Surface & Coatings Technology 304 (2016) 425–437

Contents lists available at ScienceDirect

Surface & Coatings Technology

journal homepage: www.elsevier.com/locate/surfcoat

Nanoscale modification of magnesium with highly textural lamellar nanosheets towards increasing the corrosion resistance and bioactivity

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ARTICLE INFO

Article history:
Received 15 March 2016
Revised 6 June 2016
Accepted in revised form 24 June 2016
Available online 27 June 2016

Keywords:
Nanoscale modification
Lamellar nanosheets
Microarc oxidation
Bioactivity
Absorbable implants

ABSTRACT

The present study aims to modify the surface of microarc oxidation (MAO) coated Mg using an alkaline fluoride solution so as to impart a nanoscale surface feature, which would be beneficial to improve the corrosion resistance and to promote a better bioactivity. The MAO coated Mg is modified with the formation of a highly textural lamellar nanosheet-like morphology after immersion in 0.1 M NaF (pH: 8.40) at 25 °C for 120 min, which completely covered the porous structure with the formation of nanosheets along with some agglomerated crystals. Thin film X-ray diffraction measurement, chemical composition analysis and Fourier transform infrared spectroscopy studies confirmed that the nanosheets are primarily $Mg(OH)_{2-x}F_x$ while the agglomerates are $NaMgF_3$. The complete coverage of the porous structure by the modified layer along with the formation of $Mg(OH)_{2-x}F_x$ and $NaMgF_3$ has enabled a better corrosion resistance for MAO coated Mg modified by NaF. The higher surface area of the nanosheets favoured nucleation of monocalcium phosphate anhydrous and newberyite, both of which are biologically relevant. The unique morphological feature of the modified surface helped to achieve an improved cell adhesion and proliferation of MC3T3-E1 osteoblast-like cells. The relative growth rate of both uncoated and coated Mg are >75% on all the three days, fulfilled Grade 1 specification in terms of cytocompatibility as per ISO 10993-5 standard. The formation of nanoscale surface feature, improvement in corrosion resistance, better bioactivity and acceptable cytocompatibility point out that this methodology could be of immense help to modify the surface of Mg based absorbable implants.

1. Introduction

Development of absorbable magnesium based implants is indeed challenging due to the rapid corrosion rate of Mg and its alloys that undermines their mechanical integrity [1,2]. Various surface modification methods are suggested to control the rate of corrosion of Mg and its alloys [3–5] and among these, microarc oxidation (MAO) is a viable approach. The mechanism of formation of MAO coatings and its characteristics are elaborated elsewhere [6]. The presence of micropores and cracks are inherent characteristics of the MAO coatings. A porous outer layer is considered to be beneficial to improve mechanical interlocking, to increase the bonding area, to distribute the stresses across the interface and, to achieve higher bond strength. Nevertheless, it increases the effective surface area and facilitates quicker infiltration of the corrosive medium through them down to the substrate, leading to a decrease in corrosion resistance. The strategies to improve the corrosion resistance of MAO coated Mg has been reviewed recently [7]. The pores can be sealed by various approaches to achieve an improved corrosion resistance. However, the benefits of realizing a better osseointegration could be lost due to the sealing of the pores.

In recent years, numerous attempts are made to impart nanoscale surface features on Mg for biomedical applications [8,9]. One such approach is to treat Mg with NaOH to enlarge nanoscale surface roughness that enables an increase in osteoblast adhesion, cell proliferation, alkaline phosphatase activity, and deposition of calcium phosphates [8]. It has been demonstrated that treating Mg with NaOH helps to decrease the detrimental effects of the degradation products of Mg on osteoblast density [9]. Based on their findings, Weng and Webster [9] have recommended that creating nanoscale features on Mg could improve its use for many orthopedic applications. Zhu et al. [10] have also suggested that Mg alloy modified with the formation of a $Mg(OH)_2$ film could serve as a potential material for orthopedic implants. Zhao et al. [11] have shown that an alkaline pretreatment in 5, 10 and 15 g/l NaOH at 80 °C for 30 to 90 min helps to realize a uniform, dense, crack-free 45S5 bioglass–ceramic coating on AZ31 Mg alloy with better bonding. The unevenness and presence of a number of microcracks on 45S5 coating deposited on Mg alloy surface without the alkaline pretreatment has previously been pointed out by Huang et al. [12]. Chen et al. [13] have

