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In vitro studying corrosion behaviour of biocorrodible Mg alloys

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Abstract

The idea of bioabsorbable/biocorrodible stents has gained increasing attention in the last decade. Permanent coronary stents, traditionally made from 316L grade stainless steel, are routinely used for the treatment of blocked arteries. However, these stents can cause complications such as restenosis, thrombosis and the need for the patient to undergo prolonged antiplatelet therapy. Biodegradable metal stents provide an opportunity for the stent to remain in place for a period to ensure restoration of function and then degrade through a carefully controlled bio-corrosion process. Among the number of potentially suitable materials, Magnesium alloys have shown great promise as a stent material due to their non-toxicity [1] and the corrosion rates attainable in biological environments. However, a carefully controlled corrosion process is essential in order to avoid hyper hydrogen generation and the fatal consequences that follow. In addition uniform corrosion is a basic requirement to maintain the mechanical integrity and load bearing characteristics. Work being undertaken in our laboratories focuses on controlling the corrosion behaviour of magnesium in a simulated biological environment in the presence of protein. In the investigation reported here the Mg alloy has been examined using Scanning Electrochemical Microscope (SECM) to visualize the corrosion process and identify the corrosion pattern. Complementary bulk electrochemical techniques (EIS and potentiodynamic polarization) have been used to acquire kinetic and mechanistic information. Early results obtained by SECM have revealed the tendency towards pitting corrosion in the early stages which subsequently develops in to filiform corrosion. Copyright (2012) by the Australasian Corrosion Association.

Keywords

biocorrodible, behaviour, alloys, corrosion, mg, studying, vitro

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IN VITRO STUDYING CORROSION BEHAVIOUR OF BIOCORRODIBLE MG ALLOYS

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SUMMARY: The idea of bioabsorbable/biocorrodible stents has gained increasing attention in the last decade. Permanent coronary stents, traditionally made from 316L grade stainless steel, are routinely used for the treatment of blocked arteries. However, these stents can cause complications such as restenosis, thrombosis and the need for the patient to undergo prolonged antiplatelet therapy. Biodegradable metal stents provide an opportunity for the stent to remain in place for a period to ensure restoration of function and then degrade through a carefully controlled bio-corrosion process. Among the number of potentially suitable materials, Magnesium alloys have shown great promise as a stent material due to their non-toxicity [1] and the corrosion rates attainable in biological environments. However, a carefully controlled corrosion process is essential in order to avoid hyper hydrogen generation and the fatal consequences that follow. In addition uniform corrosion is a basic requirement to maintain the mechanical integrity and load bearing characteristics.

Work being undertaken in our laboratories focuses on controlling the corrosion behaviour of magnesium in a simulated biological environment in the presence of protein. In the investigation reported here the Mg alloy has been examined using Scanning Electrochemical Microscope (SECM) to visualize the corrosion process and identify the corrosion pattern. Complementary bulk electrochemical techniques (EIS and potentiodynamic polarization) have been used to acquire kinetic and mechanistic information. Early results obtained by SECM have revealed the tendency towards pitting corrosion in the early stages which subsequently develops in to filiform corrosion.

Keywords: Bio-corrodible Mg alloy, Scanning electrochemical microscope, in vitro corrosion pattern

1. BACKGROUND

Coronary stents are mesh-like scaffolds which are placed inside the arteries following angioplasty surgery to provide mechanical support and avoid the elastic arterial recoil [1]. Currently most stents consist of a 316L grade stainless steel framework. It has been shown that the artery wall remodels within weeks after angioplasty as a consequence of the mechanical stresses generated by the deployed stent [2]. Therefore the idea of using

other biocompatible and biodegradable materials has gained increasing attraction mainly in order to eliminate the restenosis, and the need for prolonged antiplatelet therapy caused by permanent stenting [3]. Early clinical studies using bioabsorbable metal stents (BMS) have shown promise indicating good mechanical scaffolding and biocompatibility using magnesium based alloys [4]. One of the by products of magnesium alloy degradation is the Mg^{2+} cation which is an essential element and is present in large amounts (the fourth most abundant cation) in the human body [5]. The daily intake of Mg for a normal adult is about 300–400 mg and redundant magnesium cations can be harmlessly and efficiently excreted in the urine [6]. However, rapid corrosion of magnesium produces hydrogen cavities which may result in necrosis of tissues or in the extreme situation can block the blood stream and cause death. Also the local alkalization due to the fast corrosion reaction can cause disruptions to pH dependent physiological reactions in the vicinity of the magnesium implant or in the worst scenario lead to an alkaline poisoning effect [7]. Therefore, it is essential to carefully control the rate of degradation (i.e. corrosion) in order to give adequate time for artery remodelling and hence avoid extensive hydrogen evolution and alkalization. Special attention must be given to the alloy design both to avoid toxicity caused by some of the alloying components [8] and also to achieve a slower rate of corrosion [9]. It has been shown that corrosion rate of Mg alloys may alter by 3 orders of magnitude by inclusion of different alloying elements [10]. Magnesium dissolves in aqueous environments as a consequence of an electrochemical reaction with water to produce magnesium hydroxide and hydrogen gas. The overall reaction may be expressed as:

