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Controlling initial biodegradation of magnesium by a biocompatible strontium phosphate conversion coating

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ABSTRACT

A simple strontium phosphate (SrP) conversion coating process was developed to protect magnesium (Mg) from the initial degradation post-implantation. The coating morphology, deposition rate and resultant phases are all dependent on the processing temperature, which determines the protective ability for Mg in minimum essential medium (MEM). Coatings produced at 80 °C are primarily made up of strontium apatite (SrAp) with a granular surface, a high degree of crystallinity and the highest protective ability, which arises from retarding anodic dissolution of Mg in MEM. Following 14 days' immersion in MEM, the SrAp coating maintained its integrity with only a small fraction of the surface corroded. The post-degradation effect of uncoated Mg and Mg coated at 40 and 80 °C on the proliferation and differentiation of human mesenchymal stem cells was also studied, revealing that the SrP coatings are biocompatible and permit proliferation to a level similar to that of pure Mg. The present study suggests that the SrP conversion coating is a promising option for controlling the early rapid degradation rate, and hence hydrogen gas evolution, of Mg implants without adverse effects on surrounding cells and tissues.

1. Introduction

Magnesium (Mg) based biomaterials have a density and elastic modulus similar to bone, which can minimize the stress shielding effect [1,2]. Implant materials made of Mg alloys degrade *in vivo* and are eventually excreted with urine [3,4]. This eliminates the need for secondary surgery to remove the implant and also avoids interface loosening and associated inflammation [5]. In addition, it has been recognized that Mg^{2+} ions assist the growth of new bone tissues and shorten fracture healing time [6]. The rapid degradation rate of Mg implants in the physiological system (pH 7.4–7.6) can lead to accumulation of subcutaneous hydrogen (H_2) gas, which at high levels can cause separation of tissue and tissue layers [7–10]. Moreover, the high degradation rate is most deleterious in the period immediately following implantation [11]; therefore, control of the initial degradation is of paramount importance [12,13]. Applying biocompatible and protective coatings, such as hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$), a well-known bioactive material with close chemical and structural resemblance to human bones and teeth, onto metallic implants is a practical option to moderate the biodegradation process [14–18]. Such coatings have been shown to enhance biological fixation of implants to hard tissues [19,20] and facilitate functional implant deployment [21–24] without weakening the intrinsic mechanical properties. Nevertheless, HA films produced via physical deposition, such as plasma spray, present low adhesion strength and low compactness, leading to potential delamination of coatings and failure of biological fixation [25]. Impurities, such as dicalcium phosphate dehydrate and tricalcium phosphate are commonly seen within HA deposits prepared by chemical routes, with these being shown to play an inhibitor role in ossification [26,27]. Thus, engineering of a load-bearing metallic implant system with satisfactory mechanical properties, well controllable biodegradation rate, desirable bioactivity and biocompatibility remains essential.

It was reported that strontium (Sr), a trace element in the human body, is of benefit in enhancing bioactivity and biocompatibility [28–38]. Sr ions depress bone resorption, develop bone mechanical properties, enhance replication of preosteoblastic cells, and stimulate bone formation, thus eventually preventing bone loss [28]. It has been hypothesized that the presence of Sr at the interface between an implant and bone will ensure the longevity of a joint prosthesis [29]. Sr ions have the same physiological and chemical behaviour as Ca^{2+}, the major component of bone, and can be embedded into the mineral structure of bone by ionic substitution for Ca [30,31]. Sr may replace lattice sites of Ca in HA and

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form a continuous solid solution (Sr.*x*–HA, *x* is fraction of substitution) up to full substitution (Sr–apatite) with higher stability, more regular shape and stronger mechanical properties [32,33].

There have been intensive studies on the *in vitro* [34] and *in vivo* [35,36] behaviour, cytotoxicity [37] and bone formation mechanism [38] of Sr.*x*–HA cements and ceramics. Sr.*x*–HA coatings have also been implemented on metallic implants, in particular titanium (Ti), for improved bone formation. A biomimetic method was applied to deposit Sr.*x*–HA coating on Ti [29]. It was found that the fraction of Sr in HA coating depended on the concentration of Sr^{2+} ions in the coating solution, but the presence of Sr^{2+} ions was also found to reduce the overall coating thickness. Porous Sr.*x*–HA films have also been plasma sprayed onto Ti–6Al–4V [39] and demonstrated to possess satisfactory mechanical properties and *in vitro* bioactivity. Capuccini et al. [40] employed pulsed-laser to deposit Sr–HA coating on Ti and discovered that the presence of Sr in the coating reinforced the positive influence of HA on osteointegration and bone regeneration, while concomitantly reducing bone resorption. A series of Sr–HA coatings with various ratios of Sr and Ca were prepared via micro-arc treatment to elucidate the effect of Sr content on osteointegration [41]. It was revealed that the 100% Sr substituted HA played the most profound role in promoting osteoblast differentiation and inhibiting osteoclast differentiation, which is beneficial for anti-osteoporosis purposes. Furthermore, well-ordered $SrTiO_3$ nanotubes, formed on the surface of Ti implants by hydrothermal treatment, were reported to significantly enhance their osteointegration and increase the torque for removal [42,43]. This was attributed to the enhanced osteoblast differentiation and new bone apposition in both cortical and cancellous bone [43].

