Synthesis, Characterisation and Deposition of Nano Hydroxyapatite coatings on Bio-degradable Magnesium for Potential Orthopaedic Applications

Sridevi Brundavanam, M.Sc

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2015
Declaration

I declare that this thesis is my own account of my research and contains as its main content, work which has not previously been submitted for a degree at any tertiary education institution.

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Sridevi Brundavanam
Dedicated to my daughter
Gayatri
&
My husband
Ravi Krishna
Abstract

Today, Magnesium (Mg) based alloys are receiving increasing attention as potential biodegradable implant materials for orthopaedic applications. Despite advantageous properties such as density and elastic modulus that are similar to bone, magnesium’s rapid degradation rate when immersed in the highly corrosive body fluid environment has severely limited its clinical application. The focus of this thesis research was to develop biocompatible calcium phosphate coatings with tuneable biodegradable properties that are capable of extending the operational life of an Mg based implant and allow sufficient time for tissue regeneration to take place.

The research developed and examined three types of calcium phosphate coatings designed to reduce the degradation rate and prolong the life of Mg test substrates. Dicalcium phosphate dihydrate (DCPD) or Brushite coatings were formed on Mg substrates via a straightforward chemical immersion technique. While amorphous calcium phosphate (ACP) coatings were formed on Mg substrates using an electrochemical technique. Brushite coatings were characterised by widespread flower-like surface structures and the ACP coating were granular in structure with a surface covering of tube-like structures. The third coating examined was formed by the subsequent transformation of Brushite and ACP coatings to hydroxyapatite (HAP) via a low-temperature hydrothermal process. Importantly, HAP is the mineral component found in bone and is known to promote bone cell adhesion, differentiation and osteointegration.
Advanced characterisation techniques such as X-ray diffraction, field emission scanning electron microscopy, energy dispersive spectroscopy and Fourier transform infrared spectroscopy were used to investigate the size, morphology, crystalline structure, composition and topographical features of both uncoated and coated substrates. Degradation behaviour studies were carried on pure Mg substrates and the various substrate coatings using two simulated body fluid electrolytes at human body temperature (37 ºC). The first was phosphate buffer saline (PBS) solution and the second was Ringer’s solution. Corrosion rate measurements revealed DCPD coated substrates had the lowest corrosion rate in both PBS (0.126 mm/yr) and Ringer’s solution (0.1 mm/yr) compared to HAP coated [PBS (0.279 mm/yr) and Ringer’s solution (0.264 mm/yr)] and uncoated Mg substrates [PBS (1.829 mm/yr) and Ringer’s solution (3.828 mm/yr)]. The results of the research indicate that both DCPD and HAP coatings have the potential to reduce the corrosive effects produced by both PBS and Ringer’s solution. The results also suggest that the coatings have the ability to reduce the degradation rate of Mg substrates in the physiological environment.

Furthermore, because of HAPs complex hexagonal structure it offers an effective high capacity absorbent matrix and the present research has shown that like bone HAP can accumulate metallic materials such as cadmium, copper, iron and zinc. The significant improvement in corrosion resistance and the ability of HAP coatings in particular to behave as a temporary repository of metallic materials during degradation is an important step in the development of a biodegradable Mg implant for orthopaedic applications.
Publications


8. Gérrard Eddy Jai Poinern, Sridevi Brundavanam, Derek Fawcett. *Kinetic and adsorption behaviour of aqueous cadmium using a 30 nanometre scale hydroxyapatite powder synthesized via a combined ultrasound and microwave based technique* (Submitted to Journal of Materials and Environmental Science)
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Last but not least, I would like to acknowledge Murdoch University for providing a scholarship and all other facilities for me to undertake my research studies in nanotechnology.
Chapter 1 - Introduction

1.1. Overview

The quality, health and wellbeing of human life are influenced by a number of factors such as lifestyle and medical fitness. However, the majority of the population, during their life will experience medical problems ranging from damage, trauma and diseases, and ultimately complications arising from aging tissues and organs. In most cases these problems will often require surgical procedures to repair or replace the damaged or diseased tissues or organs using available replacement materials or devices. In the case of bone tissue, millions of people each year worldwide will require a bone transplant and the source of the bone tissue comes from the patient or from a suitable donor. The preferred source of bone tissue is from the patient and is known as an autograft. The advantage of an autograft is its excellent biocompatibility, osteogenic properties and furthermore delivers the patient’s own bone forming cells to the implant site. The autograft technique has been used for a long time, delivers good clinical outcomes and is considered the gold standard for bone transplantation clinical procedures. Unfortunately, the source of potential donor sites is limited and a further complication is donor site morbidity that has resulted in a search for alternative sources of bone tissue [1-4]. Alternative materials such as allogenic bone grafts (sourced from another donor) and xenograft bone grafts (sourced from another species) will result in a significant response from the body’s immune system. A further complication associated with allografts and xenografts is the serious threat of disease transmission [5]. Moreover, ethical, medical and legal concerns involved in sourcing and using allografts and xenografts has made their use difficult in many parts of the world. Importantly, all
autograft, allograft and xenograft procedures suffer from a limited supply of high quality bone tissue that is suitable for medical use.

To address the shortcomings of conventional grafts, research and development efforts have focused on using natural or creating biologically compatible synthetic materials capable of repairing or replacing damaged or diseased bone tissues. A material or a combination of materials that are used for this purpose are called biomaterials and can be used to fabricate tissue scaffolds, implantable devices and prostheses. Biomaterial development is a multidisciplinary field that covers a wide range of areas such as material science, engineering, biotechnology, and biomedical sciences. And in recent years there has been considerable effort made to produce a variety of engineered implantable biomaterials capable of promoting the repair and regeneration of human tissues such as bone, cartilage and skin [6-8]. Historically, metallic biomaterials have been used for decades to replace damaged or diseased bone tissues in load-bearing applications such as hip and knee replacements [9, 10]. Traditionally, metals used in load-bearing applications have included stainless steels, cobalt chromium and titanium alloys [11]. While materials such as polymers and ceramics with lower mechanical strength and fracture toughness have been used in a variety of low load-bearing applications [12-16]. In particular, because of their biocompatibility, calcium phosphate ceramics have been used in a variety of applications such as bone repair, bone augmentation, coating of metal implants and as filler material for both bone and teeth [17-19]. Currently, metallic implants are extensively used in load-bearing applications involving bone tissues.
Despite the many advantages offered by conventional metallic implants a number of issues relating to unfavourable inflammatory responses and mechanical compliance still exist. The adverse inflammatory response results from the slow release of toxic metallic ions produced by corrosion and wear of the metallic implant into surrounding cells and tissues [20, 21]. The ultimate detrimental result of metallic ion release is the significant reduction of the implants biocompatibility [22]. The poor mechanical compliance results from the significant difference in mechanical properties between metal implants and surrounding bone tissue. The difference in these properties results in a clinical phenomenon known as stress shielding that results in most of the load being carried by the implant. The net result of stress shielding is the significant reduction in the load being carried by surrounding bone tissues. The reduced load related stresses induce bone resorption that ultimately leads to mechanical instability and failure of the implant [23]. Another disadvantage of metal implants occurs when they are used as temporary support structures such as pins, plates and screws during the repair of damaged bone. In this case, a second surgical procedure is often needed to remove the implant after the healing process has taken place. The second surgical procedure significantly increases health costs and morbidity. These clinical issues highlight the need for new biologically compatible materials that are not only capable of providing short-term mechanical compliance and structural support during the healing process, but are also capable of safely degrading with time and avoid the issues normally associated with conventional metal implants.

Magnesium (Mg) is a lightweight, silvery-white metal and is the main constituent in a number of Mg based alloys that are currently being used in aerospace and automotive industries [24]. Because Mg’s high strength to weight ratio and mechanical properties
are similar to those of bone tissue, it is currently being investigated as a potential alternative to conventional metals used in biomedical implants. For example, the density of Mg is 1.74 g/cm$^3$ while bone varies from 1.8 to 2.1 g/cm$^3$ and the elastic modulus of Mg (45 GPa) is within the modulus range of bone (40 to 57 GPa) [25, 26]. The close similarity between the respective densities and elastic moduli have made Mg a potential biomedical material capable of significantly reducing the effects of stress shielding and bone resorption normally encountered in hard tissue engineering applications. Other attractive features include biologically degradability and absorbability. In particular, the release of Mg ions during corrosion could be considered beneficial since the body uses Mg in a number of metabolic processes and apatite formation in bone [27]. The biological importance of Mg is reflected by the fact that 30 g is stored in muscle and bone tissues in an average adult body [28]. However, the major limitation that prevents Mg’s widespread medical application as an implant material is its corrosion behaviour. In the physiological environment, Mg’s corrosion rate is too fast to be an effective biodegradable implant material. In addition, the body’s metabolic pathways cannot safely handle the rapid formation of hydrogen gas in the physiological environment. There are generally two possible routes to improve the corrosion resistance of Mg. The first involves modifying the composition and microstructure and the second involves producing a suitable surface treatment that protects the underlying metal. Most of the compositional additives and surface treatments described in the literature were not developed for medical applications and many of the additives and surface treatments are capable of also producing toxic side effects [29]. Currently, there are no specifically designed Mg based products commercially available in the biomedical sector for hard tissue engineering.
The objective of this thesis was to develop biocompatible and biodegradable coatings for Mg substrates capable of reducing and controlling the degradation rate and increasing substrate biocompatibility for potential hard tissue engineering applications. In recent years, the literature has shown a remarkable increase in the number of publications investigating the development of biologically compatible coatings. For tissue engineering applications, coatings should not only provide corrosion protection, but they should also enhance bioactivity, biocompatibility and promote osseointegration. And ideally, the coatings should also have antibiotic and drug delivery properties. Importantly, the coatings must be able to biologically degrade at a controlled rate and allow the underlying Mg substrate to slowly dissolve. Therefore, the coating should only provide an effective protective barrier for a limited timeframe, thus, allowing regenerating bone tissues to progressively replace the implant.

1.2. Scope of thesis

This thesis focuses on developing biodegradable and biocompatible calcium phosphate coatings capable of extending the operational life of an Mg based implant and allow sufficient time for tissue regeneration to take place. Calcium phosphates such as hydroxyapatite, a mineral phase found in bone, are known to promote bone cell adhesion, differentiation and osteointegration [30]. The main component of the experimental work carried out in this thesis examines the types of calcium phosphates formed on Mg substrates via chemical immersion and electrochemical techniques. Advanced characterisation techniques such as X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and Fourier transform infrared spectroscopy (FT-IR) were used to investigate the size, morphology, composition and topographical features of the uncoated and coated substrates. Extensive corrosion studies were also
carried out on various coated substrate types in phosphate buffer saline (PBS) solution and Ringer’s solution at 37 °C to simulate body fluid conditions. Also investigated was the adsorption capability of a representative coating, since a major function of bone is to store minerals routinely used by the body.

1.3. Aims of thesis

The thesis is structured around four aims, each composed of one or two individual case studies that allow a more detailed investigation into the various aspects of the research.

**Aim 1.** Chemically synthesize sufficient quantities hydroxyapatite from novel sources for potential bone cements and coatings. The synthesized hydroxyapatite will have similar chemical adsorption capabilities to natural bone. The synthesized hydroxyapatite will also have similar bioactivity and biocompatibility properties to the mineral phase found in bone (Case Study 1).

**Aim 2:** Investigate the chemical adsorption capabilities of hydroxyapatite (a representative coating material) to determine its suitability as an effective bone substitute during a tissue regeneration or tissue engineering procedure (Case Studies 2 & 3). This is of particular importance since the coating must be able to function like the mineral phase found in bone.

**Aim 3:** Develop and use chemical immersion and electrochemical techniques to synthesize calcium phosphate coatings. The use of advanced characterization techniques such as X-ray diffraction (XRD) spectroscopy, scanning electron microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared spectroscopy
(FT-IR) will be used to determine size, morphology, composition and topographical features of the various substrate coatings (Case Studies 4, 5 & 6).

Aim 4: Conduct bio-corrosion studies to investigate the degradation behaviour of the various coating types in phosphate buffer saline (PBS) solution and Ringer’s solution at 37 °C and pH 7.4 to simulate body fluid conditions (Case Study 4 & 6). The results of the corrosion studies will provide a guideline for future implant development.

The following chapters of this thesis address the above-mentioned aims, and elucidate the current state of research in this field and its relevance to developing biodegradable implants for hard tissue engineering applications.

References


Chapter 2 - Literature review

2.1. Overview and author contributions

Chapter 2 is composed of two peer-reviewed articles that survey the current literature and examine the biomedical potential of using magnesium (Mg) substrates for hard tissue bioengineering applications. The first review paper provides a brief history of Mg, its physical, chemical and mechanical properties, and its use in both medical and industrial applications. In particular, its high strength to weight ratio has made it an attractive material in industries such as transport and aerospace. The review also examines Mg biological performance and its potential application as biodegradable orthopaedic implants. However, Mg’s high corrosion rate in the physiological environment is its main disadvantage and currently prevents its use in many bioengineering applications. The metal’s low corrosion resistance, especially in electrolytic aqueous environments such as the physiological environment, where it rapidly degrades must be addressed. In recent years there has been a significant effort to improve Mg’s corrosion resistance and to slow down its degradation rate in a variety of environments. In particular, the review focuses on biological corrosion and discusses the various factors influencing Mg’s corrosion rate such as alloying elements, surface modifications and surface treatments.

The second peer-reviewed article focuses on using chemical immersion techniques for producing protective calcium phosphate coatings on Mg substrates. Chemical immersion is an economic, efficient and straightforward technique that offers a direct method of depositing calcium phosphate coatings on Mg substrates. Besides reducing the corrosive effects of the physiological environment, the coatings also have the
potential to significantly improve biocompatibility and promote bone formation at the coating-substrate interface of the Mg based implant.

The author contributions consisted of G.E.J. Poinern acting as principal supervisor who designed the overall concept for each review paper with S. Brundavanam being the major contributor to the papers. In the second review paper S. Brundavanam also acted as first author and significantly contributed to the content of the paper. All text, tables and images were carried out by S. Brundavanam under the supervision of D. Fawcett. S. Brundavanam was assisted by G.E.J. Poinern and D. Fawcett in over-coming some of the various technical difficulties encountered during the paper preparation and with the editorial changes to the manuscript as recommended by reviewers. All authors provided feed-back during the preparation of the paper which was coordinated by S. Brundavanam.

2.2. Published Review Articles

Review Article 1


Review Article 2

Biomedical Magnesium Alloys: A Review of Material Properties, Surface Modifications and Potential as a Biodegradable Orthopaedic Implant

Gérard Eddy Jai Poinern*, Sridevi Brundavanam, Derek Fawcett

Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Western Australia, 6150, Australia

Abstract Magnesium and magnesium based alloys are lightweight metallic materials that are extremely biocompatible and have similar mechanical properties to natural bone. These materials have the potential to function as an osteoconductive and biodegradable substitute in load bearing applications in the field of hard tissue engineering. However, the effects of corrosion and degradation in the physiological environment of the body has prevented their widespread application to date. The aim of this review is to examine the properties, chemical stability, degradation in situ and methods of improving the corrosion resistance of magnesium and its alloys for potential application in the orthopaedic field. To be an effective implant, the surface and sub-surface properties of the material needs to be carefully selected so that the degradation kinetics of the implant can be efficiently controlled. Several surface modification techniques are presented and their effectiveness in reducing the corrosion rate and methods of controlling the degradation period are discussed. Ideally, balancing the gradual loss of material and mechanical strength during degradation, with the increasing strength and stability of the newly forming bone tissue is the ultimate goal. If this goal can be achieved, then orthopaedic implants manufactured from magnesium based alloys have the potential to deliver successful clinical outcomes without the need for revision surgery.

Keywords Magnesium, Biological Corrosion, Biocompatibility, Alloys, Surface Modification

1. Introduction

The skeletal system of the human body is a complex three-dimensional structure that is important for two main reasons. The first arises from the need to structurally support the many body organs and other related tissues. The second is the attachment of the numerous muscle groups that are needed for body movement and locomotion. The skeleton is constructed of two types of tissue, the first is a hard tissue called bone and the second is a softer tissue composed of cartilaginous materials. The adult human skeleton consists of 206 bones[1]; some provide protection to the internal organs, while others perform specialized functions such as transmitting sound vibrations in the inner ear. The bone matrix also provides a natural reservoir for cells and mineral ions that play an important role in maintaining the biochemical balance within the body. For example, calcium is an important element involved in muscular action and nerve conduction and its level in the body is closely monitored and regulated by a process called homeostasis[2].

Bone is a natural two phase organic-inorganic ceramic composite consisting of collagen fibrils with an embedded inorganic nano-crystalline component. The primary organic phase of the bone matrix is Type I collagen, which is secreted by osteoblast cells to form self-assembled fibrils[3, 4]. The fibrils are bundled together and orientate themselves parallel to the load-bearing axis of the bone. The fibrils are typically 300 nm long, develop a 67 nm periodic pattern in which a 40 nm gap or hole is formed between the ends of the fibrils and the remaining 27 nm overlaps the bundle behind[5]. This pattern creates discrete and discontinuous sites for the deposition of plate-like nanometre sized hydroxypatite (HAP) crystals, which forms the second phase of the bone matrix. HAP is a mineral predominantly composed of calcium phosphate which has the general chemical formula of $[Ca_{10}(OH)_2(PO_4)_6]$. It is the main inorganic component of bone and teeth, accounting for up to 65% by weight of cortical bone and in the case of teeth it accounts for 97% by weight of dental enamel in mammalian hard tissue[6]. The discontinuous discrete sites limit the growth of the HAP crystals and force the crystals to grow with a specific crystalline orientation which is parallel to the load-bearing axis of the bone and collagen fibrils. The crystal plates typically have a length of 50 nm, a width of around 25 nm and on average a thickness of 3 nm[7-10]. The HAP also has
trace amounts of potassium, manganese, sodium, chloride, hydrogen phosphate, citrate and carbonate[11]. The final component of the bone matrix consists of the non-collagen organic proteins such as the phosphor-protein group which are believed to regulate the formation of the inorganic crystal phase by influencing the size, orientation and the depositional environment within the spaces between the collagen fibrils. The phosphor-protein group is also believed to be the source of calcium and phosphate ions used in the formation of the mineral phase[12].