$$Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2 \quad (1)$$

An insulating layer of $Mg(OH)_2$ forms on the surface in aqueous environments and this protects the metal from further rapid corrosion. The Pilling-Bedworth ratio (P-B ratio) is the ratio of the volume of the elementary cell of a metal oxide to the volume of the elementary cell of the corresponding metal (from which the oxide is created). Therefore a P-B ratio close to 1 corresponds with a consistent and dense oxide layer which can efficiently protect metal from further corrosion. The P-B ratio of 1.77 for $Mg(OH)_2$/Mg indicates a porous oxide film under compression which slows down the diffusion process resulting in a slower corrosion rate [11]. The natural oxide layer breaks down under physiological conditions due to the formation of the highly soluble $MgCl_2$ [12,13]. Oxide dissolution takes place at chloride concentrations exceeding 30 mmol/L promoting pitting corrosion [14,15]. Furthermore, the inclusion of alloying components with different free corrosion potentials introduces surface heterogeneity and therefore promotes non-uniform corrosion [16]. The principal rate-limiting factors in the aqueous corrosion of magnesium alloys are associated with the breakdown of the oxide film and the rate of reformation [17]. These processes are critically dependent on the composition of the Mg alloy and the (bio)chemical environment encountered [18,19].

In addition to inorganic compounds, biological environments contain organic compounds which may adhere or adsorb to the implant surface and change the corrosion behaviour. For example the presence of albumin (a protein with MWt = 66,430 Da, commonly found in biological fluids) has been found to have an effect that is dependent on the alloy composition and the protein concentration [20]. There have been studies that suggest the albumin forms a barrier film in the early stages of contact, which can be destroyed in the long term [18,21]. In some studies a rise in the corrosion rate has been reported as a consequence of adding albumin into the media due to the protein mitigating against effective formation of the passivating layer [13]. On the contrary, corrosion inhibition has been reported with the aluminium containing Mg alloy (AZ91) and a Mg-Ca alloy in SBF (simulated biological fluid) containing albumin [22,23]. It was suggested that the adsorbed layer of protein interacts with aluminium oxide and forms a protective layer which impedes the diffusion of corrosion reactants. It has been reported that protein can chelate metal ions such as Fe, Ti, Zn, Cr, Ni, Co and Cu and enhance the corrosion rate [24-28].

The aim of our study is to investigate corrosion characteristics of Mg alloys in vitro and investigate the effect of protein interaction on the corrosion behaviour. This study is expected to provide foundation for further development of an effective corrosion controlling coating.

2. EXPERIMENTAL

1.1. Material

Simulated biological fluid was prepared using analytical grade reagents consist of 5.403 $g.L^{-1}$ NaCl, 0.504 $g.L^{-1}$ $NaHCO_3$, 0.426 $g.L^{-1}$ $NaCO_3$, 0.225 $g.L^{-1}$ KCl, 0.230 $g.L^{-1}$ $K_2HPO_4 \cdot 3H_2O$, 0.311 $g.L^{-1}$ $MgCl_2.6H_2O$, 0.8 $g.L^{-1}$ NaOH, 17.892 $g.L^{-1}$ HEPES, 0.293 $g.L^{-1}$ $CaCl_2$, 0.072 $g.L^{-1}$ Na_2SO_4. The pH was adjusted to 7.40 ± 0.05 using 1 M NaOH solution. The protein was bovine serum albumin (BSA), fraction V cat no. A-3350, lot 36H0417 from Sigma chem. Co. MWt = 66,430 Da. The protein solution was prepared by dissolving BSA into SBF.

A proprietary Mg alloy coded as Alloy B containing Al and some rare earth elements was specifically formulated for Boston Scientific Co. for stent application. This was supplied in the form of a small tube.

1.2. Bulk corrosion measurement

All bulk electrochemistry experiments were performed using a CH instruments Inc. electrochemical workstation model 660D. Open circuit potential (OCP) was measured in a 2 electrode cell configuration against Ag/AgCl reference electrode. Potentiodynamic polarization technique was used in a standard 3 electrode configuration for bulk kinetic studies. 0.5 cm^2 surface area was potentiostatically scanned within ±150 mV range with respect to OCP at 4 $mV.s^{-1}$ rate. Corrosion rate was calculated by fitting Tafel cathodic and anodic slopes using the "special analysis" module integrated within the CH instrument potentiostat. Corrosion current has been reported here as a measure of corrosion rate of Mg.

