Materials Science and Engineering C 63 (2016) 341–351

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Protective layer formation on magnesium in cell culture medium

V. Wagener, S. Virtanen *

Chair for Surface Science and Corrosion, Department Materials Science, University of Erlangen-Nuremberg, Martensstr. 7, 91058 Erlangen, Germany

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 18 February 2016
Accepted 1 March 2016
Available online 3 March 2016

Keywords:
Magnesium
Calcium phosphate coating
Protein influence
Corrosion protection electrochemical impedance spectroscopy

ABSTRACT

In the past, different studies showed that hydroxyapatite (HA) or similar calcium phosphates can be precipitated on Mg during immersion in simulated body fluids. However, at the same time, in most cases a dark grey or black layer is built under the white HA crystals. This layer seems to consist as well of calcium phosphates. Until now, neither the morphology nor its influence on Mg corrosion have been investigated in detail. In this work commercially pure magnesium (cp) was immersed in cell culture medium for one, three and five days at room temperature and in the incubator (37 °C, 5% CO_2). In addition, the influence of proteins on the formation of a corrosion layer was investigated by adding 20% of fetal calf serum (FCS) to the cell culture medium in the incubator. In order to analyze the formed layers, SEM images of cross sections, X-ray Photoelectron Spectroscopy (XPS), X-ray diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX) and Fourier Transformed Infrared Spectroscopy (FTIR) measurements were carried out. Characterization of the corrosion behavior was achieved by electrochemical impedance spectroscopy (EIS) and by potentio-dynamic polarization in Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C.

Surface analysis showed that all formed layers consist mainly of amorphous calcium phosphate compounds. For the immersion at room temperature the Ca/P ratio indicates the formation of HA, while in the incubator probably pre-stages to HA are formed. The different immersion conditions lead to a variation in layer thicknesses. However, electrochemical characterization shows that the layer thickness does not influence the corrosion resistance of magnesium. The main influencing factor for the corrosion behavior is the layer morphology. Thus, immersion at room temperature leads to the highest corrosion protection due to the formation of a compact outer layer. Layers formed in the incubator show much worse performances due to completely porous structures. The existence of proteins in DMEM seems to hinder the formation of a corrosion layer. However, protein adsorption leads to similar results as concerns corrosion protection as the formed calcium phosphate layer.

1. Introduction

The scientific focus on magnesium as bio-degradable implant material in the orthopedic field is not only based on the non-toxicity of magnesium and its mechanical properties, but also on the positive influence of magnesium ions on bone formation [1–3]. Magnesium is a trace element in the human body and thus Mg ions exist abundantly in blood and several tissues. Indeed, half of the Mg ions in the body are stored in bone tissue [1]. Several studies showed that bone formation can be stimulated by the presence of Mg implants [4]. It is assumed that released Mg ions trigger the formation of calcium phosphate compounds on the implant surface that may be pre-stages to hydroxyapatite (HA), which is a main component of human bone [5,6]. The pH increase due to Mg dissolution favors the formation of calcium phosphate as well [7].

It is as well reported that calcium phosphates and especially HA act as good corrosion protective coatings on magnesium [7–10]. Thus, in several studies HA was deposited on Mg or on Mg alloys in order to increase corrosion resistance for biomedical application [9,11–13]. For the application as bone implant not only the formation of calcium phosphate in vivo but also the pre-coating with HA is of interest. Blind et al. [14] reported improved osseo-integration due to HA coatings, for example. Different studies showed that precipitation of calcium phosphate compounds on magnesium in simulated body fluids or cell culture media can occur [1,15–20]. At the same time, however, a dark grey or black layer is formed under the white calcium phosphate precipitations in most cases [17,21,22]. Degner et al. propose a corrosion protective effect of this black layer [21]. Willumeit et al. [17] found out that those layers as well mainly consist of calcium phosphate compounds. However, the morphology of the layers was not investigated in detail.

In this study, magnesium was immersed in cell culture medium under different conditions. Immersion time, temperature and CO_2 concentration

* Corresponding author.
E-mail address: virtanen@ww.uni-erlangen.de (S. Virtanen).