The organic phase gives bone its flexibility, while the inorganic phase provides bone with its structural rigidity[13, 14]. The incorporation of organic and inorganic phases in the matrix gives bone its unique mechanical properties such as toughness, strength, and stiffness. It is the combination of these properties that give bone and the skeletal system in general, its remarkable ability to withstand the various mechanical and structural loads encountered during normal and intense physical activity[15]. However, not all bone tissue in the body has the same properties and this is characterized by the presence of two types of bone. The first type consists of a hard outer layer of compact (cortical) tissue, while the second type forms the less dense and spongy (trabecular) tissue which fills the interior of the bone. This spongy interior contains marrow and the many blood vessels that supply nutrients and remove waste products from the bone tissues. Both the cortical bone and the trabecular bone are composed of the same organic and inorganic phases discussed above, but they differ in the amount of each phase present. The two bone types also differ in their respective porosities and in their structural arrangement. The amount of cortical and trabecular tissue found in bone is dependent on the external load being applied and the frequency of the load[16]. Despite its remarkable mechanical and structural properties bone can fracture from three main causes: 1) a fracture caused by sudden injury; 2) Fatigue or stress fractures resulting from repeated cyclic loads; and 3) Pathological fractures resulting from bone infections and tumours[17]. The surgical implantation of artificial biomaterials of specific size and shape is an effective solution in restoring the load bearing capacity and functionality of damaged bone tissue. The design and selection of biomaterials is highly dependent on the specific medical application. Therefore, it is imperative that new biomaterials being developed for load bearing orthopaedic implant applications should have excellent biocompatibility, comparable strength to natural bone, and produce no cytotoxicity effects[18, 19].

Metallic biomaterials have been used since the early 1900s to replace damaged or diseased hard tissues. And as early as 1907, a magnesium alloy was used by Lambotte, to secure a bone fracture in the lower leg[20, 21]. Metallic implants are generally used in load bearing applications where their high mechanical strength and fracture toughness make them superior to ceramics, polymeric materials and polymer/ceramic composites. Metallic implant materials currently used include stainless steel, cobalt-chrome alloys and titanium and its alloys. At present there are two major problems associated with using the metallic implants. The first involves the mismatch between the mechanical properties of the metallic alloy and the surrounding natural bone tissue. The elastic modulus of both stainless steel and cobalt-chrome alloys is around ten times greater than that of bone, while a titanium alloy such as Ti-6Al-4V is around five times greater[22]. Bone tissue is constantly undergoing remodelling and modification in response to imposed stresses produced by normal everyday activities. The mechanical mismatch between bone and different metallic implant materials results in a clinical phenomenon known as stress shielding. The stress-shielding phenomenon occurs when the implant carries the bulk of the load and the surrounding bone tissue experiences a reduced loading stress. The reduced loading stress experience by the surrounding bone tissue ultimately leads to bone resorption[23, 24]. The second problem stems from mechanical wear and corrosion of the implant and results in the release of toxic metallic ions such as chromium, cobalt and nickel into the body. These harmful metallic ions solicit an inflammatory response from the body’s immune system and the surrounding tissues which reduces the biocompatibility of the implant[25, 26, and 27]. This is in total contrast to the corrosion products of magnesium (Mg) which can be considered physiologically beneficial, with the adult body storing around 30 g of Mg in both muscle and bone tissue[28]. The importance of Mg to the body stems from the fact it is bivalent ion which is used to form apatite in the bone matrix and is also used in a number of metabolic processes within the body[29]. And recently, Robinson et al. reported the novel antibacterial properties of Mg metal against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus[30]. Mg is a lightweight, silvery-white metal that is relatively weak in its pure state and is generally used as an alloy in engineering applications. The density of Mg and its alloys are around 1.74 g/cm³ at 20°C, which is 1.6 and 4.5 times less dense than aluminium and steel, respectively[31]. Interestingly, the density of Mg is slightly less than natural bone which ranges from 1.8 to 2.1 g/cm³, while the elastic modulus of pure Mg is 45 GPa and human bone varies between 40 and 57 GPa[32, 33 and 34]. Because of this close similarity in the respective elastic moduli, using Mg in hard tissue engineering applications would greatly reduce the possibility of stress shielding and prevent bone resorption. Thus, Mg with its similar mechanical properties to natural bone, combined with its biocompatibility, makes it a promising material for the development of biodegradable orthopaedic implants[33, 35]. Polymeric materials have also been used in a number of tissue engineering applications since they have many attractive properties such as being lightweight, ductile in nature, biocompatible and biodegradable. Polymers are materials with large molecules composed of small repeating structural units called monomers. The monomers are usually attached by covalent chemical bonds, with cross-linking taking place along the length of the molecule. It is the
amount of cross-linking that gives the polymer its physiochemical properties. Many polymeric materials have been investigated since the body’s natural processes can easily handle the by-products resulting from their degradation, with the by-products being easily excreted in the urine. Natural polymers such as polysaccharides[36-40], chitosan[41-46], hyaluronic based derivatives[47-50] and protein based materials such as fibrin gel[51, 52] and collagen[53-56], have all produced favourable outcomes in a number of tissue engineering applications.

Similar studies using synthetic biopolymers composed of simple high purity constituent monomers, fabricated under controllable formation conditions have produced a variety of tissue scaffolds and implants with tuneable and predictable physio-mechanical properties. These biopolymers also have low toxicity reactions with the body and their degradation rate can be easily controlled. Examples of synthetic biodegradable polymers include Poly (lactic acid), PLA[57-62], Poly (L-lactic acid), PLLA[63-66], Poly (lactic-co-glycolic acid), PLGA[67-70], Polycaprolactone PCL[71-74] and Poly (glycolic acid), PGA[75-78]. These biopolymers are generally poly-α-hydroxy esters that de-esterify in the body as the polymer degrades to simple metabolites[79]. Currently available biodegradable sutures in clinical use are made from PLA and PGA. These synthetic biopolymers can also be made into different shapes and structures, such as pellets, rods, disks, films, and fibres as required for the specific application. Some of these applications include biodegradable sutures, bone and dental cement, bone grafting materials, plates, screws, pins, fixation devices and low load bearing applications in orthopaedics[80, 81]. However, even with their many attractive properties, biopolymers have low mechanical strength when compared to ceramics and metals, which has resulted in them being used in soft tissue reconstruction and low-load bearing applications. The major advantage that Mg and its alloys have over biopolymers is its superior mechanical strength, which is typically double that of biopolymers.

Ceramics are non-metallic, inorganic materials that are used in hard tissue engineering applications where they are collectively termed bioceramics. The important properties of bioceramics that make them highly desirable for biomedical applications are: 1) they are physically strong; 2) they are both chemically and thermally stable; 3) they exhibit good wear resistance, and 4) they are durable in the body environment[82]. In addition, they are readily available, can be shaped to suit the application, they are biocompatible, hemocompatible, nontoxic, non-immunogenic and can be easily sterilised[83]. But unlike Mg and its alloys, bioceramics such as HA, tend to be brittle, have low fracture toughness and are not as resilient. However, some bioceramics have found application in hip joints, coatings on implants, maxillofacial reconstruction, bone tissue engineering and drug delivery devices[81, 84-86].

A composite material consists of two or more distinct parts or phases[85]. The major advantage of using a composite biomaterial stems from the fact a single-phase material may not have all the required properties for a particular application[86]. However, by combining one or more phases with differing physical and chemical properties it is possible to create a composite material with superior properties to those of the individual components. A good example of a natural composite is bone, which is a composed of Type I collagen and HA. A typical manmade example of a biomedical composite is a bioactive coating of HAP or a bioactive glass deposited on to the surface of a titanium implant to promote bone attachment[87]. Composites, such as a 2-phase HAP-polymer mixture have also been developed to create a biomaterial with similar properties to natural bone for hard tissue engineering applications[88]. Unfortunately, as mentioned above, biopolymers biodegrade with time and as a result, the load bearing capacity and fracture toughness of the implant will decline with time.

When comparing the properties of Mg and its alloys with metals, polymers, ceramics and composites it can be shown that Mg and its alloys have many properties that are comparable, if not superior, see Table 1. However, despite its many advantages, Mg has the disadvantage of having a high corrosion rate in the body. And as a result, medical application of Mg based implants has been severely limited due to the electrolytic aqueous environment of the chloride rich body fluid (pH ranges between 7.4 and 7.6). Furthermore, there are two serious consequences of the rapid corrosion rate of Mg implants. The first is the rapid evolution of subcutaneous hydrogen gas bubbles which are produced at a rate too high for the surrounding tissues to handle[89, 90]. These bubbles usually appear within the first week after surgery and can be easily treated by drawing off the gas using a subcutaneous needle[91]. The second consequence of the high corrosion rate is the loss of mechanical integrity of the Mg implant being used in the load bearing application. The rapid decrease in mechanical properties resulting from exposure to the body fluid environment means that the implant is unable to provide the necessary support for the healing bone tissue. Generally, the implant would be expected to maintain its mechanical integrity between 12 to 18 weeks while the healing process takes place and then slowly degrade while natural bone tissues replace the implant[92].

This article reviews the biological performance, mechanical properties and potential application of biodegradable Mg based alloys for orthopaedic implants. The major disadvantage of using Mg in many engineering applications is its low corrosion resistance, especially in electrolytic, aqueous environments where it rapidly degrades. To slow the degradation rate in situ, factors influencing the corrosion rate such as alloying elements, surface modification and surface treatments are examined and discussed in the following sections.
Table 1. Some mechanical properties of selected materials

<table>
<thead>
<tr>
<th>Tissue/Material</th>
<th>Density (g cm(^{-3}))</th>
<th>Compressive Strength (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Elastic Modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Materials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial wall</td>
<td>-</td>
<td>-</td>
<td>0.50 - 1.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Collagen</td>
<td>-</td>
<td>-</td>
<td>60</td>
<td>1.0</td>
</tr>
<tr>
<td>Collagen (Rat tail tendon)</td>
<td>-</td>
<td>-</td>
<td>1.5 – 9.3</td>
<td>3.75 – 11.5</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>1.0 – 1.4</td>
<td>160 Trans.</td>
<td>1.5 – 38</td>
<td>0.01 – 1.57</td>
</tr>
<tr>
<td>Cortical bone</td>
<td>1.8 – 2.0</td>
<td>240 Long.</td>
<td>35 Trans.</td>
<td>5 - 23</td>
</tr>
<tr>
<td>Magnesium Alloys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure magnesium</td>
<td>1.74</td>
<td>20 - 115</td>
<td>90 - 190</td>
<td>45</td>
</tr>
<tr>
<td>AZ31 (Extruded)</td>
<td>1.78</td>
<td>83 - 97</td>
<td>241 - 260</td>
<td>45</td>
</tr>
<tr>
<td>AZ91D (Die cast)</td>
<td>1.81</td>
<td>160</td>
<td>230</td>
<td>45</td>
</tr>
<tr>
<td>Other metal alloys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobalt-Chrome Alloys</td>
<td>7.8</td>
<td>-</td>
<td>450 - 960</td>
<td>195 - 230</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>7.9</td>
<td>-</td>
<td>480 - 620</td>
<td>193 – 200</td>
</tr>
<tr>
<td>Titanium Alloys</td>
<td>4.4</td>
<td>-</td>
<td>550 – 985</td>
<td>100 – 125</td>
</tr>
<tr>
<td>Ceramics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic-Hydroxyapatite</td>
<td>3.05 – 3.15</td>
<td>100 - 900</td>
<td>40 – 200</td>
<td>70 – 120</td>
</tr>
<tr>
<td>Polymers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymethylmethacrylate (PMMA)</td>
<td>1.12 – 1.20</td>
<td>45 – 107</td>
<td>38 – 80</td>
<td>1.8 – 3.3</td>
</tr>
<tr>
<td>Polylethylene-terephthalate (PET)</td>
<td>1.31 – 1.38</td>
<td>65 – 90</td>
<td>42 – 80</td>
<td>2.2 – 3.5</td>
</tr>
</tbody>
</table>

Note: Table compiled from references[122, 126, 213, 218, 219, 220 and 221]

2. Biological Corrosion of Magnesium

2.1. Corrosion Mechanism

When unprotected chemically pure magnesium is exposed to humid atmospheric air it develops a thick dull gray amorphous layer composed of magnesium hydroxide [Mg(OH)\(_2\)]. The oxidation rate of this protective oxide layer is typically around 0.01 mm/yr, while the oxidation rate in salt water is around 0.30 mm/yr[93]. In magnesium alloys, controlling the alloying chemistry and the overall microstructure of the alloy can significantly reduce the corrosion rate.

Table 2. Corrosion rates for some magnesium alloys immersion in various media

<table>
<thead>
<tr>
<th>Material</th>
<th>In vitro corrosion rate (mg.cm(^{-2}).h(^{-1}))</th>
<th>In vivo corrosion rate (mg.mm(^{-2}).yr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hanks Solution Simulated Body Fluid</td>
<td></td>
</tr>
<tr>
<td>Pure Mg (99.95%)</td>
<td>0.011  0.038</td>
<td></td>
</tr>
<tr>
<td>AZ31</td>
<td>0.0065 -</td>
<td>1.17</td>
</tr>
<tr>
<td>AZ91</td>
<td>0.0028 -</td>
<td>1.38</td>
</tr>
<tr>
<td>LAE442</td>
<td>- 0.085</td>
<td>0.39</td>
</tr>
<tr>
<td>WE43</td>
<td>-</td>
<td>1.56</td>
</tr>
</tbody>
</table>

Note: Table compiled from references[92, 96, 126, 213, 214 and 215]

For orthopaedic applications pure magnesium finds the human body a highly aggressive corrosive environment, see Table 2. The body fluids are composed of water, dissolved oxygen, proteins and electrolytic ions such as chloride and hydroxide. In this environment, magnesium with a negative electrochemical potential of -2.37 V, is very susceptible to corrosion and results in free ions migrating from the metal surface into the surrounding fluid environment.

These ions can form chemical species, such as metal oxides, hydroxides, chlorides and other compounds. In thermodynamic terms, with the assumption that there is no barrier to oxidation of the metal surface, the reaction would be very rapid, evolving hydrogen gas and consuming the metal substrate surface. But in reality the electrochemical reaction results in the migration of ions from the metal surface into solution, which forms species that result in the formation of an oxide layer that adheres to the metal surface. The Mg (OH)\(_2\) layer formed on the metal surface is slightly soluble and reacts with chlorine ions to form highly soluble magnesium chloride and hydrogen gas[94, 95]. When the oxide layer fully covers and seals the metal surface, it forms a kinetic barrier or passive layer that physically limits or prevents further migration of ionic species across the metal oxide solution interface.

The corrosion of Mg in an aqueous physiological environment can be expressed in the following equations. The primary anodic reaction is expressed by the partial reaction presented in equation (1), at the same time the reduction of protons is expressed by the partial reaction occurring at the cathode (2).
Another undesirable consequence of the corrosion process in Mg and its alloys is the formation of hydrogen gas. The rapid formation of hydrogen gas resulting from the rich chlorine environment produces subcutaneous gas bubbles, which generally appear within the first week after surgery and then disappear after 2 to 3 weeks[92]. During the initial gas formation a subcutaneous needle can be used to draw off the gas. In 2007, Song postulated that a hydrogen evolution rate of 0.01 ml/cm²/day can be tolerated by the human body and does not constitute a serious threat[96]. If the Mg corrosion rate can be regulated so that the hydrogen evolution rate is below this value, then the implant will not create a gas threat. The reactions of solid Mg and the Mg (OH)₂ layer with chlorine ions in the aqueous environment are presented in equations (3) and (4).

**Solid Mg:**
\[ \text{Mg} (s) + 2\text{Cl}^- (aq) \rightarrow \text{MgCl}_2 + 2e^- \quad (3) \]

**Mg (OH)₂ layer:**
\[ \text{Mg (OH)}_2 (s) + 2\text{Cl}^- (aq) \rightarrow \text{MgCl}_2 + 2\text{OH}^- \quad (4) \]

The general reaction of the corrosion process is presented in equation (5).

\[ \text{Mg} (s) + 2\text{H}_2\text{O} (l) \rightarrow \text{Mg(OH)}_2 (s) + \text{H}_2 (g) \quad (5) \]

Corrosion in the aqueous environment of the body is not as straightforward as corrosion in the industrial environment. This is due to the corrosion rate being influenced by a variety of other factors such as: 1) the pH of body fluids; 2) variations in the pH value; 3) concentration of ions; 4) the presence of proteins and protein adsorption on the orthopaedic implant; and 5) the influence of the surrounding tissues[97, 98 and 99].

### 2.2. Types of Biological Corrosion

An important property of the oxide layer is its ability to remain fixed to the metal surface during a variety of mechanical loading situations. If the oxide layer ruptures during mechanical loading it will expose the pure Mg substrate to body fluids which will result in further corrosion. The clinical repercussion of the corrosion process is the loss of mechanical strength and the ultimate failure of the implant. Typical forms of Mg corrosion encountered within the body environment are discussed in the following sections.

#### 2.2.1. Galvanic Corrosion

Galvanic corrosion takes place between two dissimilar metals, each with a different electrochemical potential, when they are in contact in the presence of an electrolyte which provides a pathway for the transfer of electrons. The less noble metal becomes anodic, corrodes and produces a build up of corrosion by-products around the contact site. For example, if gold screws are used to attach an Mg plate to bone during reconstructive procedure, the resulting electrolytic effect of the body fluids (serum or interstitial fluid) would preferentially attack the Mg plate; see Figure 1[100]. Therefore, it would be good design practice to use metals with similar electrochemical properties when designing implant devices. For example, the fixation screws used to attach an Mg plate during a bone reconstruction procedure should be made of a titanium (Ti) alloy, since Ti is the closest metal to Mg in the electrochemical series. Mg is the most reactive metal in the electrochemical series and will always be the anode in any corrosion reaction[101]. Therefore, selection of Ti alloy fixation screws to secure the Mg plate ensures the lowest possible corrosion rate. Galvanic corrosion can also result from the presence of inter-metallic alloying elements or impurities present in the Mg matrix, see Figure 2.