Electrochemical impedance spectroscopy was performed in a standard 3 electrode set-up within 10^4 Hz-10^{-1} Hz frequency range with ±5 mV perturbation around OCP. Electrical equivalent elements were extracted from EIS spectrum by fitting an electrical equivalent circuit (EEC) using ZSimpWin v3.30 developed by Princeton Applied Research (figure 1). Pseudocapacitance was calculated using the same program. Pt mesh served as auxiliary electrode and Ag/AgCl served as reference electrode in both tafel and EIS experiments.

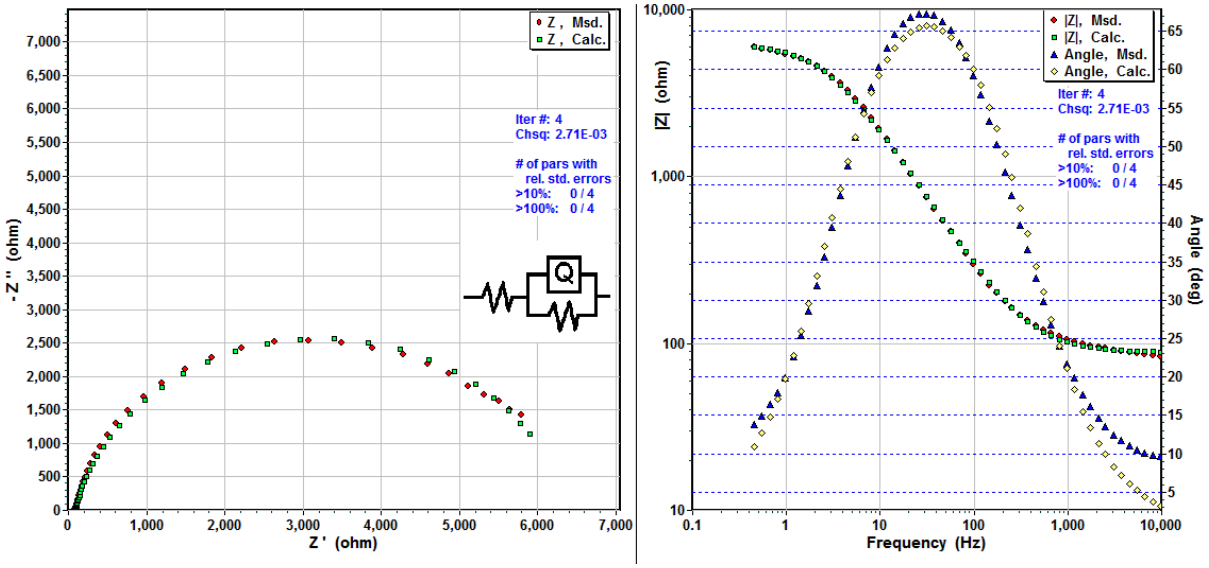

Figure 1 Fitting the EEC on the actual EIS data to extract electrical elements.

1.3. Scanning Electrochemical Microscopy (SECM)

Local electrochemical measurement was performed by scanning electrochemical microscope (SECM) model 920D (CH instruments Inc.) using a 10µm Pt microelectrode and Ag/AgCl reference electrode. A three dimensional SECM image in constant height mode was obtained by scanning the tip in the x-y plane and recording the tip current as a function of tip location. The distance between tip and substrate was adjusted to 5µm using an online digital microscope. Substrate Generation/Tip Collection (SG/TC) mode was employed where the tip potential was held at 0.4 V (vs. Ag/AgCl) to oxidize the naturally generated hydrogen (reaction 2) and locate the active dissolution sites. In this mode the Hydrogen Evolution at both Anodic (AHE) and Cathodic (CHE) can be detected.

$$H_2 \rightarrow 2H^+ + 2e \qquad (2)$$

3. RESULTS AND DISCUSSION

3.1. Bulk corrosion measurement

Open circuit potentials shown in figure 2 indicate a shift to less negative potentials due the addition of albumin to SBF. This potential shift when the solution contains BSA might be associated with a rather porous layer of adsorbed protein that confines the dissolution of Mg oxide and hence may render the surface less active. This is in line with results of Liu *et al.* [29,30]. They suggested that electrostatic bonding between negatively charged amino acid groups (present in albumin) and metal cations may form a relatively stable layer which can potentially impede dissolution of the oxide layer resulting in a more positive potential.