http://dx.doi.org/10.1016/j.msec.2016.03.003
0928-4931/

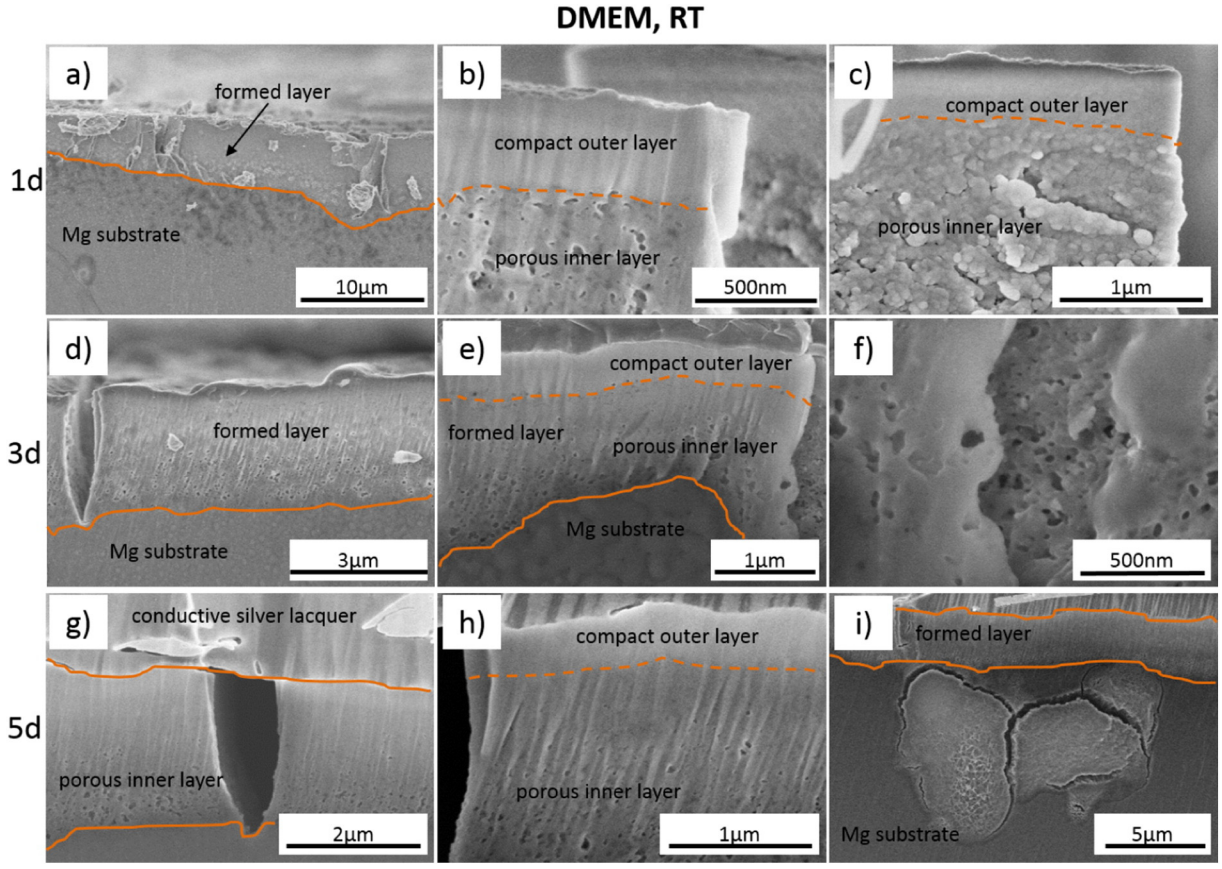

Fig. 1. SEM images after immersion of Mg in DMEM at room temperature; a)–c) immersion for one day; d)–f) immersion for three days; g)–i) immersion for five days.

were varied. The formed layers were analyzed concerning their thickness, morphology and composition and corrosion resistance. In addition, the influence of proteins in cell culture medium on the formation of a corrosion protective layer was investigated.

2. Materials and methods

2.1. Sample preparation

Samples were cut from a cp magnesium rod (25.4 mm diameter, 99.9% purity, Chempur Feinchemikalien GmbH & Co. KG), and afterwards deburred and ground with SiC abrasive paper up to a grid size of 1200. After sonication in ethanol and drying, samples were immersed in ca. 50 ml of cell culture medium for one, three and five days at room temperature and in the incubator at 37 °C (5% CO_2, ~95% humidity). As cell culture medium, Dulbecco's Modified Eagle's Medium with 1 g glucose and 3.7 g $NaHCO_3$ (DMEM, Biochrom AG) was used for the immersion at room temperature. For the immersion in the incubator 1% Penicillin-Streptomycin-Glutamine (PSG, Sigma) was added to the described DMEM, in order to prevent growth of microorganisms. For a third testing row, 20% of fetal calf serum (FCS, Gibco) was added to the cell culture medium in the incubator, in order to investigate the influence of serum proteins on the layer formation on magnesium in DMEM. After immersion in cell culture medium for the desired time, samples were rinsed with water and dried with nitrogen.

2.2. pH measurements

The pH of the cell culture medium was measured before and after immersion for all parameters. As reference, pH was measured for DMEM at room temperature, DMEM (+PSG) in the incubator and DMEM (+PSG) + 20% FCS in the incubator without immersed samples after one, three and five days.

2.3. SEM

Cross sections were prepared with help of an ion mill (IM4000, Hitachi), using an acceleration voltage of 6 V and a discharging voltage of 1.5 kV. Samples were cut in the "cross milling" mode with three repetitions per minute with an angle of ±30°. Afterwards, the cross sections were characterized with a scanning electron microscope (FE-SEM S4800, Hitachi), using an acceleration voltage between 3 kV and 10 kV. The working distance was varied between 4 and 6 mm.

2.4. XPS

XPS measurements were carried out with a high-resolution X-ray photoelectron spectrometer (PHI 5600 USA) using aluminum Kα radiation (1486.6 eV, 300 W) for excitation. The binding energy of the target elements was determined at a pass energy of 23.5 eV with a resolution of <0.4 eV (values measured every 0.2 eV for the high resolution spectra and 0.8 for survey) and a takeoff angle of 45° with respect to the surface normal. The binding energy of the C1s signal was used to correct the spectra for charging. The background was subtracted using the Shirley method in all spectra. To obtain the molar fractions of each species, the peak areas of the measured XPS spectra were corrected with the photoionization cross sections of Scofield [23] σ and the asymmetry parameter β (orbital geometry) [24], which are contained in the sensitivity factors of the acquisition software (MultiPakV6.1A, 99 June 16, copyright Physical Electronics Inc.,1994–1999). For each immersion parameter a survey spectrum and high resolution spectra for C1s, O1s, N1s, Ca2p, P2p and Mg2p signals were measured. C1s narrow scans for one day immersion were fitted with Origin (Gaussian multi-peak fitting).