**Figure 1.** Galvanic corrosion between dissimilar metals

**Figure 2.** Galvanic corrosion resulting from inter-metallic elements

#### 2.2.2. Granular Corrosion

In many metal alloys, inter-granular corrosion can occur from the presence of impurities and inclusions which are deposited in the grain boundary regions during solidification. Following solidification, numerous galvanic reactions take place between the metal matrix and the various impurities and inclusions. The ensuing corrosion rate at the various grain boundary regions exceeds that of the grains and results in an accelerated corrosion rate of the metal matrix. However, in the case of Mg alloys, inter-granular corrosion does not occur since the grains tend to be anodic, while their boundaries are cathodic in nature compared to the interior of the grains. The resulting grain boundary corrosion undercutss nearby grains which subsequently fall out of the matrix[102].
2.2.3. Pitting Corrosion

Pitting corrosion of Mg results from the rapid corrosion of small-localized areas which damage the protective surface oxide layer; see Figure 3. This form of corrosion is more serious than other forms of corrosion since the surface pits are difficult to see due to the presence of corrosion products. The pits are small, highly corrosive and continue to grow downwards, perforating the metal matrix[103]. After initial nucleation at the surface, the presence of impurities in the Mg alloy microstructure often assists in further corrosion due to the galvanic differences in the materials[104, 105]. The environment within the pit is very aggressive, with chlorides species from the body fluids and Mg⁺ ions from anodic dissolution greatly aggravating the situation. In addition, the mouth of the pit is small and prevents any dilution of the pit contents, which adds to the accelerating autocatalytic growth of the pit. During this process, electrons flowing from the pit make the surface surrounding the pit entrance become cathode-protected and the protective oxide layer is further weakened. Once pitting starts, an Mg component can be totally penetrated within a relatively short period of time and in the case of a biomedical implant, its load bearing capacity would be greatly reduced to the point of failure. Another problem associated with pitting arises from localised increase in stress produced by the pit, which has the potential to form cracks[106]. The formation of stress corrosion cracking and metal fatigue cracks in the pits can lead to failure of the implant during normal loading conditions.

2.2.4. Crevice Corrosion

Crevice corrosion is local contact corrosion that occurs between metal and metal/non metal components. For example, if a magnesium plate is to be fixed in location by a set of screws with a small gap between the screw head and plate. The gap must have sufficient width to allow the flow of the body fluids through the gap and prevent any stagnant flow, see Figure 4. The stagnant flow results in the build up of Mg⁺ ions, with an Mg⁺ ion concentration gradient soon set up between the entrance and the dead end of gap. The subsequent corrosion cell then starts to attack the metal components of the implant[107].

2.2.5. Fretting Corrosion

Fretting corrosion is the result of damage produced by metal components in direct physical contact with each other in the presence of small vibratory surface motions. The micro-motions are produced by normal every day activities experienced by the human body which result in mechanical wear and metallic debris between the surfaces of metal components making up the biomedical implant[108]. During daily activity, the micro-motions remove the passive surface layer of the metallic components in direct contact, exposing fresh metal underneath. Then both the fresh metal surfaces and the metallic surface debris undergo oxidation. The surface debris has a further detrimental effect by acting as an abrasive agent during subsequent micro-motions. The corrosion rate is dependent on the applied load, the resulting fretting motion, the microstructure of the metal or metal alloys used in the implant and solution chemistry in the region around the fretting zone[109, 110]. During the corrosion process metallic ions are produced which can form a wide range of organic-metallic complexes and some metallic implants can release toxic metallic ions such as chromium, cobalt and nickel. These harmful metallic ions significantly reduce the biocompatibility of the implant and solicit a major inflammatory response from the body’s immune system[25, 26, and 27]. In the case of magnesium, metallic ions released during fretting, can be considered physiologically beneficial since these ions can be consumed or absorbed by the surrounding tissues, or be dissolved and readily excreted through the kidneys. Fretting corrosion is common in load bearing surfaces and is also capable initiating fatigue cracks in the fretting zone. Once formed the crack can propagate into the bulk of the metal matrix and can lead to the failure of the implant.

2.2.6. Erosion Corrosion

Erosion corrosion occurs from the wearing away of the metal surface or passive layer by the impact of wear debris in the body environment surrounding the implant. The metallic debris impacts on the surface of the implant, transferring energy into the region of the collision and plastically deforming the surface. During the deformation process the surface becomes work harden to the point where the next impact exceeds the strain required for surface fracturing,
pitting or chip formation. With the passage of time, the numerous impacts result in material loss from the metal surface[111]. For example, a femoral head of a Cobalt-Chromium implant will have numerous scratches after 17 years of implantation in a patient[112]. All bio-metals used in implants inevitably corrode at some finite rate when immersed in the complex electrolytic environment of the body; even Ti alloys with the lowest corrosion rate produce corrosion debris. The debris can significantly influence the wear behaviour and erosion resistant properties of the implant. However, the effects of erosion may not be noticed until there is a significant loss of metal which ultimately leads to the clinical failure of the implant.

2.2.7. Stress Corrosion

When an electrochemical potential is formed between stressed and unstressed regions of a metal implant under load, there is an increase in the chemical activity of the metal. This stress initiated corrosion mechanism effectively increases the corrosion rate, usually by two to three times above the normal uniform rate. This usually results in the formation of small cracks that concentrate stress within the loaded implant, a mechanism known as stress corrosion cracking (SCC). Mg SCC can occur in any load stressed implant immersed in the dilute chloride environment of the body fluids. SCC initiated cracks grow rapidly and extend between the grains throughout the metal matrix[113, 114]. The progress of SCC is also influenced by the strain rate resulting from the implant loading cycles and the presence of hydrogen gas produced by the corrosion process[115, 116]. Current research suggests that chloride ions produce pitting in the protective surface layer, which ultimately leads to a break down in the layer exposing the underlying Mg matrix to the electrolytic fluids of the body environment. The resulting hydrogen diffuses into the stressed zone of the metal matrix ahead of the crack tip and allows the SCC crack to advance through the zone[117-119]. Fracture and failure of the implant will occur when the SCC is below the normal operating stress of the implant.

2.2.8. Corrosion Fatigue

Corrosion fatigue is the result of a material being exposed to the combined effects of a cycling load and a corrosive environment[120]. In general, metal fatigue is the damage caused by the repeated loading and unloading of a metal component. The cyclic stress initiates the formation of microscopic cracks on the metal surface and also damages the protective passive layer. If there are any surface imperfections such as pores or pitting from corrosion, they become crack nucleation sites which can significantly speed up crack growth rates. In the body’s environment the cracks become localized electrochemical cells that promote further corrosion. Mg in particular is susceptible to corrosion fatigue due to the presence of chloride ions in the body fluids. Corrosion within the crack promotes crack propagation and in combination with cyclic loading, the crack growth rate significantly increases. Eventually the loading stress exceeds the SCC threshold and the crack grows to a critical size resulting in the fracture of the metallic implant. The body environment can significantly reduce the fatigue life of Mg alloys, producing lower failure stresses and considerably shorter failure times.

3. Magnesium and its Alloys

For biomedical applications, the composition of the material being considered is a crucial factor since many of the elements that make up commercially available materials for industrial applications are extremely toxic to the human body. Therefore, in addition to meeting the mechanical properties needed for a particular biomedical application, the material must also be biocompatible. Ideally, a biodegradable biomedical device should be composed of materials or alloys that are non toxic or carcinogenic. It would also be very advantageous if the material was composed of elements and minerals already present and compatible within the body such as magnesium, calcium and zinc, see Table 3. Furthermore, the material should have a controllable dissolution rate or slow corrosion rate that permits the biomedical device or implant to maintain its mechanical integrity until the surrounding tissues heal and are capable of carrying the load once again. After the healing process has taken place, the load bearing properties of the biomedical implant are no longer required and the implant material should then be able to slowly dissolve away. Furthermore, the resultant by-products of the degradation process should be non-toxic; capable of being consumed or absorbed by the surrounding tissues, or being dissolved and readily excreted through the kidneys. Thus, for Mg and its alloys to be used as an effective biodegradable implant it is necessary to control their corrosion behaviour in the body fluid environment[121].

3.1. The Influence of AlloYing Elements on Physical and Mechanical Properties

There are three major groups of Mg alloys: the first group consists of pure Mg; the second group consists of aluminium (Al) containing alloys such as AZ91, AZ31 and rare earth elements (RE) such as AE21; and the final group consists of the Al free alloys such as Mg-Ca, WE, MZ and WZ. The use of alloying elements such Al, Ca, Li, Mn, Y, Zn, Zr and RE in Mg alloys can significantly improve the physical and mechanical properties of the alloy by: 1) refining the grain structure; 2) improving the corrosion resistance; 3) form inter-metallic phases that can enhance the strength; and 4) assist in the manufacture and shaping of Mg alloys.

Impurities commonly found in Mg alloys are Be, Cu, Fe and Ni and the levels of these impurities are restricted to within specific limits during the production of the alloy, see Table 3. The range of acceptable levels for Be ranges from 2 to 4 ppm by weight, while Cu is (100-300 ppm), Fe (30-50 ppm) and Ni (20-50 ppm)[122]. Since both Be and Ni are carcinogenic, their use in biomedical applications should be
avoided as alloying elements. While elements such as Ca, Mn and Zn are essential trace elements for human life and RE elements exhibiting anti-carcinogenic properties should be the first choice for incorporation into an alloy. Studies by Song have suggested that very small quantities of RE elements and other alloying metals such as Zn and Manganese (Mn) could be tolerated in the human body and could also increase corrosion resistance[123]. Mn is added to many commercial alloys to improve corrosion resistance and reduce the harmful effects of impurities[124]. Mg alloys containing rare earth elements have also been found to increase the resistance to the flow of Mg^{2+} ions out of the Mg matrix via the Mg oxide layer[125]. During the degradation process the RE elements remained localised in the corrosion layer, which also contained high levels of both calcium and phosphorous. Also during this period a thin amorphous calcium phosphate layer formed over the surface of the oxide layer[92, 126].

Recent studies by Witte et al. have investigated the degradation behaviour of Mg based alloy rods and polymer based control rods[poly (lactic acid)] in animal models. Rods of 15 mm diameter and 20 mm long were inserted into the femur of guinea pigs and the rods degradation profile monitored. The percentage compositions by weight of the Mg alloys investigated consisted of two aluminium-zinc alloys composed of 3% Al and 1% Zn [AZ31] and 9% Al and 1% Zn [AZ91] with the balance of the alloys composed of pure Mg. In addition two RE alloys were studied, the first consisted of 4% yttrium and a 3% rare earth mixture composed of neodymium, cerium and dysprosium {WE43} and the second composed of 4% lithium, 4%, aluminium and a 2% rare earth mixture of cerium, lanthanum, neodymium and praseodymium {LAE442} [92, 127]. The implants were harvested at 6 and 18 weeks, with complete implant degradation occurring at 18 weeks. During this time radiographs were regularly taken, while a micro-tomography-based technique using X-ray synchrotron radiation was used to characterize the implant’s degradation process. All Mg based alloy implants were found to be beneficial and promoted new in situ bone tissue formation, while the polymer control rods produced a less significant effect. The LAE442 alloy had the greatest resistance to corrosion, while the other alloys all had similar, but lower values of corrosion resistance and degraded at similar rates[92].

While Mg is potentially an ideal biocompatible implant material due to its non-toxicity to the human body, the safe long term use of an Mg based alloy needs to be carefully studied. Magnesium based alloys have also been used in vivo; for example an AZ91 alloy rods were implanted into the femur of a number of rabbit models and the subsequent analysis revealed that after 3 months the implant had degraded and been replaced by new bone tissue[128, 129]. At the end of this degradation process most of the alloying elements such as Al would have been released into the bodies of the rabbits. The long term health effects on the rabbits are unknown, but in the case of the human body, the release of Al into the body will create undesirable health problems[130]. In humans, Al is a neurotoxicant and its long term accumulation in brain tissues has been linked to neurological disorders such as Alzheimers disease, dementia and senile dementia[131]. In addition, the administration of RE elements such as cerium, praseodymium and yttrium has resulted in severe hepatotoxicity in rats[132]. Furthermore, using heavy metal elements as alloying components are also potentially toxic to the human body due to their ability to form stable complexes and disrupt the normal molecular functions of DNA, enzymes and proteins[133]. Therefore, there is a definite requirement to carefully select alloying elements that are non-toxic to the human body, see Table 4. Non-toxic alloying elements such as Ca[134] and Zr[135] have the potential to significantly improve the corrosion resistance of the Mg alloy and reduce the degradation rate to make the Mg metal alloy a viable implant material[33].

### Table 3. Chemical analysis of alloying elements for a selection of magnesium alloys

<table>
<thead>
<tr>
<th>Alloy</th>
<th>Nominal element component (wt. %)</th>
<th>Maximum values of trace elements (wt. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Al</td>
<td>Zn</td>
</tr>
<tr>
<td>AZ31</td>
<td>3.5</td>
<td>1.4</td>
</tr>
<tr>
<td>AZ91</td>
<td>9.5</td>
<td>0.5</td>
</tr>
<tr>
<td>AM60</td>
<td>6.0</td>
<td>0.2</td>
</tr>
<tr>
<td>LAE442</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>WE43</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Table compiled from references[92, 93, 216, 217 and 218]
bone tissues had sufficient time to heal. There are two
reduction of mechanical integrity of the implant before the
humane physiological environment would result in the

4. Surface Modifications and Treatment Processes for Biomedical Mg Alloys

The high degradation rate of Mg and Mg alloy implants in
the human physiological environment would result in the
reduction of mechanical integrity of the implant before the
bone tissues had sufficient time to heal.[26] There are two
methods of reducing the degradation rate; the first, which
was discussed in Section 3, involved alloying Mg with
biocompatible elements that can resist the corrosion process.
The second method is discussed in this section and involves
the surface modification of the implant, through a treatment
process that provides a resistive barrier against the body
environment. An important factor that needs to be taken into
account before any surface treatment is investigated is the
healing or regenerative processes of bone and other
associated body tissues. The healing process consists of three
phases; inflammatory, reparative and remodelling.

The initial inflammatory phase usually lasts between 3 to
7 days and this is the natural response of the body’s immune
system to the presence of the biomedical device or implant.
The reparative phase usually takes 3 to 4 months, during
which time integration of the implant with the new and
regenerated tissues takes place. The final remodelling phase,
which is the longest phase, can take from several months to
years to complete[136]. For Mg to be an effective
bio-absorbable implant the degradation rate must be slow
enough for the healing process to take place and the new
tissues have sufficient time to provide their own structural
support before the structural integrity of the implant is
compromised. The minimum period for this to take place is
at least 12 weeks[26]. Unfortunately, Mg alloys can
completely degrade before the end of this timeframe and as a
result there is a need to reduce the biodegradation rate. The
bulk properties of Mg based alloys dictate its mechanical
properties, but it is the surface properties that influence the
interaction between the metal and the surrounding tissue
environment of the body. As a consequence, surface

<table>
<thead>
<tr>
<th>Alloying Element</th>
<th>Mechanical Properties</th>
<th>Pathophysiology</th>
<th>Toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Adding to improve corrosion resistance in Mg-Ca alloys.</td>
<td>Blood serum level 0.919-0.993 mg/L. Levels controlled by Homeostasis of skeleton. Abundant mineral that is mainly stored in bones and teeth. Activator/stabilizer of enzymes. Involved in blood clotting.</td>
<td>Metabolic disorder of calcium levels results in the formation of excess calcium in the kidneys (stones).</td>
</tr>
<tr>
<td>Copper</td>
<td>Can increase strength of Mg casts, however, it also accelerates corrosion rate when exposed to a NaCl medium.</td>
<td>Blood serum level 74.1-131 µmol/L. Essential trace element.</td>
<td>Excessive amounts of Cu have been linked to neuro-degenerative diseases. Can produce cellular cytotoxicity.</td>
</tr>
<tr>
<td>Manganese</td>
<td>Adding to reduce the harmful effects of impurities and improve corrosion resistance.</td>
<td>Blood serum level &lt;0.8 µg/L. Essential trace element. Influences cellular functions/immune system/blood clotting/bone growth. Influences metabolic cycle of lipids/amino acids and carbohydrates.</td>
<td>Excessive amounts of Mn can produce neurological disorder. (manganism)</td>
</tr>
<tr>
<td>Lithium</td>
<td>Improvement in corrosion resistance.</td>
<td>Blood serum level 2.4-4.9 µg/l. Used in drugs to treat psychiatric disorders.</td>
<td>Overdose causes central nervous centre disorders, lung dysfunctions, impaired kidney function.</td>
</tr>
<tr>
<td>Rare earth</td>
<td>Improvement in corrosion resistance.</td>
<td>Many rare earth elements have anticancerogenic properties and are used in the treatment of cancer.</td>
<td>Accumulate in the liver and bone.</td>
</tr>
<tr>
<td>Elements</td>
<td>Improve yielding stress, Mg alloys containing Zn have an Elastic Modulus similar to bone. The presence of Zn can reduce hydrogen gas evolution during bio-corrosion.</td>
<td>Blood serum level 12.4-17.4 µmol/L. Essential trace element. Essential to enzymes and immune system.</td>
<td>In high concentrations is neurotoxic and can hinder bone development.</td>
</tr>
</tbody>
</table>

Note: Table compiled from references[122, 134, 213, 222, 223, 224, 225, 226, 227 and 228]
modifications and treatments can have a significant role to play in governing the degradation rate of the implant. To date, numerous surface modification techniques have been developed to change the surface characteristics of biomaterials. Many of these methods have been applied to modifying the surface properties of Mg bio-alloys. A brief overview of some of these surface modification processes are presented in the following four sections.

4.1. Mechanical Modifications to Induce Surface and Subsurface Properties

The surface structure of an implant is very important, since it is the initial response of the surrounding tissues to the surface of the implant material that determines whether or not there is effective tissue-biomaterial integration. Studies of conventional types of permanent implant materials have shown that surface roughness can influence both cell morphology, cell growth and implant integration. In addition, modification of the surface topography by the physical placement of grooves, columns, pits and other depressions can influence cell orientation and attachment [137-139]. In the case of Ti alloys, surface modifications such as grooves, surface sand blasting and acid etching has revealed that grooved surface features provide superior cell attachment and promote greater cell proliferation than roughened surfaces [140]. For Mg alloys, the influence of different mechanical processing operations during fabrication has the potential to greatly influence surface and subsurface properties [141, 142].

Mechanical processing techniques involve operations such as rolling, shot peening, and milling. In the case of milling, at low cutting speeds, the surface formed by honed cutting tools tends to produce a rougher surface than those of sharp cutting tools. Also, during milling and similar metal chip removing processes, the exact effect on the underlying sub-surface is not fully understood [143], while chip removal from the surface during machining can directly influence the surface topography [144]. Besides machining techniques for chip removal, the use of rolling operations can also generate high passive forces acting normal to the surface, which can induce work hardening of the sub-surface. During the rolling operation the sub-surface grain structure is changed by the compressive stresses induced and the resultant micro-topography of the surface is significantly changed [145]. A recent study by Denka et al. has revealed a significant reduction in the corrosion rate (a factor of 100 was achieved in corrosion studies) of an Mg-Ca alloy that was deep-rolled, compared to the same alloy that was machined [146]. The presence of residual compressive stresses after rolling also has the advantage of reducing micro-crack formation from pre-existing crack nucleation points within the substrate. The suppression of crack formation is also an important factor in improving the fatigue life cycle of a material being considered for biomedical applications [146, 147].

