Scanning Electron Microscopy Study of Di-Calcium Phosphate Dehydrate Coatings on Magnesium Substrates for Potential Use in Orthopaedic Implants

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Abstract: Magnesium has attracted considerable medical interest due to its mechanical properties being similar to bone. In addition, magnesium is also biocompatible and biodegradable, which makes it an ideal candidate for biodegradable orthopaedic implants. However, magnesium's high corrosion rate in body fluids makes it an unsuitable material for the manufacture of implants. The present study investigates a straightforward chemical immersion technique that deposits di-calcium phosphate dehydrate (DCPD) coatings onto magnesium substrates to increase their corrosion resistance to body simulated fluids like phosphate buffer saline solution and Ringer's solution. Scanning electron microscopy revealed the coating structures and morphologies were characterised by flower-like surface feature that were resistant to both body simulated fluids. Thus, indicating the coatings could significantly reduce magnesium corrosion rates in the body environment.

Keywords: Magnesium, Biodegradation, Protective Coating, Orthopaedic Implants

Introduction

A variety of materials have been used to manufacture orthopaedic implants for many years. Biocompatible polymers have a wide range of desirable properties that can be varied during the synthesis process [1]. However, they have low physical strength compared to both ceramic and metallic implants. Therefore, biopolymers are predominantly used in soft tissue engineering. Bioceramics have good strength, chemical stability and can promote osteointegration in bone tissue engineering. But they are also brittle and have low fracture toughness. This makes them unsuitable for load bearing applications where loading direction can repeatedly change and when loads themselves are constantly fluctuating [2, 3]. However, under changing and fluctuating loads metallic materials display superior properties such as greater ductility, higher strength, greater fracture toughness and useful anticorrosion properties [4, 5]. Metallic materials used in implants include cobalt-chromium based alloys, stainless steels and titanium alloys. However metallic implants also suffer from two major *in situ* operational problems. The first involves the elastic modulus of metallic materials being several times greater than that of bone. Typically, cobalt-chromium based alloys are about ten times greater, while titanium based alloys are around five times greater. The significant difference between elastic modulus's results in stress-shielding, which causes bone resorption and subsequent implant failure [3, 6]. The second problem results from biological corrosion and mechanical wear of the implant, which results in the release of toxic metal ions that immediately produce an unfavourable immune response. The response reduces implant biocompatibility and often leads to secondary revision surgery [7, 8]. Furthermore, from an operational perspective many implants are only needed for a short period to provide structural and mechanical support during tissue regeneration. And after the healing period an additional surgical procedure is needed to remove the implant, which can increase the risk of infection and produce further patient scarring [9].

An alternative to conventional metallic implant materials is the use of biodegradable materials [10]. The advantage of using a biodegradable implant is that during tissue regeneration the implant slowly degrades, and by the end of the healing process the newly formed tissues start carrying the load previously handled by the implant [11]. In recent years magnesium (Mg) has attracted medical interest, since its mechanical properties are similar to bone.

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And importantly, it is both biocompatible and biodegradable [12]. In terms of mechanical properties, Mg has a density of 1.74 g/cm^3 at 20°C, which is slightly lower than that of bone (1.8 to 2.1 g/cm^3). And importantly, Mg's elastic modulus is 45 GPa, which is within the range of bone (40 to 57 GPa) [13, 14]. Because of these attractive mechanical properties and its biocompatibility, Mg has become a promising biodegradable material for the manufacture of orthopaedic implants [12, 15]. However, Mg low corrosion resistance and high degradation rates in chloride rich body fluids (pH: 7.4 to 7.6) has limited its use in orthopaedic applications. The first consequence of high corrosion rates is the formation of subcutaneous hydrogen gas bubbles during the first week after surgery and the subsequent release of high levels of Mg ions during the implant life [16]. And the second consequence results from the loss of mechanical integrity between the implant and the surrounding bone during tissue regeneration [17]. However, several studies have shown reducing the corrosion rate will not only reduce hydrogen formation to acceptable levels, but will also reduce the release of Mg ions into surrounding tissues [18, 19]. An effective method for reducing the corrosion rate is to coat Mg with corrosive resistant layers to moderate the degradation rate. Thus, regulating the degradation rate is important factor in producing a viable biodegradable Mg implant. The present study used a straightforward chemical immersion process to deposit di-calcium phosphate dehydrate or brushite [DCPD; $CaHPO_4.2H_2O$] surface coatings onto Mg substrates. The study examined the relationship between immersion time and coating deposition (mg/mm^2). The influence of phosphate buffer saline (PBS) solution and Ringer's solution on substrate surface coating was investigated using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

All chemicals used in this study were supplied by Chem-Supply (Australia) and all aqueous-based solutions were produced from Milli-Q® water (10 MΩ cm^{-1}) produced from a Milli-Q® Reagent water generation system supplied by the Millipore Corporation.