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http://dx.doi.org/10.1016/j.surfcoat.2016.06.070
0257-8972/

advocated the benefits of developing a transitional layer of Mg:OH by alkaline pretreatment of Mg in 3 M NaOH at 60 °C for 12 or 24 h that helps to improve covalent bonding of phytic acid molecules, resulting in the formation of a dense and homogenous phytic acid coating. Waterman et al. [14] have reported that the formation of $Mg(OH)_2$ layer on Mg by treating it in pure H_2O at 100 °C for 15 or 30 min is beneficial for the nucleation of calcium phosphates in Hank's solution. Alkaline pretreatment by immersion in 0.05 to 4 M NaOH for 1 h has also been shown to promote apatite formation on non-woven poly(ε-caprolactone) fabrics in simulated body fluid (SBF) [15]. According to Wei et al. [16], development of a hydroxyl functionalized surface over MAO coated Ti alloy, by chemical treatment in 5 M NaOH at 60 °C for 24 h, has favoured heterogeneous nucleation of the apatite. These studies clearly indicate that alkaline pretreatment of Mg and its alloys could offer considerable benefits. Nevertheless, it is imperative to manipulate the experimental conditions to generate nanoscale surface features to achieve the desired benefits. According to Waterman et al. [14], treatment of Mg in pure H_2O at 100 °C for 15 min has resulted in the generation of $Mg(OH)_2$ layer with a uniform flake-like structure while it becomes a dense layer with many defects and cracks after 30 min. The importance of developing a $Mg(OH)_2$ layer with a specific surface structure is also recommended by Wei et al. [16].

The present study aims to modify the surface of MAO coated Mg using an alkaline fluoride solution, 0.1 M NaF (pH: 8.40), so as to impart a nanoscale surface feature. The choice of the electrolyte is made based on the ability of the alkaline solution to generate a layer of $Mg(OH)_2$ with a flake-like structure. Alkaline treatment on Mg is performed mainly using NaOH [11,13,15] whereas the use of 0.1 M NaF has been suggested for the first time in this work. The structural and morphological characteristics of the modified surface, its corrosion behaviour in Hank's balanced salt solution (HBSS) and its ability to favour the deposition of calcium phosphates from a concentrated simulated body fluid (c-SBF) under biomimetic conditions are evaluated.

2. Materials and methods

Commercially pure Mg (composition (in wt.%): Mg: 99.93; Al: 0.0032; Mn: 0.0128; Cu: 0.005; Fe: 0.0017; Si: 0.0228; Ni: 0.0003) having a dimension of 20 mm × 15 mm × 3 mm was used as substrate materials. They were surface ground using SiC coated abrasive (600 grit) paper, ultrasonically cleaned in ethanol and dried using a stream of compressed air. MAO of Mg was carried out using an alkaline silicate electrolyte that contained 5 g/l NaOH and 15 g/l Na_2SiO_3 under direct current mode at 250 V for 2 min. A large sheet of Pt (60 mm × 40 mm × 1 mm) was used as the counter electrode. During treatment, a water bath was used to control the temperature of the electrolyte <40 °C. After deposition, the MAO coated Mg samples were rinsed thoroughly using deionized water and dried. The MAO coated Mg samples were modified by immersion in 0.1 M NaF (pH: 8.40) at 25 °C for various duration of time up to 180 min. Uncoated Mg samples were also treated under similar conditions to understand the mechanism of surface modification by NaF. After the stipulated time period, the surface modified Mg samples were rinsed using deionized water and dried.