The importance of surface and sub-surface treatments on Mg alloy implants was recently investigated by Von Der Hoh et al. [148]. In their study three surface machining treatments were applied to an Mg-Ca (0.8 % wt calcium) alloy. The alloy was used to make three different geometric sample types. The first test sample was a machined 3 mm diameter smooth cylinder, the second was like the first, except that it was sand blasted for 30 s using particles ranging in size from 300 to 400 μm and the final surface topography was a threaded cylinder. The smooth cylinders were machined with no further surface treatment, so they retained the micro-surface topography produced by the cutting tool. After 6 months of in vivo implantation in adult New Zealand white rabbits, the smooth cylinders revealed good integration with the surrounding tissues and also had the least structural loss. The sand blasted cylinders had the greatest material loss with the initial cylindrical shape completely consumed, while the threaded cylinders ranged between these two extremes. The results indicated that the smoother micro-topographic surface features of the cylinders were suitable for resorbable Mg alloys, while the test samples with the rougher surfaces promoted higher degradation rates. The results of this study clearly indicated that differences in surface roughness of the test samples could significantly influence the in vivo degradation rates. The study also highlighted the need for further investigation into the effects of different surface modifications on other biocompatible Mg alloys.

4.2. Physical and Chemical Modifications

From an engineering point of view, the most effective way to prevent corrosion is to coat the metal component with a protective barrier that effectively isolates the metal from the surrounding environment. To be effective against corrosion, the protective coating must be uniform, well adhered and free from any imperfections such as pits, scratches and cracks. The major problem with Mg, as mentioned earlier, is its chemical reactivity when exposed to air or an aqueous environmental which results in the formation of an oxide/hydroxide layer over the metal surface. The presence of the oxide/hydroxide layer will have a detrimental effect on the ability of the coating to adhere to the metal surface and form a uniform protective layer. Therefore, surface cleaning and a suitable pre-treatment of the metal surface is a crucial factor in achieving an effective surface coating.

4.2.1. Physical Vapour Deposition (PVD) & Chemical Vapour Deposition (CVD)

The PVD process involves the deposition of thin layers of metal and metal alloys from atoms or molecules from the vapour phase onto a substrate surface. During the process a metal or metal alloy is heated in vacuum chamber until it evaporates and then the subsequent vapour condenses onto the cooler substrate. This process has been successfully used on a variety of metals, but in the case of Mg there are a number of problems to overcome. For example, in most PVD processes the substrate temperature range is usually between
400 and 550 °C, but in the case of Mg the substrate temperature must be kept below 180 °C for material stability reasons. The lower substrate temperature of Mg also influences the adhesive and corrosion resistance properties of the coating[149, 150]. The PVD process has successfully deposited binary alloys such as Mg-Ti, Mg-Zr and Mg-Mn along with other less biocompatible and toxic alloys. The subsequent corrosion studies have revealed that the binary-alloyed surface coating were capable of increasing the corrosion resistance of the various Mg alloys[151-153]. The chemical vapour deposition process has also been used to produce a variety of coating processes that can create a protective coating or modify the existing Mg alloy surface. During the deposition of a solid material from the vapour phase onto a (usually) heated substrate a chemical reaction over the surface takes place. This results in changes to the sub-surface of the substrate, which chemically modifies the surface properties. For example, the deposition of diamond like carbon (DLC) films on metallic implants can improve the surface properties of the implant, thus making it biocompatible with the surrounding body tissues[154].

4.2.2. Ion Implantation and Plating

Ion implantation consists of bombarding the surface of a substrate with ionized particles. The ionized particles penetrate the surface and become embedded in the sub-surface of the substrate. The ionized particles soon neutralize in the interstitial positions within the grain structure forming a solid solution. During this process physicochemical changes take place in the sub-surface of the substrate, while the bulk properties of the substrate remain unchanged. To date there have been relatively few studies carried out that have used ion implantation to enhance the surface properties of Mg alloys. A recent study by Liu et al. examined the corrosion behaviour of surgical AZ91 after it was subjected to Ti ion implantation[155]. The study revealed that a compact surface oxide layer was formed, which was predominantly composed of TiO2 with a smaller amount of MgO. Subsequent testing in simulated body fluid at 37 ± 1 °C revealed that the corrosion resistance of ion treated AZ91 alloy was improved significantly. In a similar study by Fang et al. the corrosion behaviour of a new medical grade Mg-Ca alloy was examined before and after Zn ion implantation[156]. The results revealed that after ion implantation, the Zn had improved the surface hardness and elastic modulus of the alloy. The surface oxide layer formed during corrosion testing in simulated body fluid enhanced the Mg-Ca alloys corrosion resistance. However, Wan et al. examined a Mg-Ca alloy before and after Zn ion implantation, the results found that the ion-implanted substrates had a lower corrosion resistance than the untreated substrates[157]. Subsequent analysis of the results suggests that Zn was an unsuitable metal for ion implantation with Mg-Ca alloys for biomedical applications.

Ion plating is a technique that deposits noble metal ions onto a less noble metal substrate to form a dense and well-adhered layer. The plating layers improve surface properties such as topography, roughness, surface chemistry and wear resistance. Zhang et al. used this technique to plate a pure Mg substrate with Ti ions and then subsequently studied its corrosion behaviour in a 0.9 wt % NaCl solution[158]. The results not only revealed a substantial improvement in corrosion resistance, but also found that an interdiffusion layer had formed between the Ti coating and the Mg substrate.

4.2.3. Thermal Spray Coatings

During this coating process, materials such as metals, metal alloys, ceramics, polymers and composites are feed (powder or wire form) into a gun. The material is then heated to a molten or semi molten state within a gas stream. The resulting micrometer size droplets are accelerated in the gas stream, which is directed towards the surface of the substrate[159]. This technique was successfully used by Zhang et al. to deposit an Al layer on an AZ91D substrate[160]. To ensure adhesion of the coating to the substrate, a post heat treatment process was carried out 450 °C. There was significant diffusion of Al and Mg around the interface of the coating, which enhanced both the corrosion resistance and anti-wear properties of the coating. The disadvantage of using an Al coating on the AZ91D substrate for a possible biomedical implant application is the negative effect of Al3+ ions being released into the surrounding tissues during subsequent corrosion. Ceramic coatings such hydroxyapatite (HAP), TiO2, Al2O3, and ZrO2 have also been successfully applied to Ti alloys to improve their corrosion resistance, wear resistance and biocompatibility[161]. However, in a study by Zeng et al., thermally sprayed TiO2 onto an Mg alloy (AM60) revealed that the subsequent coating showed no improve in its corrosion resistance compared to the untreated Mg alloy when they were both immersed in Hank's’ solution[162]. The study also revealed that galvanic corrosion occurred between the surface of the Mg alloy and the coating layer, which effectively reduced any protective properties offered by the coating. This highlights the weakness of thermally sprayed ceramic coatings which have rough surfaces, high porosity and poor adhesion properties[163].

4.2.4. Laser Surface Melting, Alloying and Cladding

The high-density energy of a laser beam can be effectively used to modify the surface region of Mg alloys. The surface region of the alloy can be melted to create a meta-stable solid solution. This is then followed by rapidly cooling the substrate, which results in the refinement of the surface microstructure. This technique can also be used to improve the surface properties of an Mg alloy substrate by melting a metallic coating and the underlining sub-surface. During the rapid melting process both the coating and sub-surface mix before re-solidifying during subsequent cooling to form a new surface alloy which coats the bulk of the substrate. Furthermore, if the appropriate alloying metals are
incorporated into this surface modification technique it is possible to significantly improve surface properties such as corrosion resistance[164, 165]. For example, improved surface properties of a Mg alloy (AZ91) have been achieved with the dispersion of hard metallic particles such as TiC and SiC in the molten pool generated by laser melting[166, 167]. Also, laser cladding of an Al-Si alloy onto a number of Mg alloys such as AS41, AZ91 and WE54 have also been attempted, but unfortunately, the surface properties were not significantly improved[168, 169].

4.3. Wet Chemical Processes

4.3.1. Electrochemical Deposition of Metallic Coatings

The corrosion resistance of Mg and its alloys can be increased by an electroplating technique. In this technique a metal salt is reduced in solution to its metallic form, the electrons for reduction are supplied from an external source and the resulting metallic ions are deposited on to the surface of the substrate. However, most metals are more electrochemically noble than Mg, which can cause serious problems if there are any imperfections in the deposited layer. Such imperfections will expose the underlying substrate and result in the formation of small localized areas of corrosion. The corrosion sites form highly corrosive pits that tunnel down into the Mg substrate and seriously weaken the substrate[141]. From an industrial point of view, electroplating is a highly effective technique for coating Mg and its alloys with metallic coatings such as nickel, chrome and aluminium coatings[142]. These coatings have good mechanical properties and provide effective corrosion protection. Unfortunately, these metals are also harmful to human tissues, which make them highly unsuitable for biomedical applications.

4.3.2. Chemical Conversion Coatings

Chemical conversion coatings are formed by chemically treating the surface of Mg and Mg alloys to produce a thin outer coating of metal oxides, phosphates or other compounds that are chemically bonded to the surface[170]. The conversion coating acts as a protective barrier that isolates the substrate from the surrounding environment and prevents the corrosion.

Industrially, there are several different types of conversion coatings such as chromate, phosphate-permanganate, rare earth, stannate and hydrides. Many of the processes used to produce conversion coatings involve the use of toxic materials that are detrimental to human health. For example, the presence of hexavalent chromium (CrVI), that is used in chromate coatings.

An alternative treatment to chromate conversion coatings are: 1) phosphate; 2) phosphate-permanganate; and 3) stannate coatings. All three of these conversion treatments have comparable corrosion resistant properties to those of chromate treatments. Xu et al. have investigated the behaviour of a phosphate treatment on an Mg alloy (Mg-Mn-Zn) that was subsequently immersed in a simulated body solution (SBF). During the treatment process a biocompatible brushite layer [CaHPO4.2H2O] was formed on the surface of the substrate. Subsequent immersion in SBF revealed that the brushite layer transformed into a coating of HAP, with the excess phosphate ions being released into the surrounding environment. The released phosphate ions were also found to neutralize the alkalization effect produced by the corrosion process. The treatment process did not prevent corrosion, but it did significantly slow down the degradation rate[171]. And a phosphate-permanganate process developed by Han et al. using Mn3(PO4)2 was able to produce a resilient surface coating on a Mg alloy (AZ31D) that was self-healing in saline solutions[172]. While a stannate treatment developed by Gonzalez-Nunez et al. was able to deposit a 2 to 3 µm thick layer of MgSnO3 on an Mg alloy (ZC71). The coating was adherent, continuous, and crystalline which produced a passivating effect on the substrate surface[173]. Unfortunately, no degradation rate data was reported, indicating that more studies are needed to indeed gauge the effectiveness of this process for in vivo applications.

Magnesium fluoride (MgF2) conversion coatings on Mg alloys have produced mixed results in providing corrosion protection. Degradation studies carried out by Zeng et al. reported that an MgF2 conversion layer formed on an Mg alloy (AZ31) provided marginal corrosion resistance in a 0.9 wt % NaCl solution[174]. While in a similar study Hassel et al. found that a conversion layer of MgF2 formed on an Mg alloy (ZM21) could provide reasonable corrosion resistance[175]. And an in vivo study carried out by Witte et al. revealed that a conversion layer thickness between 150 and 200 µm formed on the surface of a RE based Mg alloy (LAE442) was able to reduce the degradation rate and reduce the release of alloying elements[176]. In a similar study by Gao et al., the feasibility of forming RE conversion layers on pure Mg to improve corrosion resistance was examined[177]. The two RE elements under investigation were Ce and Y, with each element being used individually to form a surface treatment solution. The first solution contained CeCl3 which formed a conversion layer consisting of Mg(OH)2, CeO2 and MgO, while the second solution contained Y(NO3)3 which formed a conversion layer consisting of Mg(OH)2, Y2O3 and MgO. The study revealed that the second conversion layer had improved corrosion resistance compared to the first. However, both coating provided limited corrosion resistance due to their thin thickness and soft structure, which was incapable of withstanding minor mechanical damage. Furthermore, both the toxicology and metabolic pathways within the human body of RE elements such as Ce and Y are still unclear and need to be fully investigated before they can be used in biomedical applications.

4.3.3. Calcium Phosphate Surface Coatings

A more biocompatible form of conversion coating can be derived from a variety of calcium phosphate compounds. In
particular, HAP [Ca (PO$_4$)$_6$(OH)$_2$], which has been widely used as a bone substitute and replacement in several biomedical applications [178-180]. There are three major advantages in using HAP in hard tissue engineering applications: 1) it has good biocompatibility and bioactivity properties with respect to bone cells and other body tissues; 2) it has a slow biodegradability in situ; and 3) it offers good osteoconductivity and osteoinductivity capabilities [181, 182]. These properties are very important because bone tissue constantly undergoes remodelling, a process in which bone tissue is simultaneously replaced and removed by the bone cells, (osteoblasts and osteoclasts respectively). It is these advantages that make HAP and TCP (tri-calcium phosphate) compounds attractive for coating metallic orthopaedic implants. In this application, both HAP and TCP coatings promote bone formation which enhances bonding between the implant and the surrounding tissues.

It is also due to these positive biological responses within the human body that has made calcium phosphate coating an attractive option for potentially reducing the biodegradation rate of Mg orthopaedic implants. Several techniques have been used to deposit calcium phosphate coatings onto Mg substrates, these range from anodization [183], bio-mimetic coatings [184-186], electro-deposition [187, 188], hydrothermal [189] and wet chemical methods [190, 191].

Xu et al. has investigated using an immersion technique that involves soaking an Mg-Mn-Zn alloy in an alkaline solution to form a brushite (CaHPO$_4$.2H$_2$O) surface coating. Unfortunately, the layer formed was porous and did not prevent corrosion in a simulated body fluid. However, the degradation rate was significantly reduced and provided the Mg alloy substrate with reasonable protection against the corrosive effects of the simulated body fluid. The study also found that the brushite coating was able to improve the surface biocompatibility of Mg alloy substrate, since the brushite coating transformed into a HAP phase with time. Also during this transformation, acidic phosphate ions were released into solution, which tended to have a neutralizing effect on the alkalization process [192]. Furthermore, the surface treatment enhanced the bioactivity of the Mg-Mn-Zn alloy and promoted bone formation [193].

A similar calcium-phosphate coating was produced by Wang et al., which involved immersing a Mg substrate into a solution containing Ca and P[Ca (NO$_3$)$_2$ and Na$_2$HPO$_4$] to create a di-calcium phosphate di-hydrate (DCPD) surface layer [194]. The DCPD layer was effective in providing protection for the Mg substrate during the first 21 days of immersion in a simulated body fluid.

Recently, Yanovska et al. investigated the influence of low magnetic fields during a one-step dipping technique [195]. The involved dipping a Mg substrate into an aqueous solution containing Ca (NO$_3$)$_2$.4H$_2$O and Na$_2$HPO$_4$.12H$_2$O. Deposition of both DCPD and HAP phases under the influence of magnetic fields lead to crystal orientation during the formation of the phases. The technique also produced coatings with enhanced corrosion resistance, which in turn reduced the degradation rate [195]. In an alternative method, Song et al. used an electro-deposition technique to produce three types of coating namely; DCPD, HAP and fluorapatite (FHA). The study found the FHA coating had long-term stability and remained intact even after 1 month of immersion in a simulated body fluid and provided effective corrosion resistance to the Mg alloy [196].

4.3.4. Alkali Heat Treatments

Heat treatment can be a beneficial way of improving the microstructure and enhance the surface properties of Mg and Mg alloys. The corrosion behaviour and cytotoxicity of alkali heat-treated pure Mg samples immersed in simulated body fluid (SBF) were investigated by Li et al. [197]. The samples for treatment were placed into a super saturated NaHCO$_3$-MgCO$_3$ solution and then heat-treated. SBF solutions with and without chloride ions were used to study the influence of chloride ions on the corrosion behaviour of treated Mg samples. All the treated samples showed a significant improvement in corrosion resistance in both SBF (CT) and SBF solutions compared to the untreated Mg samples. In addition, after 14 days of immersion in the SBF fluids, a calcium phosphate compound with a molar ratio of 1.858 was detected on the surface of the samples. While the subsequent cytotoxicity testing revealed no signs of morphological changes in the cells and no inhibitory effect of the surface treatments on cell growth could be detected.

In a similar study by Liu et al., the corrosion behaviour of a heat-treated Mg-Al alloy (AZ63) immersed in a SBF solution for 14 days was investigated [198]. During heat treatment (solution treatment at 413°C for 24 h followed by aging at 216°C for intervals of 1 h, 5.5 h and 12 h), the microstructure of the Mg-Al alloy changes as the Al atoms in the alloy diffuse towards the grain boundaries and subsequently precipitate out of solution to form the β phase. And as a consequence of the diffusion process (Aging), the concentration of Al atoms remaining in the matrix (α phase) decreases and results in the matrix having a reduced corrosion rate. In addition, the study found that samples microstructure significantly influenced the overall corrosion morphology. For example, the surface of the untreated samples displayed deep and uniform corrosion, while the surface of the treated samples had only shallow pitting [198].

4.3.5. Anodization

The anodization of magnesium is an electro-chemical process that changes the surface chemistry of the metal, via oxidation, to produce a stable anodic oxide layer. The structure of this layer is characterized by a thin barrier layer at the metal-oxide interface, followed by a less dense porous oxide layer. The porous layer can display a variety of different structures and properties which are dependent on the composition, substrate micro-structure and processing parameters [142]. The processing parameters that influence oxide layer formation include: 1) the type, temperature and concentration of electrolyte; 2) current density; and 3) the applied anodization voltage. These parameters can also
significantly influence the resulting corrosion behaviour of the substrate[199]. The anodization process can also produce an oxide layer consisting of pores, whose size and density is dependent on the selection of the appropriate processing parameters. Industrially, porous oxide layers are usually coloured and then sealed or form part of a pre-treatment process prior to painting or coating. Many of the industrial coating and surface treatments used on anodized Mg alloy components to reduce corrosion are toxic to the human body. And as a result, research efforts have focused on searching for biocompatible surface treatments and processes that are non-toxic. For example, Hirohoto et al. have studied the effects of controlled calcium phosphate (Ca-P) precipitation on pure Mg substrates by anodization and then thermally treating the substrates in an autoclave[200]. After thermal treatment, the subsequent immersion studies in Hanks’ solution revealed that the Ca-P coated substrates had very little corrosion[201]. The advantage of this technique comes from the Ca and P elements being deposited on the substrate, since both bioactive materials are known to induce osteoinduction and promote new bone tissue growth[201].