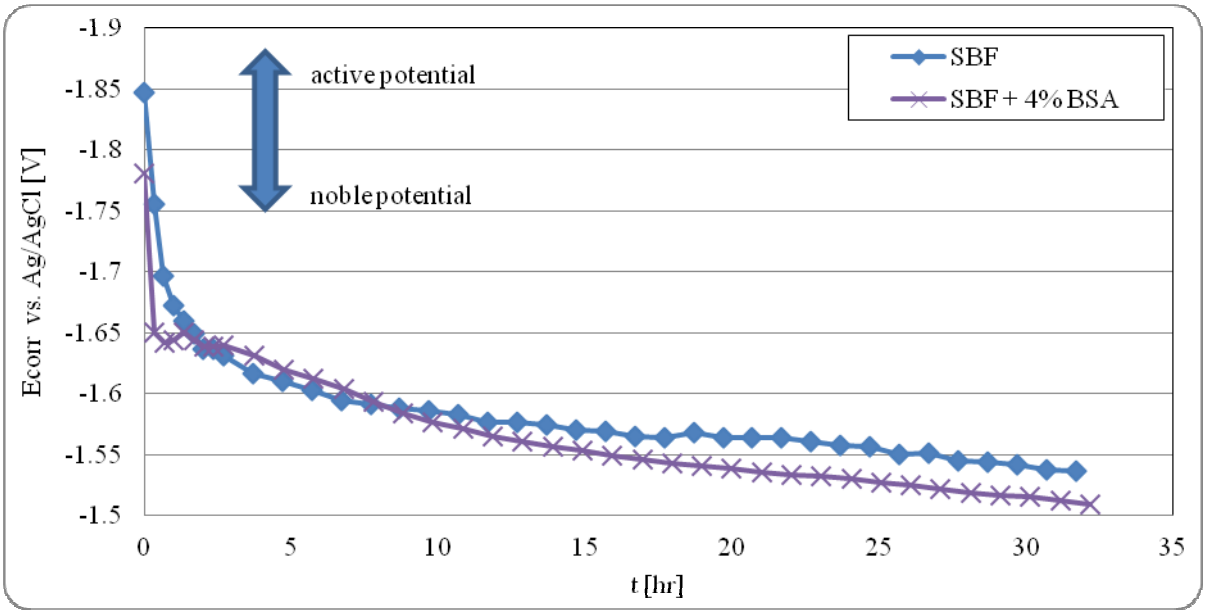

Figure 2 Open circuit potential in contact with simulated biological fluid containing complexing agents and protein.

The corrosion rate data in figure 3 indicate that addition of 4% BSA significantly retards the corrosion in the early stages. However, after about 3 hrs under these conditions, the corrosion rate for the BSA containing solution increases rapidly reaching values close to the neat SBF solution. This behaviour suggests that the protein quickly forms a resistive layer on the Mg surface in the presence of BSA thereby hindering the diffusion of corrosive species and thus suppressing the initial corrosion rate. In contrast, the more rapid corrosion initially seen in the SBF system causes a build up of corrosion deposits which eventually leads to a lower corrosion rate, comparable to the BSA containing system. Similar effect has been reported by Wang *et al.* indicating rapid adsorption of albumin on Mg surface in early stages resulting in a lower corrosion rate [31]. They have revealed that albumin can effectively chelate the corrosion product in the long term leading to a higher corrosion rate.