The morphological features of the MAO coating and those modified by NaF were evaluated by a scanning electron microscope (SEM) with field emission source (Hitachi – Analytical UHR Schottky Emission Scanning Electron Microscope SU-70). Before SEM analysis, all the samples were sputter coated with a thin layer of Pt to reduce charging as well as to increase the amount of secondary electrons that can be detected from the surface, which would help to enhance the signal to noise ratio. During SEM analysis, the images were acquired using an accelerating voltage of 5 and 10 kV while the working distance was varied between 4.9 mm and 16.3 mm, depending on the type of sample. The chemical composition of the coated surfaces was assessed by energy dispersive spectroscopy (EDS) attached with SEM. Background correction in EDS analysis was performed by applying background filtering technique. The structural characteristics as well as the nature of functional groups were determined by thin film X-ray diffraction (TF-XRD) (PANalytical X'pert MRD) measurement and Fourier transform infrared (FT-IR) spectroscopy (Perkin–Elmer, Spectrum GS) in attenuated total reflectance (ATR) mode.

The 3-dimensional surface topography of the MAO coated Mg and those modified by NaF was assessed using atomic force microscopy (AFM) (Bruker NanoScope V multimode 8 scanning probe microscopy). The AFM imaging was performed in tapping mode over a scanning area of 5 μm × 5 μm. The corrosion resistance of the uncoated Mg, MAO coated Mg and MAO coated Mg modified by NaF in HBSS at 25 °C was evaluated by potentiodynamic polarization and electrochemical impedance (EIS) studies. The details of the characterization studies and evaluation of corrosion behaviour were elaborated in our earlier paper [17]. The uncoated, MAO coated Mg and MAO coated Mg modified by NaF were also subjected to immersion test in HBSS at 37 °C for 168 h. After completion of the immersion test for 168 h, the samples were taken out, rinsed with de-ionized water and dried. The corrosion products as well as the remnant coating present on the samples were removed by immersion in a mixture of 200 g/l CrO_3 with 10 g/l $AgNO_3$ for 30 min, washed thoroughly with de-ionized water and dried. The surface morphology of the corroded region was assessed by SEM with field emission source to compare the extent of corrosion attack and to understand the corrosion mechanism.

In order to evaluate the ability of the MAO coated Mg and those modified by NaF to promote deposition of calcium phosphates, they were subjected to immersion in c-SBF under biomimetic conditions at 37 °C for 24 h. The c-SBF solution contains 1.65 g/l $CaCl_2$; 0.30 g/l KH_2PO_4; 7.0 g/l NaCl; 0.40 g/l $Na_2HPO_4 \cdot 4H_2O$; and 0.35 g/l $NaHCO_3$. The constituents of the c-SBF essentially comprised of ions that are commonly present in physiological solutions. However, the concentrations of these ions are relatively higher in c-SBF so as to facilitate an early deposition of calcium phosphates. A similar composition was used previously by Waterman et al. [14]. After deposition, the coated samples were heat-treated at 300 °C for 1 h to promote crystallization of the calcium phosphate. A similar procedure was followed in a previous study by Yang et al. [18].

The cytocompatibility of uncoated Mg, MAO coated Mg and those modified by NaF was evaluated by an indirect assay method as per ISO 10993-5 standard using MC3T3-E1 osteoblast-like cell line. Minimum Essential Medium Eagle (α-MEM) (Sigma, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco Co., USA) and 1% antibiotic served as the cell culture medium. The uncoated Mg, MAO coated Mg and those modified by NaF were incubated in cell culture medium for 72 h under physiological conditions (37 °C; 5% CO_2; 95% relative humidity) as per ISO 10993-12 standard. The ratio of surface area of the sample to the cell culture medium was maintained at 1.25 cm^2/ml. After incubation for 72 h, the samples were removed from the cell culture medium, washed with deionized water and dried. The extracts were centrifuged at 14,000 rpm for 15 min and supernatant solution was separated for further use (100% extract). It is diluted with α-MEM to prepare 10% extracts. MC3T3-E1 cells were seeded with a cell density of 1×10^4 cells/ml of the medium in 24-well plates and incubated for 24 h under physiological conditions. Subsequently, the medium was replaced with 100 μl of 10% extracts and 1 ml of negative control (cell culture medium alone) and the MC3T3-E1 cell lines were incubated again under physiological conditions for 1, 2 and 3 days. 10 μl of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2.4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) solution was added into each well at the end of each time interval and they were again incubated for 2 h. The absorbance of the solution mixture in each well was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader. The relative growth rate (RGR) of the cells was calculated using the following equation:

$$RGR(\%) = (OD_t/OD_n) \times 100\%$$