Micro-arc oxidation (MAO) is an electrochemical process which uses a high anodic voltage and high current density to create an intense micro-arc (plasma) near the metal surface to induce oxidation. The oxide layer formed during this process is substantially thicker than conventional anodization, since the sub-surface of the metal substrate is also oxidized[202]. This technique can be used to deposit ceramic coatings on valve metals such as Al, Mg, Ta, Ti, W, Zn and Zr and their alloys. And by selecting the appropriate process parameters, the (MAO) technique can produce high quality coatings with superior adhesion, corrosion resistance, micro-hardness, wear resistance and strength. In a corrosion and wear study by Zhang et al., die cast Mg alloy (AZ91D) substrates were treated with a MAO coating. Then both treated and untreated substrates were immersed in the Hanks’ solution to determine the effectiveness of the MAO coating in reducing the corrosion rate[203]. Immersion testing revealed that the untreated substrates lost 15 times more mass due to corrosion than the MAO treated substrates. In addition, the mass loss from the untreated substrates during wear tests was 1.5 times greater than those of the MAO treated substrates. The tests clearly indicated that the MAO surface treatment was effective in improving both the corrosion and wear resistance of the Mg alloy. Unfortunately, there is no current data available describing the combined effects of corrosive and wear on MAO treated Mg alloys in Hanks solution.

In a recent study examining the combined effects of corrosion and wear on an Mg Alloy (AZ91), Chen et al., investigated untreated and MAO treated substrates immersed in different composition based solutions composed of NaCl and NaHCO₃[204]. The study revealed that the MAO coating did improve the corrosion resistance of the Mg alloy. However, the wear resistance of the treated substrate tended to decrease with time. The decreasing wear resistance was caused by wear debris, which consisted of abrasive particles produced by the breakdown of the MAO coating. Both anodization and MAO oxide coating can effectively reduce the corrosion rate of Mg and Mg alloys by producing a strong resilient oxide layer that provides an effective protective barrier between the metal substrate and the fluid environment. For example, Song et al. has demonstrated that the oxide layer produced during the anodization of a pure Mg substrate was capable of providing an effective barrier to corrosion for 1 month in Hanks’ solution. During this time, no hydrogen evolution was detected, indicating that the degradation had been delayed[205, 206]. This is an important factor for a biodegradable implant, since controlling the dissolution rate allows matching of the degradation rate of the implant with the growth of new replacement bone tissue.

4.4. Polymer Coatings

Many implantable biomedical devices and implants are coated with a thin adherent polymeric material that effectively isolates the device from the fluidic environment of the body. Polymer coatings are frequently used to modify the surface properties of biomedical implants to improve their biocompatibility, performance and therapeutic effectiveness. The interface between implant surface and body environment is critical in soliciting the appropriate immunological response. Therefore, selecting the correct polymer coating is crucial in determining the biocompatibility of the implant and also provides a wider range of design options that can be used to improve the surface properties of the original implant surface. For example, selecting a polymer coating which slowly biodegrades can potentially delay the corrosion of an Mg implant and maintain its mechanical integrity over a longer timeframe.

Biomedical coatings can be divided into two primary categories: 1) short term, which include disposable or single patient use; and 2) long term application of prosthetic implants and reusable laboratory equipment[207]. To achieve the bio-functional requirements and protection, a successful polymer coating must adhere to the Mg implant and be strong and flexible enough to withstand the normal movement of the implant. The coating should also be capable of being sterilised and be sufficiently durable to perform its protective function under the expected conditions of the particular application[208]. The advantage of polymer coatings is that they can be chemically, physically and mechanically customized to suit a specific application. Thus, being able to select and fine-tune the various properties has enabled polymeric materials to be used in a wide range of coating applications such as protection, improved lubricity, antimicrobial, adhesion resistance, ultrasonic imaging and blood compatible coatings for drug delivery[209].

Before coating, an appropriate pre-treatment process is required to effectively clean the surface of the Mg implant. This process should produce a clean, dry and contaminant free surface capable of providing the maximum possible
adhesive strength between the polymer and implant surface. The presence of entrapped air and moisture on the surface could lead to degassing and the formation of holes in the coating during the curing process. The coating process and associated coating parameters can influence the resulting microstructure and morphology of the polymer coating. This explains why coatings with similar compositions can have different surface properties[210]. A polymer coating can be applied to an Mg implant using a solvent, an aqueous solution or from the vapour phase. Water-based coating techniques have the advantages of eliminating or reducing solvent effects on the substrate and are more environmental friendly, since there are no toxic solvent wastes produced. Polymer coating produced by vapour-deposition can produce both uniform and defect-free coatings. The quality and chemical structure of these coatings combine to provide an effective barrier to the body environment and also enhance the protective properties of the coating. For example, Parylene (poly (p-xylylene), is a polymer coating that has been used to protect implanted sensors and other biomedical devices[210].

In addition, Mg needs a suitable surface primer or binding agent to improve the adhesion between the polymer coating and the implant surface. Unfortunately, primers composed of metallic ions, components of binding agents and almost all organic solvents found in paints and similar surface preparations are toxic or detrimental to the human body and therefore cannot be used in biomedical applications. Recently, Huang et al. primed a pure Mg substrate using a silane coupling agent before using dip coating technology to coat the substrate with degradable poly (lactic acid)[211]. The silane coupling agent was found to improve the adhesion between the poly (lactic acid) and the substrate. Similarly, PLGA, a polymer with good blood compatibility was also coated onto a pure Mg substrate using the same process. Subsequent corrosion testing in Hank's solution revealed that the PLGA could provide an effective coating and protect the underlining Mg substrate. However, corrosion protection of the Mg substrate was dependent on the degradation of the PLGA. And in a bone replacement application, the coating was unable to provide an adequate protective barrier[211]. In a similar study, Xu et al. was able to show that producing different surface modifications on chitosan coatings applied to Mg alloy substrates were able to influence the biodegradability of the coating[212].

Polymeric coatings have the potential to modify the surface properties of an Mg based implant and significantly improve the implants usability, durability and performance. Polymeric materials used in coating an implant have the potential to be specifically designed to provide physical, chemical and mechanical responses that can be fine-tuned to solicit and enhance specific biochemical responses within the body environment. However, a significant research effort is still needed to find polymeric materials that can produce thin, dependable, multi-functional coatings with controllable degradation rates that can be used to prolong the mechanical effectiveness of Mg based implants.

5. Conclusions

Mg and Mg based alloys are extremely biocompatible and have similar mechanical properties to natural bone. This makes them an attractive material for the manufacture of biodegradable, with the capability to replace many currently used orthopaedic materials such as biodegradable biopolymers. And despite having the potential to function as an osteoconductive and biodegradable substitute in load bearing applications, the practical application of Mg based alloys faces the serious challenge of overcoming the rapid corrosion rates that occur within the physiological environment of the body. The types of biological corrosion occurring within the body environment and the influence of body fluid pH, concentration of ions, protein adsorption on the implant surface and the influence of the surrounding tissues was discussed. To overcome the effects of biological corrosion, a number of treatment methods designed to reduce the corrosion rate, such as the addition of alloying elements and surface modification techniques were discussed. The biological consequences of adding alloying elements to reduce the corrosion rate was explored and the need for careful selection was discussed. For example, alloying elements such as Al and Li can be used to improve the corrosion resistance of an alloy, but the release of their ions in the body can create undesirable health problems. Therefore, careful selection of the appropriate alloying elements and the resulting corrosion by-products were discussed in this article. Both these issues are critical for any material being considered for a biomedical application within the human body.

Furthermore, the development of new bioactive surface modifications and coatings, with superior physicochemical and mechanical properties has the potential to enhance the performance of Mg alloy implants by improving both their corrosion and wear resistance. The first type of surface modification discussed used conventional mechanical processes such as machining and rolling to enhance both surface and sub-surface properties. The second type of surface modification discussed examined the types of physical and chemical surface treatments that could deposit a metallic or ceramic coating or produce a surface conversion coating. The corrosion resistance and biocompatibility of the various surface modifications were discussed. In the case of ion implantation, the technique gave mixed corrosion resistance values when Zn was used to treat an Mg-Ca alloy.

While a conversion coating technique using a calcium phosphate compound produced a coating that not only reduced the corrosion rate, but also improved the biocompatibility and promoted bone formation at the surface of the Mg alloy. Whilst the use of polymeric coatings to protect implanted sensors and biomedical devices is well established, further studies are needed to examine the viability of using a polymeric material with suitable biodegradable properties to extend the operational life of a Mg alloy implant.

The combination of new Mg alloys and evolving surface
modification processes, has presented the opportunity to design and develop a biocompatible material that has the potential to be used in an orthopaedic implant. The ability to select alloying elements and surface modifications provides the opportunity to design a specific Mg alloy implant with mechanical properties and biodegradation profile that can be tailored to the specific orthopaedic application. However, more biomedical studies are needed to investigate the interaction between the material surface and the surrounding tissue environment. Also, more in situ experimental studies are needed to examine the long-term effects of alloying elements released during the biological corrosion of Mg based alloys. The biomedical, materials science and engineering research presented in this review article has clearly demonstrated the potential of using Mg based alloys to manufacture orthopaedic implants, but there are still challenges to be overcome. The first challenge is to improve the corrosion resistance of Mg base alloys by using only biocompatible alloying elements and to design biocompatible surface treatment that permits the controlled degradation of the implant. While the biomedical challenge consists of more clinical trials to establish the long-term biocompatibility of Mg based alloys and their corrosion products within the body environment.

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Chemical immersion coatings to improve biological degradability of magnesium substrates for potential orthopaedic applications

Sridevi Brundavanam, Gérrard Eddy Jai Poinern, Derek Fawcett
Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Western Australia 6150, Australia

Email address: g.poinern@murdoch.edu.au (G. E. J. Poinern)

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Abstract: Historically, cobalt-chromium, stainless steel and titanium alloys have been the main principal materials used in a variety of medical procedures for load-bearing implants in the body. Magnesium and magnesium-based alloys have the potential to be used as short-term structural support during the healing process of damaged hard tissues and diseased bone. Unlike traditional biologically compatible metals, which are not biologically degradable, magnesium based alloys offer both biological degradability and biological absorbability. Despite the many advantages offered by magnesium, its rapid degradation rate in the highly aggressive and corrosive body fluid environment has severely limited its present day medical application. This article reviews the chemical immersion technique for producing calcium phosphate coatings on magnesium substrates for slowing down the degradation rate while maintaining the biological compatibility and absorbability.

Keywords: Magnesium, Biodegradability, Chemical Immersion, Bone Tissue Engineering

1. Introduction

Biologically compatible materials have been engineered into a variety of medical devices and implants to assist in healing, replace diseased tissues and in some drastic cases completely replace damaged tissues. The repair and replacement of diseased or damaged hard tissues presents a major challenge to patient health, wellbeing and quality of life. A major consequence of tissue damage or loss resulting from accidents or diseases is the psychological impact on patient wellbeing. For example, even minor injuries to fingers or toes that interfere with function and usually heal without much trouble can have a significant impact. While severe bone diseases and injuries that result in the loss of a limb creates functional problems for patients for many years. In many cases the device or implant is temporary and only needs to remain in the body during the healing process. Once the healing process is complete, a second surgical procedure is required to remove the device or implant which significantly increases patient site morbidity and associated health costs [1-3]. Alternatively, when an implant needs to remain in the body permanently, as in the case of a total joint replacement, long-term biocompatibility, mechanical strength and structural stability become important factors that must be addressed.

Biologically compatible polymers have been extensively investigated since the 1950's for a variety of potential tissue engineering applications. Natural polymers such as collagen [4, 5], chitosan [6, 7], hyaluronic based derivatives [8, 9], polysaccharides [10, 11], and a variety of protein based materials such as fibrin gel[12, 13] have all been extensively studied and found to be suitable for a wide range of tissue engineering applications. Synthetically manufactured biodegradable and biologically absorbable polymers such as Poly (lactic acid), PLA [14, 15], Poly (L-lactic acid), PLLA [16, 17], Poly (lactic-co-glycolic acid), PLGA [18, 19], Poly-caprolactone PCL [20, 21] and Poly (glycolic acid) PGA [22, 23] have all being investigated and used in a variety of biomedical applications. For example, biodegradable sutures currently in clinical use are made from PLA and PGA. These have also been extensively investigated for the controlled delivery of drugs to specific organs within the body [24-26]. Advantages of using biological compatible polymers arise from their low toxicity within the body and the ability to...
control their degradation rate. Furthermore, the by-products of degradation can be easily handled by the body’s natural processes and excreted in the urine [27]. Polymers can also be produced in a variety of shapes and structures such as disks, films, fibres and pellets to meet the specific requirements for a particular application. In addition, polymers can be produced with micrometre and nanometre scale typographical surface features to enhance cell-substrate interactions with the surface of the implant [28].

Unfortunately, polymers with all their many advantages are limited by their low mechanical strength, which severely restricts their use in load bearing and hard tissue supporting applications. Metals have more desirable mechanical properties due to their relatively high strength, elastic modulus, fracture toughness and resilience and as a result several metallic biomaterials such as cobalt-chromium-based steel alloys, titanium-based alloys, nickel-based alloys and stainless steels have been widely used as implant materials [29-31]. However, studies have shown that conventional surgical metal alloys are not biologically absorbable and because of corrosion and wear, there is a release toxic metallic ions into surrounding cells and tissues [32-34]. These detrimental metallic ions induce an unfavourable inflammatory response from the body’s immune system and the surrounding tissues, which significantly reduces the biocompatibility of the implant [33]. Furthermore, the significant difference in mechanical properties between metal implants and surrounding bone tissue results in a clinical phenomenon known as stress shielding. For example, the elastic modulus of both cobalt-chrome alloys and stainless steel is approximately ten times larger than that of bone, while a titanium alloy such as Ti-6Al-4V is around five times greater [35]. Normally, bone tissues are constantly undergoing remodelling and modification in response to the stresses produced during everyday activities. However, the presence of a metal implant creates stress-shielding, which results in a major portion of the load being carried by the implant. Thus, with most of the load being carried by the implant, the surrounding bone tissues experience significantly less load related stress and as a result leads to bone resorption, mechanical instability and the ultimate failure of the implant [36]. In addition, metallic implants used as temporary structural supports, such as pins, screws, and plates often need to be removed by a second surgical procedure once the healing process has taken place. The increased health costs and morbidity associated with the second surgical procedure highlights the need for new biologically compatible materials that can provide short-term structural support during the healing process. Then after healing has taken place to an acceptable level, the material would then biologically degrade and safely be reabsorbed and metabolized by the body.

One interesting alternative to conventional metals used as current bio-implants is magnesium. Magnesium (Mg) is a lightweight, silvery-white metal that has been extensively used in alloy form in a wide range of engineering applications such as aerospace and automotive [37]. The density of Mg and its alloys are around 1.74 g/cm³ at 20°C, which is 1.6 and 4.5 times less dense than aluminium and steel, respectively. Interestingly, the density of pure Mg is 1.74 g/cm³, while natural bone ranges from around 1.8 to 2.1 g/cm³ and the elastic modulus of Mg and human bone are 45 GPa and 40 to 57 GPa respectively [38, 39]. It is because of the close similarity in the respective densities and elastic moduli that have made Mg a promising candidate for hard tissue engineering applications. The mechanical properties of Mg being similar to natural bone means that it has the potential to significantly reduce the possibility of stress shielding and prevent the associated bone resorption problems. Mg is also biologically degradable and biologically absorbable, with both Mg and its corrosion products considered physiologically beneficial, with as much as 30 g stored in the bone tissues and muscles of an adult body [40]. The body uses Mg, a bivalent ion, in a number of metabolic processes and to form apatite in the bone matrix [41]. And recent studies by Robinson et al. have shown that Mg has novel antibacterial properties against pathogens such as _Escherichia coli_, _Pseudomonas aeruginosa_ and _Staphylococcus aureus_ [42]. Because of these advantageous properties Mg has gained significant interest as a potential biologically degradable material that removes the need for additional surgeries to reclaim pins, screws, and plates used in the short term while the healing process takes place. Figure 1 presents an ideal life span of an Mg implant that slowly degrades and allows regenerating bone tissues to progressively carry the load. The use of Mg as an implant material also has the potential to avoid the long-term complications associated with conventional metal implants in the body.

![Graphical representation of an ideal load carrying transition between a slowly degrading Mg implant and progressively regenerating bone tissue](image)

**Figure 1.** Graphical representation of an ideal load carrying transition between a slowly degrading Mg implant and progressively regenerating bone tissue

### 2. Biological Degradation of Magnesium

The main limitation that prevents Mg being used in orthopaedic applications is its low corrosion resistance in body fluids, which are composed of water, dissolved oxygen, proteins and electrolytic ions such as chloride and hydroxide. In this highly corrosive aqueous environment results in the rapid release of ions from the metal surface which combine...
with ions in the fluid to form chemical species, such as metal oxides, hydroxides, chlorides and other compounds \[43\]. Generally metals have a tendency to corrode in electrolytic environments with some metals having a greater propensity to degrade more rapidly than others as seen in Table 1. However, the interfacial region has also a significant bearing on the overall performance of metallic bio-implants. This can be shown in Table The formation of metal oxides results in the creation of an oxide layer composed of Mg(OH)\(_2\) that adheres to the metal surface. The oxide layer is slightly soluble and reacts with chloride ions to form highly soluble magnesium chloride and the rapid production of hydrogen gas bubbles \[44, 45\]. The localised formation of gas bubbles generally begins just after surgery and continues for periods as long as three weeks. During this post surgery period, the pH around the implant increases and results in alkalization of the surrounding tissue environment. The presence of hydrogen bubbles and local alkalization can severely affect pH dependent physiological processes in the vicinity of the implant and delay tissue healing \[46\]. However, the use of a subcutaneous needle can be used to prevent significant build up of gas around the implant. Furthermore, in his study Song has suggested that small hydrogen evolution rates of around 0.01 ml/cm\(^2\)/day can be easily handled by the human body and does not constitute a serious threat \[47\]. The high initial corrosion rate produces a thick oxide layer, which fully covers and seals the metal surface to form a passive layer, which physically protects the metal surface from further corrosion. Unfortunately, the high corrosion rate during the first two to three weeks can cause a significant reduction in the mechanical and structural integrity of the implant before the bone tissues have had sufficient time to fully heal.