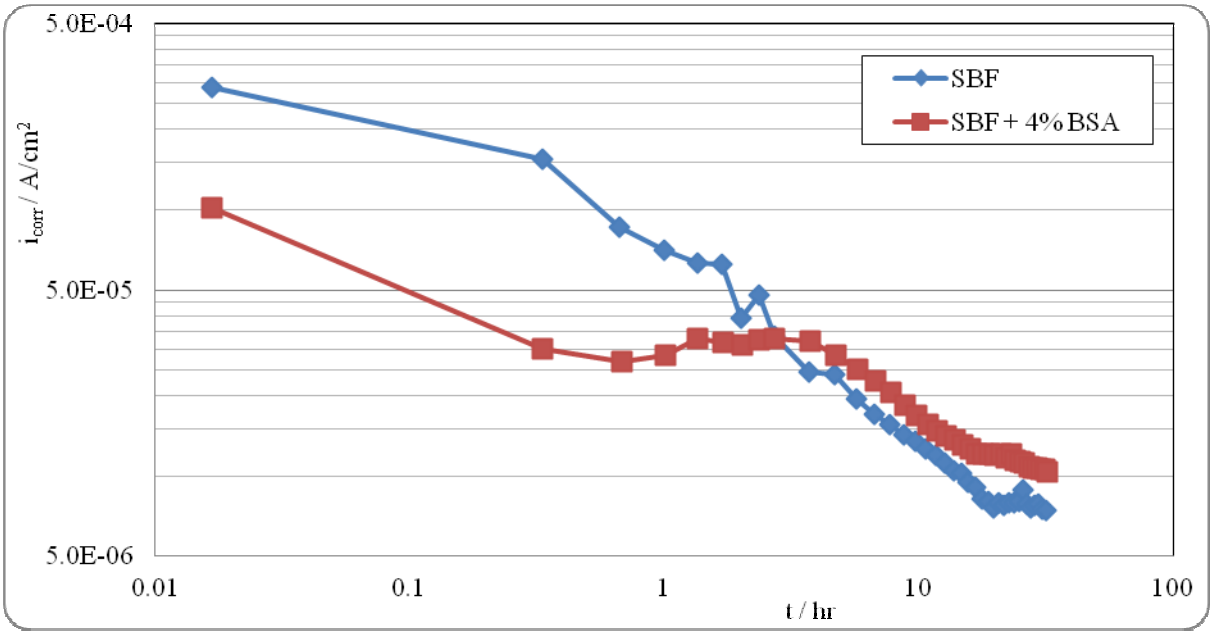

Figure 3 Corrosion rate of Mg alloy in contact with simulated biological fluid containing complexing agents and protein.

Figure 4 shows increase of capacitance coefficient within the diffusion layer formed by corrosion deposits on the Mg surface when exposed to SBF. This is probably due to the rapid corrosion process in the early stages which may result in either roughening of the surface or the growth of a more dielectric film in the first 8 hours. This is followed by a thickening of the corrosion product leading to a decrease in the capacitance value. In contrast, the capacitance value in the presence of BSA initially decreases, consistent with a thickening of the surface layer, and then is relatively unchanged over 30 hr period, consistent with the BSA hindering the extensive build up of corrosion deposits.

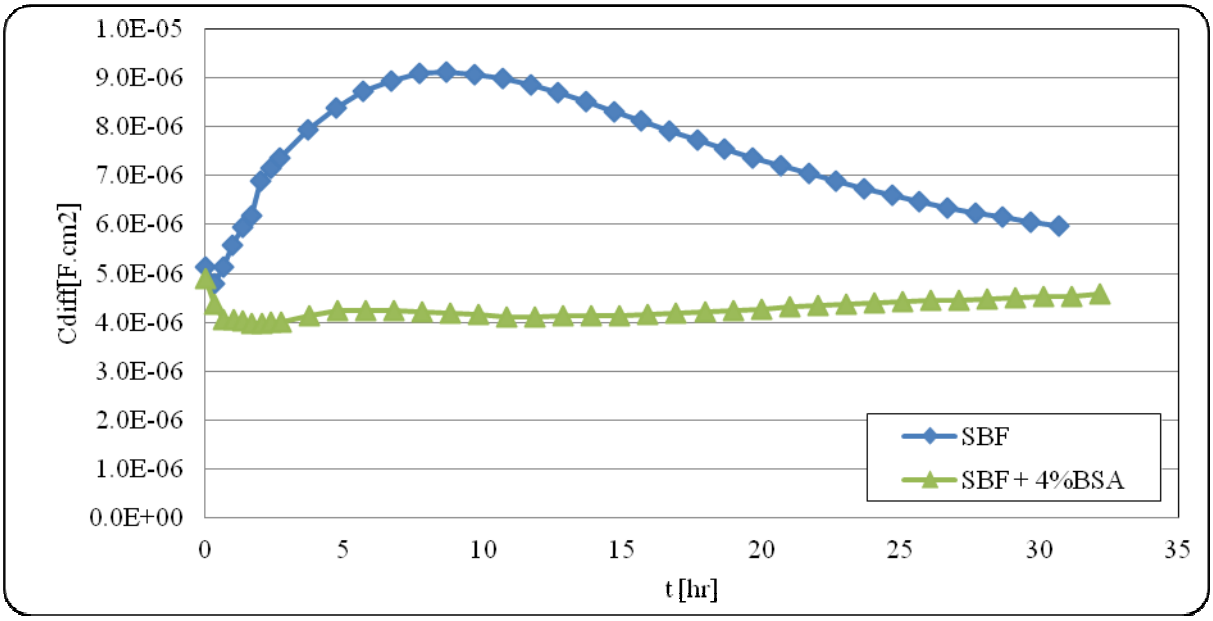

Figure 4 Capacitance value associated with diffusion layer, Cdiff, on Mg surface in contact with simulated biological fluid containing 4% BSA.