Though the beneficial effects of Sr on bone regeneration are clear, studies exploring the effect of Sr coated Mg-based biomaterials either on inhibiting biodegradation or osteointegration are rare, to date. In the present study, a new group of Sr phosphate (SrP) conversion coatings were developed, characterized and optimized to retard the initially high dissolution rates of Mg. Biodegradation was monitored by immersion in minimum essential medium (MEM), electrochemical tests and inductively coupled plasma–time of flight mass spectrometry (ICP-ToF MS). Biocompatibility was assessed by investigating the proliferation and differentiation of hMSCs *in vitro*, exposed to the biodegradation extracts.

2. Materials and methods

2.1. Preparation of SrP conversion coatings

High purity (>99.9%) Mg specimens (⩽40 ppm Fe) with dimensions 10 × 10 × 5 mm were ground to 1200 grit finish and used as substrate materials. Prior to the coating process, Mg blocks were ultrasonicated in acetone at room temperature for 15 min, followed by absolute ethanol for 10 min and then rinsed with deionized water. In this study, SrP coatings were only applied to pure Mg and, as a result, coating pre-treatment, which is critical in the case of (any) conversion coating for Mg alloys, was not discussed [44,45]. A coating solution contained 0.1 M $Sr(NO_3)_2$ and 0.06 M $NH_4H_2PO_4$ and pH was adjusted to 3.0 by HNO_3. A one-step conversion coating process was conducted by immersing Mg specimens in the coating solution at various temperatures from, 40 to 80 °C. Coated specimens are assigned names according to the coating temperature (i.e. Mg40C was coated at 40 °C); while uncoated Mg is used as the "control". Coated Mg samples were dried in air and kept in a desiccator for further characterization. All chemicals used in this work are analytical grade from Sigma–Aldrich (Sydney, Australia).

2.2. Microstructural analysis of SrP conversion coatings

Imaging was carried out using scanning electron microscopy (SEM; FEI Nova Nano). The profile of the surface of various Mg specimens was analysed with a VECCO WYKO NT1100 optical profilometer. The structure and phase composition of various Mg surfaces before and after coating were identified by X-ray diffraction (XRD; Philips PW1140), using Cu K_α radiation ($\lambda = 1.5418$ Å, 40 kV, 25 mA) at a scanning speed of 0.01°min^{-1} at a 2θ range of 20–60°. Surface chemistry was analysed by X-ray photoelectron spectroscopy (XPS; Thermo K-alpha) with a hemispherical "analyzer"? and the core level XPS spectra for Sr3d, Mg2p, P2p, O1s and C1s were recorded. The measured binding energy values were calibrated by the C1s (hydrocarbon C–C, C–H) of 285 eV. The photoelectrons were generated by Al K_α (1486.6 eV) primary radiation (20 kV, 15 mA).

2.3. Adhesion strength

The adhesion strength of Mg80C SrP coating was measured by the modified ASTM C-633 method [46]. Both sides of the substrates (SrP coating was left on one side only) were attached to cylindrical stainless steel jigs 16 mm in diameter and 15 mm in length with Rapid-type® Araldite glue (Ciba-Geigy Ltd., Switzerland). A tensile load was applied to the substrates with a Lloyd tensile tester machine (LR 30K, Lloyd Instruments Ltd.) at a crosshead speed of 1 mm min^{-1} until fracture occurred. Three specimens were used to calculate the mean tensile bond strength and standard deviation according to the fracture load and surface area ($\pi \times 82$ mm^2).

2.4. Immersion testing

Immersion tests were carried out in MEM [12,47–49] (Invitrogen Australia Pty. Ltd.) in a sterile Sanyo incubator maintained at 37 °C and an atmosphere of 5% CO_2 and 95% air. The chemical composition of MEM and human plasma can be seen in Table 1 [47,50]. Immersion was carried out up to 14 days. The samples with an exposure surface area of 4 cm^2 were placed into vented containers containing 20 ml of MEM buffered with 2.2 g l^{-1} of sodium bicarbonate. A physiological pH value of 7.4 was maintained during the test [51]. The MEM medium was refreshed every 2 days to maintain the ion levels. The H_2 volume evolved during exposure was collected using a funnel placed over the specimen to ensure the H_2 gas originated from the specimen surface. A burette was mounted over the funnel and filled with MEM. The connections of funnel–burette and burette–peleus ball were sealed using Para film to avoid leakage. The Sr^{2+} ion release into MEM after

Table 1
Details of human plasma and MEM medium.

Components	Human plasma	MEM
Na^+	142	117.4
Cl^-	103	123.5
K^+	5.0	5.4
Ca^{2+}	2.5	1.8
Mg^{2+}	1.5	0.4
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.4
D-Glucose	5	5.5
Bicarbonate (HCO^{3-})	22–30	26.2
Phenol red	–	0.03
Albumin (g l^{-1})	34–54	–

All concentrations in mmol l^{-1} unless otherwise stated.
Concentration of inorganic blood contents given as in Ref. [50].
A11900-024, Invitrogen.