Table 1. Electrochemical series of selected metallic ions and their voltage potential \[78\]

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Potential (Volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au(^{3+})</td>
<td>+1.420</td>
</tr>
<tr>
<td>Pt(^{2+})</td>
<td>+1.200</td>
</tr>
<tr>
<td>Cu(^{2+})</td>
<td>0.345</td>
</tr>
<tr>
<td>Cd(^{2+})</td>
<td>-0.402</td>
</tr>
<tr>
<td>Fe(^{2+})</td>
<td>-0.440</td>
</tr>
<tr>
<td>Cr(^{3+})</td>
<td>-0.710</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>-0.762</td>
</tr>
<tr>
<td>Al(^{3+})</td>
<td>-1.670</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>-2.340</td>
</tr>
</tbody>
</table>

In atmospheric air at room temperature Mg corrodes to form a thin grey oxide layer over its surface. The oxide layer then reacts with atmospheric moisture to form a more stable magnesium hydroxide \[\text{Mg(OH)}_2\] and hydrogen gas \[43\]. Under standard environmental conditions, the Mg(OH)\(_2\) layer is able to provide some degree of protection and is also capable of slowing down the corrosion rate even under aqueous conditions \[48\]. When Mg is exposed to an aqueous environment the corrosion process can be expressed by the following equations. The primary anodic reaction involves metallic Mg being converted to Mg\(^{2+}\) ions as seen in equation (1), meanwhile the reaction occurring at the cathode, presented in equation 2, involves the reduction of protons.

**Anodic reaction:**

\[
\text{Mg} \rightarrow \text{Mg}^{2+} + 2e^- (1)
\]

**Cathodic reaction:**

\[
2\text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{H}_2 (2)
\]

The general reaction of the overall corrosion process is represented by equation (3) below.

\[
\text{Mg}^{(s)} + 2\text{H}_2\text{O}^{(l)} \rightarrow \text{Mg(OH)}_2^{(s)} + \text{H}_2^{(g)} (3)
\]

However, when Mg is exposed to chloride ions present in the physiological environment, the Mg(OH)\(_2\) interfacial layer reacts with the chloride ions to form highly soluble MgCl\(_2\). The high solubility of MgCl\(_2\) and the significant reduction in corrosion resistance provided by the reacting Mg(OH)\(_2\) layer results in rapid dissolution of the underlying Mg substrate and the formation of hydroxide ions and hydrogen gas \[49\]. The resulting dissolution and corrosion rate are important factors in the use of Mg as a biomaterial, since corrosion is likely to result in mechanical failure of an implant. Therefore, the corrosion rate must be taken into account when considering Mg and Mg based materials for hard tissue engineering and surgical applications. Ideally, the corrosion rate should be at a rate that allows temporary support of tissues during the recovery period. During the recovery period, initial mechanical strength would be maintained until the effects of corrosion start to occur. This would be followed by a gradual decrease in strength over the period of tissue recovery and finally the implant would be absorbed leaving the recovered tissues to carry the full load \[50\]. Other corrosion related factors that need to be considered is the increase in local pH and hydrogen evolution, both of which could have significant effects on tissues surrounding the implant. If Mg and Mg based materials are to be successfully used as an orthopaedic biomaterial, then the degradation behaviour and related factors of these materials need to be effectively controlled.

### 3. Controlled Degradation Via Chemical Immersion Treatment

In spite of magnesium’s many advantageous material properties, its high chemical reactivity and poor corrosion resistance has prevented its widespread use in orthopaedic applications. In general, materials used in orthopaedic applications such as titanium alloys will only experience load induced stresses in the inner core of the implant, while its surface will be exposed and interact with the surrounding physiological environment. Because the interfacial properties between the implant surface and the physiological environment are very important, different processing techniques such as alloying, thermal spray coating, ion implantation, micro-arc oxidation, anodizing and surface coating treatments have been widely used to improve the biocompatibility of the underlying material \[43, 51, 52\]. In the
case of Mg, the processing techniques have primarily focused on improving the corrosion resistance of the metal when exposed to the biological environment [53, 54].

Bioactive coatings such as calcium phosphate materials have been successfully applied to a variety of metallic implants in order to improve biocompatibility, promote attachment to surrounding hard tissues and to suppress the release of corrosion products into the human body [31]. Calcium phosphate (CaP) coating can be applied to a variety of substrate materials of varying sizes and shapes using a relatively straightforward technique known as chemical immersion [55, 56]. Apart from convincing experimental results of a number of independent studies that indicate CaP coatings can significantly improve corrosion resistance [57-59], the coatings also have the advantages of being non-toxic, displaying good biocompatibility and having enhanced bioactivity properties with respect to bone cells and other body tissues [60]. Despite these many advantages, a number of studies have also shown a number of shortcomings such as poor coating adherence, surface cracking and effective control of the CaP phases formed during immersion [54]. Regardless of these shortcomings, biologically mimicking CaP coating formed via the chemical immersion technique have the potential to control the corrosion rate and enhance the biocompatibility of Mg and Mg based alloys for orthopaedic applications.

The use of HAP coatings has a number of advantages besides improving the corrosion resistance of Mg and Mg.
based alloys in the physiological environment. HAP is a major inorganic component found in natural bone tissues, therefore using HAP as a biological coating on Mg offers a number of attractive properties such as its good biocompatibility and bioactivity properties with respect to bone cells and other body tissues [67]. Other desirable properties include slow biodegradability in situ and its ability to promote osteoconductivity and osteoinductivity, which can accelerate the in-growth of surrounding tissues [68-70]. These properties are of particular importance in the case of bone tissue that are constantly being replaced and removed by bone cells such as osteoblasts and osteoclasts, via a process known as remodelling. Studies have also shown that HAP displays an excellent biocompatible response to soft tissue such as skin, muscle and gums [71]. However, due to its low mechanical strength HAP is restricted to low load bearing clinical applications. Typical examples include coating the surface of conventional metallic implants to improve their biocompatibility and bioactivity, bone augmentation, drug delivery, and as filler material for both bone and dental implants [72-76].

HAP coatings can be directly deposited directly onto Mg substrates using chemical immersion techniques or by chemically converting modifying a pre-existing calcium phosphate coating. For example Tomozawa et al. have treated pure magnesium substrates with Calcium-EDTA and KH$_2$PO$_3$ based solutions [77]. The solution concentrations were varied from 0.01 M/L to 0.25 M/L, while the treatment temperatures were varied from 313 K to 373 K. Optimisation of their immersion process revealed that by adjusting the concentration and thermal treatment time to 0.25 M/L and 2 h, respectively, dense rod-like HAP crystals grew along the c-axis. HAP formation produced a dense, crystalline and uniform coating without the formation of a Mg(OH)$_2$ intermediate layer. The HAP coating formed by this chemical immersion technique was found to be stable and capable of significantly improving the corrosion resistance of the Mg substrates. Alternatively, a two-step immersion technique can be adopted to synthesize HAP coating on an Mg substrate. During the first step a CaP coating is deposited onto the substrate. Then during the second step the process converts the calcium phosphate into HAP. For example, the authors have produced a dicalcium phosphate dehydrate (brushite DCPD) coating on Mg substrates from aqueous solutions containing Ca(NO$_3$)$_2$ and KH$_2$PO$_4$ at 298 K. During the second step DCPD coated substrates are immersed into a solution of sodium hydroxide (NaOH) at 80 ºC for 2 h. At the end of this low temperature thermal treatment the DCPD is converted to HAP. The authors are currently carrying out degradation studies to determine the corrosion resistance of DCPD and DCPD converted to HAP coatings, Figure 4. Preliminary results indicate that significant improvement in corrosion resistance can be achieved by applying CaP coatings to Mg substrates. The chemical immersion technique is a straightforward and economic technique for synthesizing CaP coatings such as HAP on magnesium substrates. The coatings produced by chemical immersion were capable of slowing down the degradation rate by significantly improving corrosion resistance of the coated substrate.

![Figure 4. Polarization curves showing the improvement in corrosion resistance of an Mg substrate after receiving a DCPD coating. The testing solution used was a phosphate buffer saline solution at 37 ºC and a pH of 7.4](image)

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### 4. Conclusion

In recent years, there has been significant research into developing novel biologically absorbable materials for orthopaedic applications. Mg has demonstrated that it has some attractive properties and the potential to be used as a biologically degradable implant material. Mg is an extremely biocompatible material with mechanical properties similar to bone tissue. However, in spite of having good biocompatibility and bioactivity properties, Mg’s poor corrosion resistance to the physiological environment has prevented its successful use in orthopaedic applications. Chemical immersion is an economic, efficient and straightforward technique that offers a direct method of depositing CaP coatings such as DCPD and HAP on Mg substrates. Not only do CaP coatings have the potential to reduce the corrosive effects of the physiological environment, but they also offer the potential to significantly improve biocompatibility and promote bone formation at the surface of an Mg based implant. Coating Mg with a dense CaP layer that significantly reduces the corrosion rate makes this an attractive material for the manufacture of biodegradable orthopaedic implants. While a significant amount of research has been conducted in recent years investigating the potential medical use of Mg and commercially available Mg alloys, further research is needed to fully evaluate methods of reducing the degradation rate in the physiological environment. Chemical immersion is one method that has the potential to deliver CaP coated Mg based materials with the potential to be used in the manufacture of biodegradable implants. However, further research is needed to fully optimize the operational parameters of the chemical immersion technique so that there is greater control of the resulting coating properties. In addition, further in vitro and in vivo studies are needed to verify the mechanical integrity of
the coatings and their biological compatibility with bone tissues.

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**References**


placement – a materials science perspective. Biomat erials, biomaterials, the ultimate choice for orthopaedic i mplants – A properties of calcium phosphate reinforced ZK60A magnesium


2.3. Chapter summary

Chapter two was composed of two peer-reviewed papers discussing the biomedical potential of using calcium phosphate coated Mg substrates as a potential scaffold material in hard tissue engineering applications. The first paper consisted of a major literature review of Mg as a potential biomaterial capable of being used in hard tissue applications. The high corrosion rate of Mg in the physiological environment was discussed and various current industrial treatment processes examined. Unfortunately, many of the current treatment processes use toxic materials as additives or form part of the coatings and surface modifications. The potential use of calcium phosphate coatings was discussed and found to be a safe alternative to conventional surface treatments and alloying elements. The second review paper examined the potential use of chemical immersion as an economic, efficient and straightforward technique for reducing the degradation rate and extending the life of an Mg based implant in the physiological environment.
Chapter 3 – Case Study 1: Synthesis of Hydroxyapatite Nanometre Scale Powders

3.1. Overview and author contributions

Chapter three addresses the first aim of the research project; namely, chemically synthesize hydroxyapatite (HAP) in sufficient quantities from a number of sources for chemical adsorption studies and for the development of bone cements and coatings. A recent study by Poinern et al. has clearly demonstrated that ceramic pellets from compacted and sintered HAP nanometre scale ultrafine powders have the capability to significantly enhance both bioactivity and biocompatibility for potential tissue regeneration applications [1]. Following surgical implantation into sheep M. latissimus dorsi, the sponge-like porous structure of the pellets promoted mixed cell colonisation and matrix deposition. Importantly, after 12 weeks their study revealed there was extensive cellular activity present in the pellets that supported collagenous and bony matrix deposition. The study also revealed that the pellet’s extensive three dimensional inter-connecting pore structure promoted connective tissue and inflammatory cells to infiltrate. The presence of different cell types, other than bone cells also confirmed HAP’s wider biocompatibility towards other cell types. Based on the encouraging results of Poinern’s study and similar studies reported in the literature [2-5], the present research focused on using HAP and similar calcium phosphate materials as the primary coating material to protect Mg substrates.

In addition to HAP’s bioactivity and biocompatibility, its complex hexagonal structure offers an effective high capacity absorbent matrix capable of delivering a slow and sustained release of pharmaceutical products such as antibiotics, drugs, enzymes,
hormones and steroids. The use of HAP as a delivery platform for pharmaceutical products has proven to be effective in the treatment of diseases such as osteomyelitis, osteoporosis and osseous cancer [6-9]. HAPs absorbent matrix also means that in orthopaedic applications, like bone, it can accumulate substances like minerals. Hence, there was a need in this research to effectively and economically produce quantities of high quality HAP that could be used in coating and for metal ion adsorption studies (Case Studies 2 and 3 in Chapter 4). The first part of this chapter looks at optimising a chemical synthesis route developed by the Murdoch Applied Nanotechnology Research Group (MANRG) at Murdoch University. And the second part looks at a new and novel technique for converting calcium hydroxide directly into HAP (Case Study 1).

S. Brundavanam carried out all of the experiment work and conducted advance characterisation techniques [(XRD), (SEM), (EDS) and (FT-IR)] to determine the various physical and chemical properties of the synthesized nanometre scale HAP powders. S. Brundavanam was also actively involved in the subsequent data analysis. G.E.J. Poinern acted as principal supervisor and designed the overall concept for the research forming Chapter 3 with S. Brundavanam and D. Fawcett (MANRG - Research Fellow) acting as technical consultant. S. Brundavanam was assisted by G.E.J. Poinern and D. Fawcett in over-coming the various technical difficulties encountered during the research and assisted with editorial changes recommended by reviewers for the research article presented as Case Study 1. All authors provided feedback during the preparation of the paper.
3.2. Optimising Laboratory Procedure for Synthesizing Hydroxyapatite via a combined Ultrasound and Microwave based technique.

In the present research, the MANRG developed HAP synthesis procedure was optimised to improve its overall efficiency and product outcome. Exhaustive descriptions of the various aspects of the MANRG procedure can be found in the literature [1, 10, 11]. However, in the interests of completeness a brief description of the procedure follows. Synthesis begins by adding a 40 mL solution of 0.32 M calcium nitrate tetra-hydrate into a small glass beaker. Then approximately 2.5 mL of ammonium hydroxide is added and mixed until a solution pH of 9.0 is achieved. The solution is then subjected to ultrasonic irradiation for a 1 h using a Hielscher UP50H 50 W Ultrasound Processor. During the second hour of ultrasonic irradiation, a 60 mL solution of 0.19 M potassium di-hydrogen phosphate was slowly added drop-wise. During the second hour, the solution pH and Calcium/Phosphate [Ca/P] ratio was maintained at 9 and 1.67 respectively. After ultrasonic treatment the solution underwent centrifugation (15,000 g) for 20 minutes at room temperature (24 °C). The resultant white precipitate was collected, washed and then subject to a further centrifugation for 10 minutes. The precipitate was collected and deposited into a fused silica crucible. The loaded crucible was then thermally treated at 100 % power for 40 minutes in a domestic microwave (1100W at 2450 MHz-LG® Australia). After thermal treatment, the dry agglomerated powder was ball milled to produce an ultrafine nanometre scale HAP powder. The synthesis process is presented schematically in Figure 1 to given an overall appreciation of the procedure.
3.3. Case Study 3: An alternative technique for synthesising hydroxyapatite directly from calcium hydroxide.

Because of the advantageous biocompatible properties of HAP there is a definite need to produce large quantities economically. Case Study 1 investigated the effects of ultrasonic irradiation on the size, morphology and crystal structure of HAP synthesised directly from calcium hydroxide and phosphoric acid. The study details the experimental procedure for synthesising the nanometre scale powders and then uses advanced characterisation techniques such as X-ray diffraction (XRD), scanning
electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) to investigate crystallite size, morphology and powder structure.

Published Research Article

Synthesis of a Hydroxyapatite Nanopowder via Ultrasound Irradiation from Calcium Hydroxide Powders for Potential Biomedical Applications

Sridevi Brundavanam, Gérard Eddy Jai Poinern*, Derek Fawcett

Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Australia

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Abstract  Nanoscale hydroxyapatite based ceramics are a relatively new form of materials that are currently being investigated for a number of potential biomedical applications. This study reports on a straightforward wet chemical method that uses calcium hydroxide and phosphoric acid as precursors. After chemical synthesis a conventional thermal treatment was used to produce an ultrafine hydroxyapatite nanopowder. Varying ultrasonic power between zero and 400 W during the synthesis process produced crystallite sizes ranging from 15.4 nm down to 12.2 nm. The morphology of particles synthesized under the influence of ultrasonic irradiation was predominantly spherical and granular. Also present were a small number of irregular shaped plates. Energy dispersive spectroscopy revealed the samples had a Ca:P ratio of 1.66, which was very close to the ideal value of 1.67. FT-IR studies identified functional groups and confirmed the results of the X-ray diffraction data that the powders were indeed composed of nanoscale hydroxyapatite.

Keywords  Hydroxyapatite, Biomaterials, Nanometer Scale Materials, Ultrasound

1. Introduction

The efficient repair of hard tissues that form the human skeletal system continues to be a challenging goal in biomedical engineering. For many years, a variety of biologically compatible ceramics such as alumina, bioactive glasses, calcium phosphates and zirconia have been used in reconstructive and regenerative hard tissue procedures with varying degrees of success [1-3]. Importantly, any ceramic being considered for a biomedical application must be completely biologically compatible. Specifically, it must be biologically stable [4], nontoxic and non-immunogenic to body tissues [5, 6]. Among the biologically compatible ceramics, calcium phosphates are compositionally similar to the mineral phase found in bone and consequently have been used in a variety of bone replacement therapies [7, 8]. Besides being compositionally similar, calcium phosphates are lightweight, chemical stable and are composed of ions commonly found in physiological environment [9]. The two most studied and used forms of calcium phosphates in the biomedical field are tri-calcium phosphate [Ca$_3$(PO$_4$)$_2$, TCP] and hydroxyapatite [Ca$_{10}$(OH)$_2$(PO$_4$)$_6$, HAP]. For example, HAP is ideal for many biomedical applications since it is the most thermodynamically stable calcium phosphate in the physiological environment [10]. Furthermore, studies have shown that HAP has good biocompatibility and bioactive properties with body tissues. In particular, for bone tissue engineering applications it offers good osteoconductivity and osteoinductivity capabilities. While it’s slow biodegradability in situ allows tissue regeneration and tissue replacement to take place [3, 11, 12]. These properties are of particular importance since bone continually undergoes cellular remodeling and as a result tissue is simultaneously deposited by osteoblasts cells and removed by osteoclasts cells [3, 13]. It is due to the advantageous bioactive properties of HAP that has made it a successful biomaterial for a number of clinical applications such as dental repair procedures, repairing bone defects, bone augmentation and coatings on metallic implants [14, 15]. The positive results achieved in these clinical applications has encouraged further research into HAP and its potential use in a variety of new tissue regeneration and tissue engineering applications [16, 17]. To date several ceramic processing techniques have been used to synthesize HAP such as wet precipitation, sol-gel, hydrothermal and ultrasonic [18-22]. However, there is a current need to develop more efficient processing techniques that have the potential to deliver large quantities of HAP nanopowders for future biomedical applications. In the present study, a straightforward synthesis process was used to precipitate HAP seeds from a solution containing calcium hydroxide and phosphoric acid. The process was conducted at room temperature, while the solution pH was maintained at 10 via the addition of
ammonia. Synthesis was carried out with and without ultrasonic irradiation to examine its influence on particle size and morphology. After a straightforward thermal treatment, particle size, composition, crystalline structure and morphology of nanopowders were studied using advanced characterization techniques such as powder X-ray diffraction (XRD) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared spectroscopy (FT-IR).