3.2. SECM in Generation/collection mode

Substrate generation/tip collection (SG/TC) mode of SECM was utilized to directly sense the hydrogen evolution from the corrosion process. In this mode an electroactive specie generated at the substrate can be oxidized or reduced at a certain potential at the microelectrode tip, therefore giving direct information from the kinetic of the reaction at the substrate. Hydrogen oxidation was the target reaction to directly sense the hydrogen generated on Mg surface as a bi-product of corrosion process. Reaction (2) expresses the oxidation reaction being sensed at the SECM microelectrode tip. The hydrogen is a bi-product of corrosion process at either the anodic and cathodic sites.

$$H_2 \rightarrow 2H^+ + 2e \qquad (2)$$

Reaction (2) in alkali condition near the Mg surface may change to the reaction (3):

$$H_2 + 2OH^- \rightarrow 2H_2O + 2e \qquad (3)$$

At higher pH where a reasonable amount of OH^- is present the reaction (3) will dominate the hydrogen consumption at the microelectrode. The Nernst equation for either of these reactions will result in the equation (4) where the pH dependence of the reaction is defined:

$$e_{H^+/H_2} = e^{\circ}_{H^+/H_2} - 0.059\ pH \qquad (4)$$

According to the equation (4) at pH as high as 8.5 [32] in the close proximity of the Mg surface the oxidation potential of hydrogen is -0.5 V SHE (~ -0.7 V vs. Ag/AgCl). Therefore any potential higher than -0.5 V SHE may be set as tip potential to sense the hydrogen production. However, as a result of the oxidation reaction of water interfering with the anodic current from the target reaction (oxidation of hydrogen) at low potentials, the potentials as low as -0.7 V vs. Ag/AgCl was not approached. A good resolution and sensitivity was observed at +0.4 V (Ag/AgCl) at which the SECM experiments in SG/TC mode were performed.

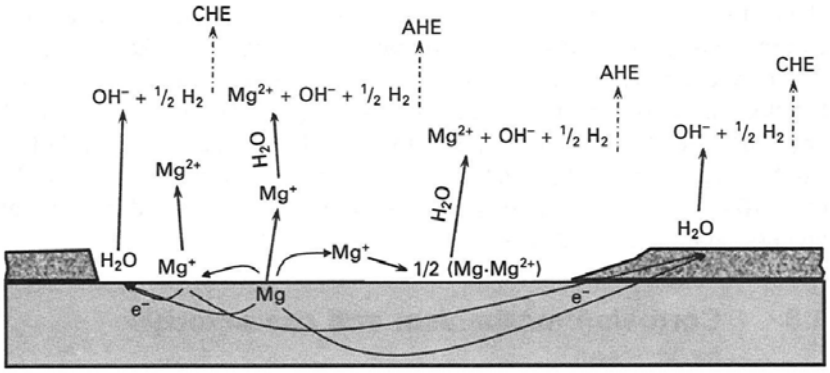

Figure 5 Schematic illustration of primary anodic and cathodic reactions involved in the self-corrosion of Mg [33].

3.2.1. Corrosion regime

Figures 6a-6c show initiation of the filiform corrosion after 11 hr of contact with simulated biological fluid and its development across the Mg surface in approximately 5 hr. Corrosion proceeds through “pitting” pattern with stable pits forming within the first 10 hrs without any sign of filiform corrosion (figure 6a), but subsequently converts to a filiform corrosion regime (figure 6b), possibly due to the build up of a resistive layer over active areas. Filiform or wormtrack corrosion is caused by an active corrosion cell which moves across a metal surface. Usually the head is the anode and the tail the cathode. Filiform corrosion occurs under protective coatings and anodized layers. It has been shown that filiform corrosion can occur on uncoated AZ91 and this indicates that a relatively resistant oxide film can naturally be formed on this alloy [16]. The filaments are covered by an oxide film which is then fractured by evolving hydrogen gas. The initiation site of filament is shown in the figure 6b at

the top-right corner of the image in a dashed square. Relatively rapid growth of this filament in 5 hrs results in a developed filiform pattern highlighted by the dashed box (figure 6c).