2.2. Magnesium substrate preparation

Magnesium (Mg: 99.9% pure) ribbon 5 mm wide and 0.5 mm in thickness was cut into 42mm long rectangular strips. Surface cleaning of strips started with polishing using 120pp silicon carbide (Si C) paper to remove any major surface oxides and contaminants. This was flowed by finer polishing using 240pp paper to remove any surface texturing produced during the previous polishing step. After polishing, the strips were first cleaned in a 5% wt. nitric acid (HNO_3) solution, followed by washing in acetone, then rinsed with Milli-Q® water and then allowed to air dry. After drying the weight of each substrate was recorded using an Ohaus Pioneer PA214C analytical microbalance.

2.3. Electrolyte solution for surface treatment of substrates

Di-calcium phosphate dehydrate (DCPD) coatings were produced from an electrolyte prepared at room temperature (25 ± 1ºC). The electrolyte preparation consisted of adding 0.32 M of Ca $(NO_3)_2$ and 0.19 M of KH_2PO_4 to 100 mL solution of Milli-Q® water contained in a volumetric flask. The content of the flask were then thoroughly mixed for 10 min at 400 rpm. After mixing the resulting electrolyte pH was 4. Coatings were produced by immersing individual substrates in the electrolyte contained in separate 10mL sample vials. The substrates were clamped in position using a 15 mm fold-back clip as seen in Figure 1 (A). After immersion, the substrates were removed from the electrolyte at pre-determined time intervals (7.5, 15, 30, 60, and 180 min) and washed in Milli-Q® water, and then allowed to dry for a least 12 h under vacuum to remove all traces of moisture. The substrates were then reweighed using the Ohaus Pioneer PA214C analytical microbalance.

2.4. Solutions and substrate degradation evaluations

Two liquid agents were used to evaluate the corrosion resistance of treated and untreated Mg substrates. The agents used were phosphate buffer saline (PBS) solution and Ringer's solution. The PBS solution was made up of (in g/L) 8.006 NaCl, 0.201 KCl, 1.420 Na_2HPO_4 and 0.240 KH_2PO_4 that were added to 500mL of Milli-Q® water contained in a volumetric flask. Ringer's solution was made up of (in g/L) 8.6 NaCl, 0.6 KCl and 0.66 $CaCl_2. 2H_2O$, which was added to 500mL of Milli-Q® water contained in a volumetric flask. Both solutions were thoroughly mixed and their respective pH's adjusted to 7.4. During the degradation studies, the substrates were clamped in a vertical orientation, and immersed in the respective test mediums for the test period (1, 2 and 3 days). After the specific test period, substrates were removed from test mediums and washed with Milli-Q® water before being dried for at least 12hr under vacuum.

2.5. Scanning electron microscopy (SEM) study

A JEOL JCM-6000, NeoScope™ electron microscope generated images that were used to evaluate the degree of substrate degradation. SEM images were also used to examine the size, morphology and topographical features of the deposited DCPD coatings. Before microscopy analysis, dried substrates were attached to SEM holders using carbon tape and then gold coated (1 minute period) in an SPI-Module™ Sputter Coater to prevent charge build up. In addition, Microsoft® Photoshop was used to colourise SEM images to highlight substrate surface features.

3. Results

3.1. Degradation of untreated substrates in PBS and Ringer's solutions

Visual inspection of untreated Mg substrates exposed to PBS and Ringer's solutions revealed high rates of degradation. Ringer's solution was found to be the most aggressive towards the untreated substrates as seen in Figure 1(B). SEM images revealed the corrosion resistance of the untreated substrates was poor, with extensive regions completely degraded and large areas of lost material as seen in Figure 1(C). Similar image analysis also confirmed PBS had a similar effect on untreated substrates. Thus, highlighting the need for a coating to reduce the degradation rate.
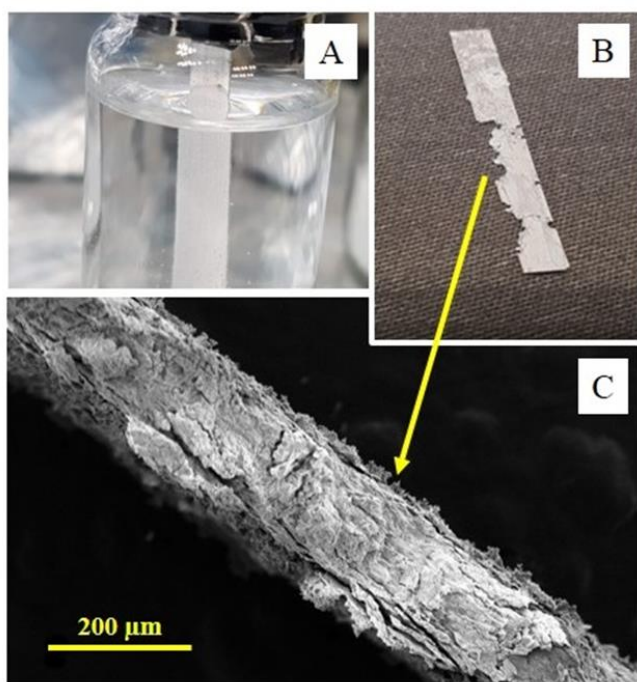