2. Materials and Methods

2.1. Materials

HAP powders were synthesised from analytical grade calcium hydroxide \([\text{Ca(OH)}_2]\) supplied as the source of Ca\(^{2+}\) ions and analytical grade phosphoric acid \([\text{H}_3\text{PO}_4\ (86.2\%)]\) supplied as the source of phosphate ions. Calcium hydroxide was purchased from Ajax Finechem (New South Wales, Australia) and phosphoric acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Solution pH was achieved by the addition of ammonia that was supplied by CHEM-SUPPLY (New South Wales, Australia). All aqueous solutions were made using Milli-Q® water (18.3 MΩ cm\(^{-1}\)) produced by an ultrapure water system (Barnstead Ultrapure Water System D11931; Thermo Scientific, Dubuque, IA). The ultrasound processor used during ultrasonic assisted synthesis of HAP powders was an UP400S [400 W, 24 kHz, H22 Sonotrode (22 mm diameter, 45 mm maximum submerged depth)] supplied by Hielscher Ultrasound Technology. Ultrasonic outputs were controlled by operating the rotary regulator as per the manufacturer’s specifications (20% to 100%).

2.2. Synthesis of Nanometre Scale HAP Powders

The synthesis procedure begins by preparing a 100 mL aqueous solution of 0.32M Ca(OH)\(_2\) in a glass beaker. The solution was then heated to 40 °C under vigorous stirring for 15 minutes on a standard laboratory hotplate/magnetic stirrer. Then a 100 mL solution of 0.19 M H\(_3\)PO\(_4\) was slowly added to the Ca(OH)\(_2\) solution. The mixture was further stirred for 15 minutes while the mixture pH was adjusted to 10 by the drop-wise addition of ammonia. At the end of the mixing period, excess fluid was decanted from the beaker and the precipitate was deposited onto a glass dish. The dish was then placed onto a hotplate which was progressively heat up to 300 °C. The precipitate was then thermally treated for 3 h.

The synthesis procedure is schematically presented in Figure 1 (a). Ultrasonically assisted synthesis follows a similar procedure except for two 30 minutes ultrasonic treatment periods as indicated in the schematic procedure presented in Figure 1 (b). At the end of each synthesis procedure the resulting powders were examined using advanced characterisation techniques.

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Figure 1. Schematic of experimental procedures used for synthesizing HAP nanopowders: (a) without ultrasound and (b) with ultrasound
2.3. Advanced Characterisation

2.3.1. Powder X-ray Diffraction (XRD) Spectroscopy

X-ray diffraction (XRD) spectroscopy was used to study the phase’s present and crystalline sizes in the powder samples. Spectroscopy data was recorded at room temperature, using a GBC® eMMA X-ray Powder Diffractometer \([\text{Cu } K\alpha = 1.5406 \text{ Å radiation source}]\) operating at 35 kV and 28 mA. The diffraction patterns were collected over a 2θ range of 20° to 60° with an incremental step size of 0.02° using flat plane geometry with 2 second acquisition time for each scan.

2.3.2. Transmission Electron Microscopy (TEM)

The size and morphology of particles formed by the synthesis process were investigated using TEM. Sample preparation consisted of collecting a portion of the synthesized powder and then placing it into a small tube containing Milli-Q® water. The tubes were then sealed and placed into an ultrasonic bath for 10 minutes. Then a single drop from the suspension was deposited onto a carbon-coated copper TEM grid using a micropipette and then allowed to slowly dry over a 24-hour period. After sample preparation a bright field TEM study was carried out using a Phillips CM-100 electron microscope (Phillips Corporation Eindhoven, The Netherlands) operating at 80kV. TEM was also used in conjunction with FESEM images to measure particle sizes and determine particle morphology present in the powders samples.

2.3.3. Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform infrared spectra (FT-IR) spectroscopy was used to identify species, functional groups and vibration modes present in the respective powder samples. Powder analysis was carried out using a Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment. FT-IR spectra were recorded in the range from 525 to 4000 cm\(^{-1}\) in steps of 4 cm\(^{-1}\).

2.3.4. Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive Spectroscopy (EDS)

All micrographs were taken using a FE-SEM Zeiss Neon 40 EsB with an attached energy dispersive X-ray spectrometer. The micrographs were used to study the size, shape and morphological features of the synthesized powders. Samples were mounted on individual substrate holders using carbon adhesive tape before being sputter coated with a 2 nm layer of platinum to prevent charge build up using a Cressington 208HR High Resolution Sputter coater. The EDS technique was used to verify the results of the XRD analysis and to calculate the Ca/P ratio of the synthesized powders. In addition, FESEM was also used in conjunction with TEM images to measure particle sizes and determine particle morphology present in the powders samples.

Figure 2. XRD patterns of nanometre scale hydroxyapatite powders synthesized under the influence of ultrasonic irradiation ranging from 0 to 400 W.
3. Results and Discussions

3.1. XRD Spectroscopy and TEM Analysis

Analysis of powder XRD patterns was used to identify the purity and crystalline size of HAP nanopowders produced with and without ultrasonic power. Figure 2 presents representative XRD patterns for nanopowder samples. Maximum ultrasonic power used was 400 W (100 %) and lower power level measurements were carried out at pre-determined power levels (0 %, 20 %, 40 %, 60 %, 80 % and 100 %). All patterns in Figure 2 match the known phases for pure HAP and are consistent with the phases listed in the ICDD database. In addition, each pattern identifies the main (h k l) indices associated with peaks found in the HAP samples, namely (002), (211), (300), (202), (310), (222) and (213). All patterns show characteristic HAP peaks and no evidence of non-HAP related phases were seen. Further analysis was carried out to determine the crystalline size, t (hkl), of each powder sample using the Debye-Scherrer equation [23-25].

\[
 t_{(hkl)} = \frac{0.9 \lambda}{B \cos \theta_{(hkl)}}
\]

where, \( \lambda \) is the wavelength of the monochromatic X-ray beam, \( B \) is the Full-Width at Half Maximum (FWHM) of the peak at the maximum intensity, \( \theta_{(hkl)} \) is the peak diffraction angle that satisfies Bragg’s law for the (h k l) plane and t(hkl) is the crystallite size. The crystallite size for each sample was calculated from the (002) reflection peak. Analysis of crystallite size data revealed increasing ultrasonic power tended to produce smaller crystallite sizes. Synthesis of powders without ultrasound power gave a mean value of 15.4 nm and powders synthesized at 400 W produced crystallites with a mean value of 12.2 nm as seen in Figure 4 (a).

Figure 3 (a) presents a representative TEM image of Ca(OH)\(_2\) particles prior to synthesis. The particles are spherical and range in size from 0.1 \( \mu \)m up to 0.3 \( \mu \)m. Figure 3 (b) presents a typical image of HAP powders produced without the presence of ultrasonic power. The particles are rod-like to needle-like in shape and indicate growth in the c direction. The particles tend to be around 10 nm in diameter, but range in length from 50 nm upwards. However, this is not the case for powders synthesized under the influence of ultrasonic irradiation as seen in Figure 4 (b). The TEM image shows a completely different particle size and morphology. The morphology is predominantly spherical and granular in nature, but present were a small number of irregular shaped platelets which can also be seen in the high resolution FESEM image presented in Figure 4 (c).

3.2. FESEM, EDS and FTIR Spectroscopy

FESEM in conjunction with TEM microscopy was used to study particle size and morphology of nanopowders synthesized under the influence ultrasonic irradiation. Both Figure 4 (b) and (c) reveal the powder sample synthesized at 400 W is highly agglomerated. The particle morphology seen in this study is similar to morphologies previously reported in the literature [22-25]. Particle size analysis based on TEM and FESEM images for the 400 W power setting reveals a narrow particle size distribution. Particle sizes range from 20.0 nm up 66.7 nm, with spherical particles having a mean diameter of 22.8 nm and granular particles having a mean diameter of 36.7 nm as seen in Table 1. Close examination of Figure 4 (c) also reveals the presence of a small number of tube-like and irregular shaped platelets. Both of these particle features are within the narrow particle distribution as indicated in Table 1. This narrow particle distribution has also been seen in similar studies involving the sonochemical synthesis of HAP [26]. Figure 4 (d) presents an EDS spectrum of a typical powder sample showing peaks corresponding to Ca, P, and O. The presence of these peaks confirms the chemical composition of HAP. Analysis of EDS data indicated that the powder had a Ca:P ratio of 1.66, which was very close to the ideal value of 1.67.
Figure 4. (a) Graphical results of XRD analysis showing the crystallite size dependence on ultrasonic power used during synthesis, Representative TEM micrograph (b) and high resolution FESEM (c) showing the spherical and granular shaped HAP particles synthesized.

Table 1. Particle size distribution and morphology from TEM and FESEM analysis for a HAP nanopowder synthesized under the influence of 400 W.

<table>
<thead>
<tr>
<th>Shape</th>
<th>Profile</th>
<th>Size Range (nm)</th>
<th>Aspect Ratio</th>
<th>Mean (nm)</th>
<th>Std. Dev. (nm)</th>
<th>% of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical (smooth)</td>
<td>Dia.</td>
<td>20.0 – 33.4</td>
<td>NA</td>
<td>22.8</td>
<td>3.7</td>
<td>44.9</td>
</tr>
<tr>
<td>Spherical (angular)</td>
<td>Dia.</td>
<td>26.7 – 46.7</td>
<td>NA</td>
<td>36.7</td>
<td>7.5</td>
<td>26.1</td>
</tr>
<tr>
<td>Irregular</td>
<td>Width Length</td>
<td>20.0 – 40.2</td>
<td>NA</td>
<td>27.4</td>
<td>7.4</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>33.4 – 66.7</td>
<td>1.7</td>
<td>47.3</td>
<td>4.8</td>
<td>17.4</td>
</tr>
<tr>
<td>Rod like</td>
<td>Dia. Length</td>
<td>20.0 – 33.3</td>
<td>NA</td>
<td>21.7</td>
<td>4.4</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.0 – 66.7</td>
<td>1.2</td>
<td>43.4</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 5 presents representative FT-IR spectra of three HAP nanopowders synthesized under various levels of ultrasonic irradiation. The first spectrum (Blue) is a powder synthesized without ultrasonic irradiation. The other two spectra are powders synthesized under different ultrasonic power levels [Red (80 W ~ 20%) and Green (400 W ~ 100%)]. All three spectra have similar band locations and intensities normally associated with nanoscale HAP. Considering all three spectra together, starting from the right hand side of Figure 5 and moving to the left the various bands are identified. The first two bands encountered are 562 cm\(^{-1}\) and 601 cm\(^{-1}\), which are the result of ν\(_4\) vibrations produced by the O-P-O mode. The next significant bands located at 631 cm\(^{-1}\) and 880 cm\(^{-1}\) are associated with the carbonate groups and clearly indicate the presence of carbonates in all three sample spectra. The next band located at 964 cm\(^{-1}\) is the result of ν\(_1\) symmetric stretching vibrations normally associated with the P-O mode.

The very strong bands located at 1024 cm\(^{-1}\) and 1090 cm\(^{-1}\) indicated the presence of PO\(_4^{3-}\) functional groups (P-O mode) and the weaker bands at 1415 cm\(^{-1}\) and 1460 cm\(^{-1}\) corresponds to CO\(_3^{2-}\) functional groups. The next band located at 1654 cm\(^{-1}\) also corresponds to a CO\(_3^{2-}\) group. The presence of carbonates in the samples most likely results from atmospheric carbon dioxide interacting with the alkaline HAP precursor solution during the synthesis process [27-29]. The band located at 2978 cm\(^{-1}\) indicates the stretching vibrations associated with the C-H mode, while the band located at 3376 cm\(^{-1}\) indicates the presence of absorbed water. The final peak located at 3569 cm\(^{-1}\) corresponds to OH vibrations in the HAP lattice. The results of the FT-IR analysis have clearly identified functional groups normally associated with HAP and confirm the results of the XRD data.
The results of the study have shown that synthesis of nanoscale HAP from an aqueous solution containing calcium hydroxide and phosphoric acid without the aid of ultrasonic irradiation produced particles with a needle-like morphology. However, aqueous solutions subjected to ultrasonic irradiation during synthesis tended to produce a different particle size and morphology. Ultrasound assisted synthesis produced particles with a morphology that was predominantly spherical and granular in nature. Also present in the samples were a small number of irregular shaped platelets. The particle size distribution was narrow and ranged from 20.0 nm up 66.7 nm. Both XRD and FT-IR characterization studies confirmed the powders were nanoscale HAP. Further studies are needed to examine the influence of larger ultrasonic powers using this method and biological compatibility studies via in vitro cell studies needs to be undertaken.

Acknowledgements

Mrs. Sridevi Brundavanam would like to acknowledge Murdoch University for providing her PhD Scholarship to undertake the hydroxyapatite synthesis studies as part of her PhD project.

REFERENCES


3.4. Chapter summary

This chapter examined optimising the MANRG procedure for producing HAP and developing an alternative route for producing HAP. The alternative procedure manufactured HAP from calcium hydroxide precursor solid powders and phosphoric acid. The procedure examined the effect of ultrasonic irradiation during the synthesis and revealed that increasing ultrasonic power produced smaller particle sizes. The morphology of the particles produced was spherical, granular and agglomerated. The smallest mean particle size produced was 12.2 nm. Furthermore, the straightforward synthesis technique has scale up potential that makes it an attractive method for producing large quantities of HAP for commercial applications.

References


Chapter 4 - Case Studies 2 and 3: Adsorption of Selected Heavy metals using Hydroxyapatite absorbers

4.1. Overview and author contributions

This chapter addresses aim 2 of the research project, namely, investigating the chemical adsorption capabilities of hydroxyapatite (HAP) to determine its suitability as an effective bone substitute during a tissue regeneration or tissue engineering procedures (Case Studies 2 & 3). This is of particular importance since the coating must be able to function like the mineral phase found in bone tissue and have the ability to act as an adsorption matrix.

S. Brundavanam carried out all of adsorption studies and conducted the advanced characterisation techniques [(XRD), (SEM), (EDS) and (FT-IR)] needed to fully investigate the properties of the HAP absorbers. S. Brundavanam was also actively involved in the subsequent adsorption data analysis and interpretation of the characterisation results. G.E.J. Poinern acted as principal supervisor and designed the overall concept for the research forming Chapter 4 with S. Brundavanam and D. Fawcett (MANRG - Research Fellow) acting as technical adviser. S. Brundavanam significantly contributed to resolving various technical difficulties encountered during the adsorption studies and assisted with editorial changes recommended by reviewers for the two peer-reviewed research articles as presented in Case Studies 2 and 3. All authors provided feedback during the preparation of the two articles.
4.2. Published Research Articles

Case Study 2

- Gérrard Eddy Jai Poinern, Sridevi Brundavanam, Derek Fawcett. Kinetic and adsorption behaviour of aqueous cadmium using a 30 nanometre scale hydroxyapatite powder synthesized via a combined ultrasound and microwave based technique (Currently under review - submitted to Journal of Materials and Environmental Science)

Case Study 3

Kinetic and adsorption behaviour of aqueous cadmium using a 30 nm hydroxyapatite based powder synthesized via a combined ultrasound and microwave based technique

Gérrard Eddy Jai Poinern*, Sridevi Brundavanam¹, Suraj Kumar Tripathy², Mrutyunjay Suar³, Derek Fawcett¹

¹ Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology School of Engineering and Energy, Murdoch University, Murdoch, Western Australia 6150, Australia.
² Chemical & Bioprocess Engineering Lab Center of Industrial Technology & School of Biotechnology KIIT University, Campus-11, Bhubaneswar 751024, Odisha, India.
³ School of Biotechnology, KIIT University, Bhubaneswar-751024, Odisha, India

Received 30 January 2015
*Corresponding Author. E-mail: g.poinern@murdoch.edu.au Tel: (+61 8 9360-2892)

Abstract
The removal of heavy metals such as cadmium from contaminated waterways and soils is a very important aspect of environmental remediation. This study investigated the kinetic and adsorption performance of a nanometre scale hydroxyapatite (HAP) absorber synthesised from a combined ultrasound and microwave based technique for the removal of cadmium from an aqueous salt solution. Parameters such as contact time, initial pH, initial cadmium concentration and temperature were investigated. The Freundlich isotherm resulted in a more precise modelling of the communicated experimental data. Maximum monolayer adsorption capacity of absorber was found to be 123.45 mg/g at 298 K. Kinetic studies established cadmium adsorption tended to follow a pseudo-second order model and intra-particle diffusion played a significant role in determining the rate. Adsorption was endothermic, spontaneous and resulted in structural changes to the absorber. HAP was found to be an effective absorbent material for the removal of cadmium loaded aqueous solutions.