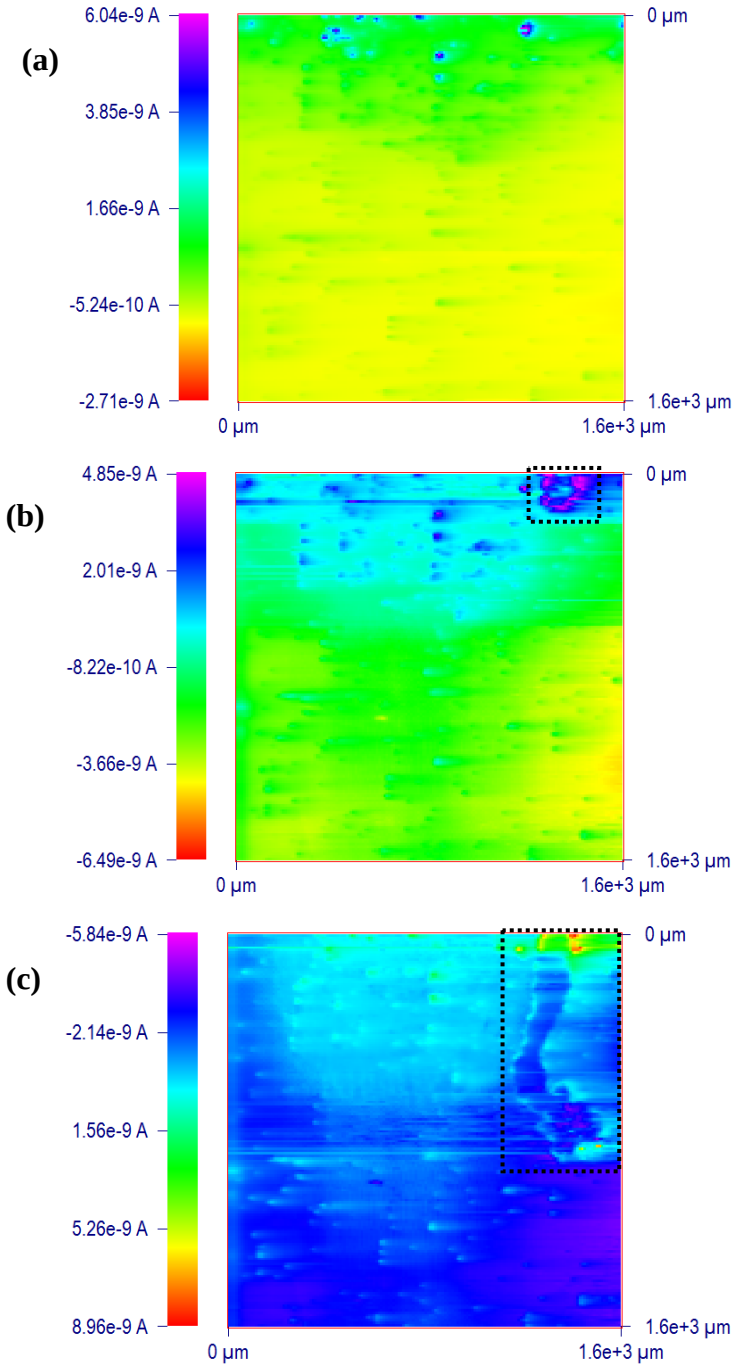

Figure 6 SECM images taken after (a) 10 hr (b) 11 hr (c) 16 hr contact with simulated biological fluid.

3.2.2. The effect of protein

The effect of addition of bovine serum albumin to SBF was studied in the early stages of corrosion. Figure 7a and 7b show the electroactivity of Mg surface in SBF and SBF containing 4% BSA, respectively. Upper scale colours (e.g. blue) represent electroactive sites and lower scale colours (e.g. green, red) represent relatively passive sites. It can be seen that the number and extent of electroactive sites has been significantly reduced in presence of albumin. In addition, protein has significantly altered the corrosion regime with the Mg surface

becoming significantly less active and corrosion regime has moved towards a less localized pattern due to adsorption of protein on the surface. Similar behaviour had been observed by Retting et al. [18] and Wang et al. [21] using bulk corrosion measures. It is believed that protein adsorbs to Mg surface in the early stages of corrosion resulting in a hindered diffusion process and reduced corrosion rate. However, surface alkalization in the long term may decompose protein structure and release amino acids that can act as effective chelating agents [18, 21]. Consequently this can remove the corrosion deposits and increases the corrosion rate by activating the surface (figure 3). Further study will be required to elucidate the effect of protein in the long term exposure.