Figure 1. (A) Electrolyte vial with substrate clamped in position, (B) Substrate corroded by Ringer's solution and (C) SEM image showing the degree of corrosion.

3.2. Formation of DCPD coatings on substrates

Immersion of substrates in surface treatment electrolyte solutions resulted in the deposition of a DCPD coatings. The deposition rate varied over the 180 minute immersion period as seen in Figure 2(A). In the early stage there was a rapid deposition rate, then between 30 and 60 minutes the formation rate started slowing. And then from 60 minutes onwards the deposition continues to slow and by 130 minutes it starts levelling off. During this later period there was a noticeable decline in gas bubbles being formed. This decline was credited to the newly deposited coating, which prevented the electrolyte reaching the underlining substrate. Representative SEM images of treated substrates are presented in Figure 2(B) and

Figure 2(C). An edge image is presented in Figure 2(B) and clearly shows the DCPD flower-like structures completely covering the entire region. Similarly, the other surfaces of the substrate were completely covered by a flower-like DCPD coating as seen in Figure 2(C).
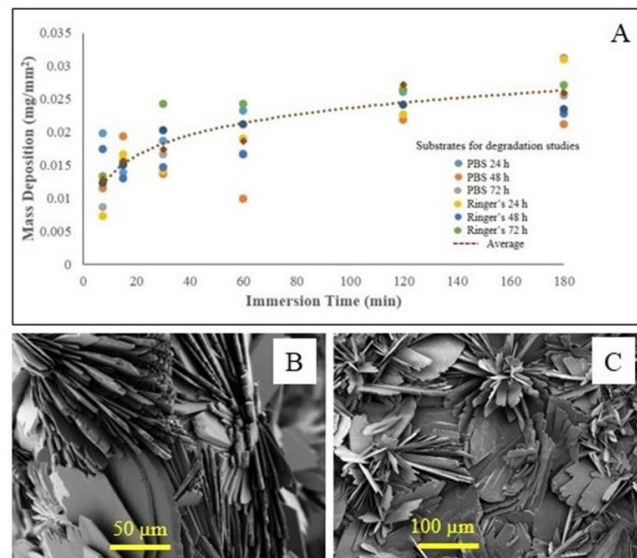

Figure 2. (A) DCPD deposition rates on substrates needed for degradation studies and DCPD coatings composed of flower-like structures (B) Edge view and (C) Surface view.

3.3. Evaluation of substrate coating degradation

Both PBS and Ringer's solution can severely corrode unprotected Mg substrates. The presence of DCPD coatings on all of the substrates improved their corrosion resistance compared to uncoated Mg substrates. In the case of PBS, SEM image analysis revealed coating could significantly increase substrates resistance to corrosion over the 72 h test period as seen in Figures 3 (A), (B) and (C). The study revealed the bulk of the coating remains intact, but there were indications of coating separation and charges in coating morphology as seen in Figure 4. In the case of Ringer's solution, the colourised SEM images presented in Figure 3 (D) and (E) show the dramatic reduction in corrosion over a 24 h period. The uncoated Mg substrate in Figure 3 (D) shows extensive corrosion, while the coated substrate in Figure 3 (E) reveals no corrosion damage to the substrate. Thus, highlighting the increase in corrosion resistance provided by the coating. Closer examination of SEM images also reveals some micro-fractures between the coating and the substrate as seen in Figure 4 (A). Also in the case of PBS there was also evidence of partial coating dissolution and reforming taking place which resulted in the formation of acicular crystal growth as seen in Figures 4 (B) and (C).
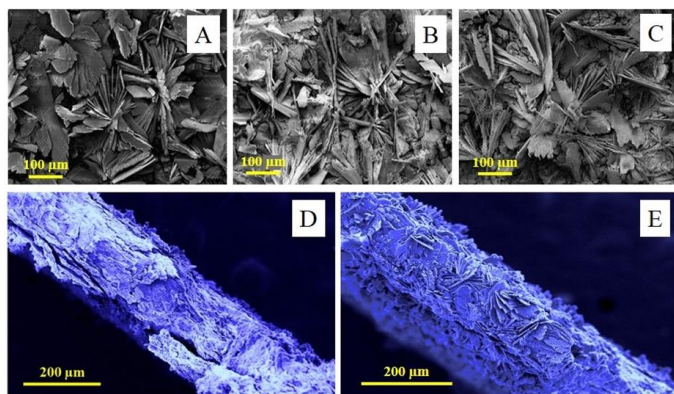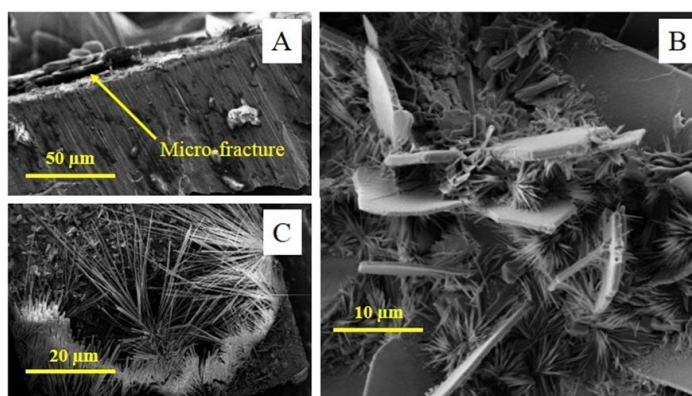
Figure 3. PBS degradation of DCPD coating after (A) 24 h, (B) 48 h and (C) 72 h. Ringer's solution (D) degradation of pure Mg substrate after 24 h and (E) degradation of DCPD coating after 24 h.

