surface microstructure and morphology [10]. For example, previous studies have found that a bioglass $45S_5$ (Na_2O–CaO–SiO_2–P_2O_5)-coated Mg alloy has better activity towards apatite formation in SBF solution due to its potential bond with living bone through the calcium phosphate layer [11,12]. In addition, metallic oxides such as TiO_2, SiO_2 and ZrO_2 can be used to form apatite on its surface in SBF solution. Zirconia (ZrO_2), has already been applied in the heads of hip joint prostheses, and it has desirable properties, such as high strength and fracture toughness[12]. The human body contains about 300 mg of Zirconium, the amount taken by adult peoples is about 3.5 mg per day orally, moreover, it was also detected in blood and urine [13]. Furthermore, none of the insoluble materials was found to be extremely toxic up to 30 ppm [14]. Biocompatibility of zirconia as ceramics biomaterials were investigated *in vivo* by implanting them in bone and in soft tissues before performing the *in vitro* studies [15].

Anodization is a well-known surface modification/treatment that can produce bioactive pores on the outer layer of the Mg alloy, improving its anticorrosion activity and biocompatibility [16]. In the initial time of implantation, the non-porous inner dense layer of the anodized film inhibits corrosion and hydrogen release while the porous layer can increase the surface area for cell adhesion and proliferation. The porous outer film surface can be adapted with bioactive constituents, such as growth factors or bone morphogenic proteins, then the scaffold would provide even better conditions for cell interaction [17]. The coated magnesium samples show improved cell viability as the incubation time increasing for the implant surface in an *in vitro* test. In contrast, naked samples usually exhibit a lower cell viability and no significant changes resulting through an increase in the incubation time due to the fast corrosion and hydrogen release [18]. Calcium (Ca^{+2}) ions are considered to be essential elements for cell signaling because these allow for better absorption of proteins, such as fibronectin and vitronectin, that can promote cell attachment [19].

The objective of the this study is to increase both the biodegradability resistance and *in vitro* bioactivity of the AZ31B Mg alloy using nanostructure apatite coating produced through anodization with SBF solution with and without ZrO_2 NPs electrolyte. A full characterization of the resulted coatings were studied by our group [20,21]. Herein, a detailed *in vitro* biodegradation and cytocompatability study of AZ31B magnesium alloy and coating were evaluated in this study to achieve the desired objectives. The resulted nanosize apatite can mimic that of natural extracellular matrices (ECM) morphology. In addition, adding ZrO_2 NPs to the SBF electrolyte could improve the corrosion resistance and bioactivity of the new surface.

2. Experimental

2.1. Sample preparation

A commercial AZ31B magnesium alloy (Alfa Aesar Company, South Korea) with elemental composition Al (2.5–3.5) wt%; Zn (0.7–1.3) wt%; Mn (0.2–1.0) wt%; Si ≃ 0.05 wt%; Cu ≃ 0.01; Mg is balanced, was used in this study. The samples were cut into 12 mm × 12 mm × 6.35 mm (total surface area 5.93 ± 0.2 cm^2) then grinding was applied using SiC paper (600–2000 grit). The samples were subsequently cleaned ultrasonically in acetone for 5 min to remove residual grease, then rinsed in distilled water for 5 min and finally dried in warm air.

2.2. Anodizing procedure

GP-7202GT, INTEC anodizing machine was used connected to a DC auto range power supply (IT6723H 300 V/10A/850W) with a platinum cathode connected to the anodization cell. SBF solution was used as the electrolyte with and without 10 wt% ZrO_2 NPs (Zirconium (IV) oxide nanoparticles, dispersion, <100 nm particles size (BET), 5 wt% in H_2O, Ph: 5–6, Sigma–Aldrich, South Korea). The treatment process was carried out with a constant current of 30 mA at 50 V for 5 min at room temperature. The SBF solution was simply prepared by our research group [22]. A commercial Hank's balanced salt (Aldrich, H2387-1L) was dissolved in 1 L distilled water followed by the addition of $MgSO_4$ (0.097 g), $NaHCO_3$ (0.350 g), and $CaCl_2$ (0.185 g) the pH value was about 7.0. Hereafter, the terms B, SB, and ZSB are used to refer to bare, SBF anodized, and 10 wt% ZrO_2 NPs/SBF anodized samples, respectively.

2.3. Characterization

The surface morphology of the samples before and after the immersion test was observed using both a digital camera and field emission scanning electron microscope (SUPRA 55VP) with an electron beam of 0.8 nm at 15 kV and connected with EDS analysis.