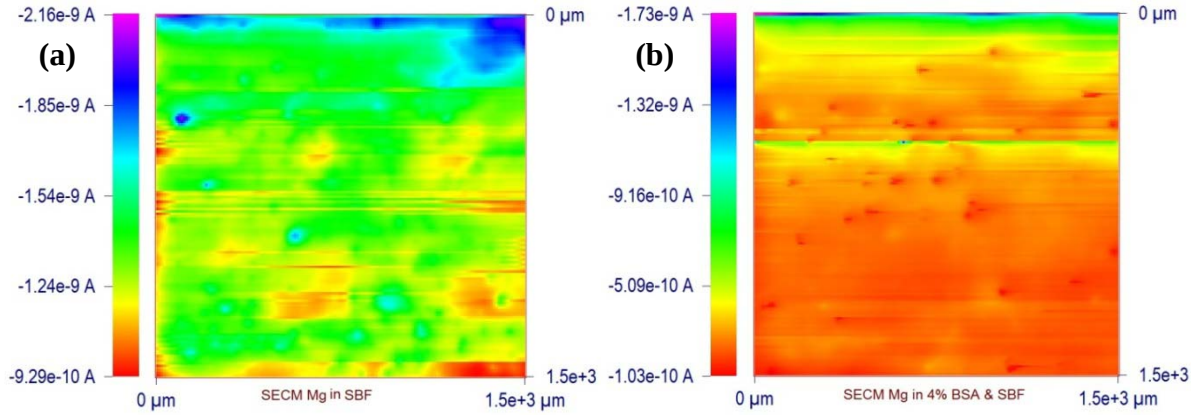

Figure 7 SECM images of Mg surface in (a) SBF and (b) SBF+4% BSA after about 1 hr.

4. CONCLUSION

Effect of protein on kinetic and regime of corrosion was studied. It has been shown that a layer of protein adsorbs on the active surface of Mg and forms a relatively resistive layer which slows down the extensive rate of corrosion in the early stages. Also it was shown that a high rate of corrosion in the early stages may result in extensive build-up of corrosion products thus generating a more nobel surface.

Corrosion regime was investigated using scanning electrochemical microscope (SECM) in simulated biological fluid (SBF) and the effect of protein was studied. It was shown that corrosion regime changes from local pitting corrosion to filiform or wormtrack corrosion pattern in the long term contact with SBF. This is due to resistive corrosion product on the surface. Also it was evidenced that protein forms a relatively protective layer on Mg surface which significantly passivates electroactive dissolution sites in the early stages of corrosion.

5. ACKNOWLEDGMENT

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7. AUTHOR DETAILS

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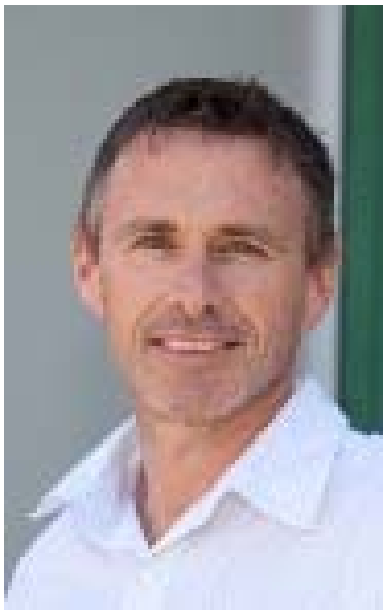

A/Prof. Simon Moulton is Associate Leader, ACES Bionics Program at the Intelligent Polymer Research Institute, University of Wollongong. Since completing his PhD in 2002, Simon has been with IPRI as a post-doctoral researcher (post doc). He has published a book on Organic Bionics (2012) as well as 52 journal articles in international journals such as Science, Advanced Materials and Advanced Drug Delivery Reviews. Currently Simon supervises 7 PhD students and oversees research projects in drug delivery for epilepsy control, as well as materials development for muscle, nerve and bone regeneration.

Professor Maria Forsyth, Chair of Electromaterials and Corrosion Science is the Associate Director of ARC Centre of Excellence for Electromaterials Science and ARC Australian laureate fellow. Prof. Forsyth's primary focus is in energy storage and materials characterization. Her research focuses on developing an understanding of charge transport at metal/electrolyte interfaces and within electrolyte materials. Such materials have included a range of novel ionic liquids, polymer electrolytes and plastic crystals. Using this understanding, her team collaborates very productively with colleagues within academia, CSIRO, DSTO as well as industry to design new materials and processes to control and optimise these phenomena in two key areas – corrosion and electrochemical devices. Professor Forsyth's group has also developed a range of unique characterisation approaches including multinuclear, variable temperature pulsed field gradient NMR, with which they investigate ion transport mechanisms in electrolyte materials and surfaces.

Prof. Gordon G Wallace is Director of the Intelligent Polymer Research Institute, University of Wollongong. Gordon completed his undergraduate (1979) and PhD (1983) degrees at Deakin University. And was awarded a DSc from Deakin University in 2000. He was appointed as a Professor at the University of Wollongong in 1990. He was awarded an ARC Professorial Fellowship in 2002; an ARC Federation Fellowship in 2006 and ARC Laureate Fellowship in 2011. He has published more than 600 refereed publications; a monograph (3rd Edition published in 2009) on Conductive Electroactive Polymers: Intelligent Polymer Systems and co-authored a monograph on Organic Bionics (published 2012). Gordon has supervised more than 65 PhD students to completion.