4. Discussion

For a biodegradable Mg orthopaedic implant to be effective, it must slowly degrade and allow regenerating bone tissues to progressively take over the mechanical and structural function of the implant. However, in spite of being biodegradable and having similar mechanical properties to bone, Mg's rapid corrosion rate in chloride rich body fluids has limited its use in orthopaedic applications. The present SEM study evaluated the effectiveness of DCPD coatings to improve corrosion resistance and slow down degradation rates of Mg substrates. The first part of the study found that both PBS and Ringer's solutions were highly corrosive towards untreated Mg. With Ringer's solution in particular causing significant structural damage to the substrate as seen in Figure 1(C). Thus, highlighting the need to slow down the corrosion rate, which has been identified as the major hurdle preventing the use of Mg to produce orthopaedic implants [12, 15].

Figure 4. (A) micro-fracture between coating and substrate, (b) formation of acicular crystal growth between flower-like structures after 48 h and (C) a close up of a cluster of acicular crystals

The chemical immersion technique used to deposit a protective DCPD coating on Mg substrates was a straightforward technique. The resulting coatings displayed flower-like surface structures that were widespread across substrate surfaces as seen in Figures 2 (B) and (C). Comparable coatings and surface structures have been reported by other researchers using similar chemical immersion process [20, 21]. The DCPD coatings were found to be effective in reducing degradation, as seen in Figures 3 (D) and (E) for substrates exposed to Ringers solution. Similarly, substrates exposed to PBS also exhibited higher rates of corrosion resistance. Comparable studies have also found similar calcium phosphate coating can also increase corrosion resistance and reduce degradation rates [22]. However, SEM analysis also found cases where coating separation from the substrate occurred as seen in Figure 4 (A). Separation of surface coatings has also been reported in the literature and highlights the importance of ensuring a strong attachment between coating and the underlining substrate [12, 23]. The present study also found morphological changes occurred after 48 h to coatings exposed to PBS, which resulted in the formation of acicular crystal growth between the flower-like structures normally present on the substrates as seen in Figure 4 (B). The growth of the acicular crystals did not appear to compromise the coating integrity, but further studies are needed to fully investigate their formation and their influence on coating integrity. Also, further studies are needed to investigate the mechanical integrity of the coatings and their longevity. The present study has established that DCPD coating can significantly improve the durability of Mg substrates in PBS and Ringer's solution. And is an important step in developing a robust and straightforward surface coating technique that can be used to control the corrosion rate of Mg implants.

Conclusion

A straightforward chemical immersion technique was developed, optimised and used to deposit DCPD coatings on Mg substrates. The corrosion rate of DCPD coated Mg substrates in PBS and Ringer's solutions were significantly reduced. Since the chemical behaviour of PBS and Ringer's solutions are similar to body fluids, the results of the present study indicate the coatings could reduce Mg corrosion rates in the body environment. However, further studies are needed to fully investigate the mechanical integrity of the substrate-coating interface. In addition, the presence of micro-fracturing between coating and substrate surfaces exposed to PBS exposure was also detected. Also present was acicular crystal growth between the flower-like structures after 48 hours of exposure to PBS. Both these features need further investigation before this chemical immersion technique can be effectively used for coating Mg implants.

Disclosure

The authors claim no conflict of interest in this work.

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