Keywords: nanohydroxyapatite, adsorption, cadmium, ultrasound, microwaves
Introduction

Accumulation of heavy metal contaminants in freshwater supplies, effluents, wastewater, and soils is an extremely important environmental problem that threatens the world. Even in small concentrations, heavy metals are extremely hazardous to living organisms due to their toxicity and tendency to accumulate in the food chain [1, 2]. Therefore, the removal of heavy metals from contaminated water sources is a priority issue in many environmental remediation programs worldwide. Heavy metals such as cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn) are generally considered the major contaminants of surface water, ground water and soils. The main sources of these contaminants are metal plating industries, mining industries and drainage from abandoned disposal sites. In particular, cadmium is highly toxic, very hazardous for aquatic and soil life and is a known carcinogen in humans [3]. Although cadmium can be found in very small quantities in the natural environment, a significant level of this particular harmful metal is potentially released during industrial and mining processes [4]. Three most common modes of non-ecologically friendly metal uptake by the human metabolic system are via nutritional intake, drinking water and inhalation. Inhalation of cadmium produces significant irritation to the respiratory tract and all three modes of consumption lead to anaemia, osteoporosis, osteomalacia, kidney damage and Itai-itai disease [5-7]. In the case of osseous related diseases, it is the remarkable ion exchange capacity of natural hydroxyapatite (HAP) present in bone tissue that permits the replacement of calcium ions with cadmium ions.

It is due to this extraordinary ability of HAP to accumulate and bind with heavy metals ions such as cadmium within the body, which produces the serious detrimental effect on the health and well-being of an individual. In the case of environmental remediation of contaminated wastewaters, several processes such as filtration, chemical precipitation, electrochemical deposition, ion exchange, adsorption and solvent extraction have all been
extensively used with varying degrees of efficiency and cost effectiveness [8-10]. Chemical immobilization by adsorption is one method for reducing the bioavailability of toxic metals via the formation of new stable minerals with lower solubilities in the environment. Several studies have identified the high adsorption capacity of HAP and its ability to combine especially with divalent heavy metals. It is this property that enables HAP to significantly reduce metal ion concentrations in aqueous solutions [11-14]. HAP is a naturally occurring mineral with a hexagonal crystal structure composed of calcium phosphate groups and has the general formula of \([\text{Ca}_{10}(\text{OH})_2 (\text{PO}_4)_6]\) for the unit cell. Both chemical and crystallographic studies have also shown that synthetic HAP is similar to the chemical composition of the naturally occurring inorganic component found in teeth and bone tissue [15]. Synthetic forms of HAP have been successfully used in a wide range of applications such as bioceramics for dental and bone repair procedures, absorbents to separate enzymes and proteins during chromatography and as catalysts for dehydrogenation and dehydration of alcohols [16, 17]. The wide range of applications stem from the advantageous surface properties of HAP that include a hydrophilic nature, surface charge, pH, porous structure and 2.6 P-OH surface groups per nm\(^2\) which act as sorption sites [18, 19]. It is the sorption properties of HAP that makes it an attractive absorbent material for potential remediation of heavy metal contaminated water, which adds up from the environment naturally or synthesized and disposed as waste industrial process by-products into the ecosystem.

The synthesis of nanometre scale crystalline HAP (nano HAP) for use as an adsorbent material has been extensively studied and has resulted in techniques such as emulsions, hydrothermal and solution-gelation being used to produce the material [20-22]. However, wet chemical synthesis offers many advantages due to its straightforward and economically efficient protocols during the process. In particular, one advantage is being able to control size and morphology of the forming particles by varying experimental parameters. It is the control of experimental parameters that directly regulates particle nucleation, aging and growth kinetics.
Recent studies have also investigated the use of ultrasonic irradiation during wet chemical synthesis to enhance the manufacture of nanometre scale materials [23, 24]. During ultrasonic irradiation, acoustic cavitations create bubbles that grow and then implosively collapse creating localized hot spots with temperatures as great as $5300 \text{ K}$ and pressures around 500 atmospheres. The acoustic cavitations also produce very rapid cooling rates that often exceed $1010 \text{ K/s}$ [25]. It is the extreme pressures and temperatures experienced during ultrasonic irradiation that promotes the physical effects and chemical reactions which directly influence the size and morphology of the forming particles [26]. Another technique that has been successfully used to improve the synthesis route is microwave heating that significantly reduces reaction times and increases product yields when compared to conventional heating methods [15, 26, 27]. Therefore, incorporating ultrasonic and microwave based techniques into the wet chemical method offers greater efficiency in manufacturing nanometre scale materials. In this study, solutions containing calcium, hydroxyl and phosphate ions were subjected to ultrasound irradiation to form the initial calcium phosphate compounds. This was followed by thermally treating the compounds in a microwave oven to produce the nanometre scale HAP powders used in characterisation and adsorption studies.

The objectives of this study were to first synthesize a nanometre scale HAP powders via a combined ultrasonic and microwave heating based wet chemical method. And secondly, investigate the potential use of the powders as an adsorbent material for the removal of $\text{Cd}^{2+}$ cations from aqueous solutions. The synthesized powders were characterized using X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), energy dispersive spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR). The adsorption capacity of the powders was investigated via the removal of $\text{Cd}^{2+}$ cations from cadmium contaminated water using a batch equilibrium procedure. Both Langmuir and Freundlich adsorption isotherms were used to model the experimental data. While the kinetic behaviour of the adsorption mechanism were studied using Lagergren’s pseudo-first order, McKay & Ho’s
pseudo-second order and intra-particle diffusion models. Furthermore, the influence of initial 
Cd\(^{2+}\) cation concentration, contact time, temperature, solution pH and thermodynamic 
parameters were all evaluated from the adsorption measurements.

2. Materials and methods

2.1. Materials

HAP powders were synthesized from high purity calcium nitrate tetra-hydrate [Ca(NO\(_3\))\(_2\)\(\cdot\)4H\(_2\)O] and potassium di-hydrogen phosphate [KH\(_2\)PO\(_4\)], while solution pH was controlled by the 
addition of ammonium hydroxide [NH\(_4\)OH]. An Ultrasound Processor [UP50H: 50 W, 30 kHz, 
MS7 Sonotrode (7mm diameter, 80 mm length)] supplied by Hielscher Ultrasound Technology 
was used to deliver ultrasound irradiation during HAP synthesis. The source of Cd\(^{2+}\) ions used 
in the adsorption studies was high purity cadmium chloride [CdCl\(_2\)]. All chemicals used in this 
work were supplied by Chem-Supply (Australia) and all aqueous solutions were made using Milli-Q\(^\circledR\) water (18.3 M\(\Omega\) cm\(^{-1}\)) produced by an ultrapure water system (Barnstead Ultrapure 
Water System D11931; Thermo Scientific, Dubuque, IA).

2.2. Synthesis of nanometres scale hydroxyapatite

The synthesis procedure used to produce the nanometre scale HAP powders was developed by 
the authors and a detailed description is given elsewhere [15, 24 &28]. However, in the interest 
of completeness a brief description is presented. The procedure in brief consists of adding a 40 
 mL solution of 0.32 M calcium nitrate tetra-hydrate into a small glass beaker and then adjusting 
the solution pH to 9.0 using approximately 2.5 mL of ammonium hydroxide. The solution was 
then sonicated for 1 h using the Ultrasound Processor set to 50 W and maximum amplitude. 
During the second hour of ultrasound treatment 60 mL of 0.19 M potassium di-hydrogen 
phosphate solution was slowly added while the solution pH was maintained at 9.0 and the 
Calcium/Phosphate [Ca/P] ratio was maintained at 1.67. On completion of the ultrasound
treatment, the solution was then subjected to 20 minutes of centrifugation (15,000 g) at room temperature to produce a solid white precipitate. The precipitate was collected, washed and centrifuged for a further 10 minutes. After the second centrifugation, the precipitate was deposited into a fused silica crucible before being placed into a domestic microwave oven for thermal treatment [Set at 100 % power for 40 minutes: 1100W at 2450 MHz-LG® Australia]. After thermal treatment, the resultant agglomerated powder was then ball milled until an ultrafine nanometre scale HAP powder was produced.

2.3. Advanced characterisation techniques

2.3.1. X-ray diffraction (XRD) spectroscopy

Powder X-ray diffraction (XRD) spectroscopy was used to examine and identify the purity, crystalline size and phases present in synthesized nanometre scale HAP powders. Spectroscopy data was recorded at room temperature, using a Siemens D500 series diffractometer [Cu Kα = 1.5406 Å radiation source] operating at 40 kV and 30 mA. The diffraction patterns were collected over a 2θ range of 20° to 60° with an incremental step size of 0.04° using flat plane geometry with 2 second acquisition time for each scan. The crystalline size of the particles in the powders was calculated using the Debye-Scherrer equation [Equation 1] from the respective spectroscopy patterns.

2.3.2. Field emission scanning electron microscopy (FESEM)

FESEM was used to study the size, shape and morphological features of the HAP powders before and after the adsorption studies. All micrographs were taken using a high resolution FESEM [Zeiss 1555 VP-FESEM] at 3 kV with a 30 µm aperture operating under a pressure of 1.333×10⁻¹⁰ mbar. Samples were mounted on individual substrate holders using carbon adhesive tape before being sputter coated with a 2 nm layer of gold to prevent charge build up using a Cressington 208HR High Resolution Sputter coater.
2.3.3. Energy dispersive spectroscopy (EDS)

EDS was used to provide an elemental analysis of the powder samples using an Oxford Instruments energy dispersive system (133 eV resolution), via a 10 mm² SATW detector. The analysis was carried out to verify the results of the XRD analysis and to calculate the Ca/P ratio of the synthesised nano-HAP powders.

2.3.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy of synthesized nanometre scale HAP powders and cadmium loaded HAP powders was carried out using a Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment. Both FT-IR spectra were recorded in the range from 525 to 4000 cm⁻¹ in steps of 4 cm⁻¹.

2.4. Batch adsorption studies

All adsorption experiments were carried out using the batch equilibrium technique. During experiments the adsorption capacity of absorber for Cd²⁺ ions was investigated. The study also examined the influence of the initial Cd²⁺ ion concentration, contact time, temperature and pH of the test solutions.

The influence of contact time on Cd²⁺ ion adsorption on absorber was examined using aqueous solutions containing 100 mg/L of Cd²⁺ ions (100 ppm) prepared from cadmium chloride [CdCl₂]. A sample of 0.1 g HAP taken from the stock solution (1g/L) was added to a 100 ppm Cd²⁺ prepared solution. The magnetic stirring speed was set to 400 rpm, while the temperature of the suspension was maintained at 298 ± 1 K. The pH of the suspension was adjusted by adding drops of 0.1 M NaOH to the suspension and then maintaining the value of 7 throughout the experiment. Sample volumes were taken from the suspension during the mixing
process at pre-determined time intervals (10, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min) so that Cd$^{2+}$ ion concentration in the solution could be measured. After each specified time, the sample solution was first filtered using a Whatman® 0.22μm membrane syringe filter and then centrifuged at 15,000 g for 20 minutes. Each time a sample volume was taken, an equal volume was then added to the solution to maintain the initial volume. The concentration of Cd$^{2+}$ ions in the sample solution was determined by inductively coupled plasma atomic emission spectrometer (Varian Vista Axial CCD ICP-AES). All experiments were carried out in triplicate.

The effect of solution pH on adsorption was studied by adjusting the pH from 2 to 12. This was done by treating the solution with either 0.1M HCl or 0.1M NaOH. The temperature was normally set to 298 K, except where temperature variation studies were carried out. After 300 min, the solid was separated from the suspension using a Whatman® 0.22μm membrane syringe filter and the residual cadmium level was measured. The influence of initial Cd$^{2+}$ ion concentration was studied by first preparing a series of Schott reagent bottles containing 100 mL aqueous solutions consisting of varying concentrations of Cd$^{2+}$ ion (50 to 175 mg/L) with an initial pH of 7. Then 1 g/L stock solution containing nanometre scale HAP powder was added (100 mL) into each bottle. The bottles were then sealed and the magnetic stirring speed was set to 400 rpm, while the bottles were thermostatically maintained at the respective isotherm (283, 293, 303, 313 and 323 K). Measurement of Cd$^{2+}$ concentration levels was carried out at predetermined time intervals (10, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min). All initial Cd$^{2+}$ ion concentration experiments were carried out in triplicate. The data collected from the adsorption experiments was then used in the subsequent kinetic and adsorption isotherm modelling studies.

3. Results and discussion

3.1. XRD spectroscopy analysis
Analysis of respective powder XRD patterns was used to identify the purity and crystalline size of the synthesised nanometre scale HAP powders used in the adsorption studies. A XRD pattern of a representative pure HAP powder before being used in the adsorption studies is presented in Figure 1. Inspection of the HAP powder (blue pattern in Figure 1) reveals that the reflection pattern matches the known phases present in pure HAP and is consistent with the phases listed in the ICDD database. The pattern also identifies main (h k l) indices found in the HAP sample, namely (002), (211), (300), (202), (310), (222) and (213). The pattern only shows characteristic HAP peaks and there was no evidence of non-HAP phases. In addition, the crystalline size, \( t_{(hkl)} \), of each sample was calculated from the respective XRD patterns using the Debye-Scherrer equation \([29-31]\)

\[
\frac{1}{t_{(hkl)}} = \frac{0.9\lambda}{B \cos \theta_{(hkl)}}
\]

where, \( \lambda \) is the wavelength of the monochromatic X-ray beam, \( B \) is the Full Width at Half Maximum (FWHM) of the peak at the maximum intensity, \( \theta_{(hkl)} \) is the peak diffraction angle that satisfies Bragg’s law for the (h k l) plane and \( t_{(hkl)} \) is the crystallite size. The crystallite size calculated from the (002) reflection peak for the sample gave a mean value of 30 nm.

Studies have shown that HAP has a strong selectivity towards divalent metal cations via an ion-exchange mechanism \([19, 32]\). The results of this study confirm HAPs selectivity towards \( \text{Cd}^{2+} \) cations and the substitution of \( \text{Cd}^{2+} \) cations for lattice \( \text{Ca}^{2+} \) cations. The ion-exchange mechanism was also assisted by the slight difference between the respective ionic radius’s of \( \text{Cd}^{2+} \) (9.7 nm) and \( \text{Ca}^{2+} \) (9.9 nm). After each adsorption procedure the solid residue was collected and subjected XRD analysis. Inspection of the XRD pattern for a representative \( \text{Cd}^{2+} \) loaded sample is presented in Figure 1 and reveals structural changes resulting from \( \text{Cd}^{2+} \) absorption. The ion-exchange that takes place between the \( \text{Cd}^{2+} \) and \( \text{Ca}^{2+} \) cations also results in a very small decrease in the unit cell dimensions and hence volume of the unit cell. Thus, the associated small XRD shifts reported in the literature were also seen in this study and support
the ion-exchange mechanism [11, 33]. In this study, the ion-exchange mechanism between Cd\(^{2+}\) and Ca\(^{2+}\) can be represented by the equivalent molar exchange in general HAP formula Ca\(_{10-x}\) Cd\(_{x}\) (PO\(_4\))\(_6\) (OH)\(_2\), where x can vary from 0 to 10 depending on experimental parameters and reaction time [11].

**Figure 1:** XRD pattern of synthesized nanometre scale HAP powder after milling and drying (Blue), while the second XRD pattern (Red) reflects the maximum uptake of Cd\(^{2+}\) of the solid residue at the end of the adsorption study. The study used 1 g/L HAP absorbent, a 100 mg/L Cd\(^{2+}\) ion solution, with a pH of 7 and stirred at 400 rpm for a total contact time of 300 min.
3.2. FESEM and EDS analysis

FESEM was used to study particle size and morphology before and after adsorption studies. A typical micrograph of a pure HAP powder sample is presented in Figure 2(b) and reveals a sphere like particle morphology that is highly agglomerated. The spherical morphology seen is similar to particle morphologies previously reported in the literature [15, 29-31]. Particle size analysis of micrographs revealed a mean particle diameter of 28 ± 5 nm and compared favourably to the XRD determined value of 30 ± 5 nm. After adsorption studies, FESEM analysis was also performed on samples to determine any changes in size or morphology. Figure 2 (d) presents a typical micrograph of the Ca_{10-x}Cd_{x}(PO_4)_{6}(OH)_{2} structure displaying the same agglomerated spherical morphology seen in samples prior to adsorption studies. The mean particle size seen in the Cd^{2+} loaded samples was estimated to be 26 ± 5 nm. The studies have revealed that there was no significant difference in particle size and morphology between unloaded and loaded powder samples. Figure 2 (a) presents a typical EDS spectrum of an unloaded sample showing peaks corresponding to Ca, P, and O, and confirms the chemical composition of HAP. In addition, a number of Au and Cu peaks were also seen in the EDS spectrum. The Au peaks were the product of the Au coating used on the samples, while the Cu peaks are a consequence of X-rays being scattered from the copper grid. Figure 2 (c) presents an EDS spectrum of a representative Cd^{2+} loaded sample and confirms the chemical composition of the sample. The presence of two cadmium peaks (indicated by red arrows) also confirms the results of the XRD analysis discussed above.
Figure 2: EDS spectrum (a) and FESEM micrograph (b) of unloaded nanometre scale crystalline HAP powder and EDS spectrum (c) and FESEM micrograph (d) of a typical Cd$^{2+}$ loaded HAP residue.

3.3. FT-IR studies

FT-IR spectroscopy and subsequent analysis was used to identify species, functional groups and vibration modes associated with peaks seen in sample spectra taken before and after adsorption studies. Figure 3 presents the results of FT-IR spectroscopy of representative powder samples taken before and after adsorption studies. Starting from the right hand side of Figure 3 with a powder sample prior to adsorption testing. We see three peaks occurring at 561 cm$^{-1}$, 600 and 631 cm$^{-1}$ that are consistent with $\nu_4$ vibrations normally associated with O-P-O modes. The weaker peak located at 832 cm$^{-1}$ is associated with a carbonate group and clearly indicates the presence of carbonates in the sample. The presence of carbonate ions in the sample is a consequence of atmospheric carbon dioxide interacting with HAP precursors in the synthesis solution and has been reported in the literature by other researchers [34, 35]. The small peak
located at 963 cm\(^{-1}\) indicates \(v_1\) symmetric stretching vibrations normally associated with a P-O mode. The strong peak located at 1027 cm\(^{-1}\) and the smaller peak located at 1091 cm\(^{-1}\) correspond to \(\text{PO}_4^{3-}\) functional groups. While the weaker peak located at 1374 cm\(^{-1}\) corresponds to a \(\text{CO}_3^{2-}\) functional group Moving further leftward we encounter a smaller peak located at 1644 cm\(^{-1}\) which indicates the presence of a \(\text{CO}_3^{2-}\) group. The next peak located at 3215 cm\(^{-1}\) indicates the presence of absorbed water, while the last identified weak peak located at 3570 cm\(^{-1}\) corresponds to \(\text{OH}^-\) ion vibrations in the HAP crystal lattice. The second pattern presents the FT-IR analysis of a representative powder sample after adsorption testing. The results are very much the same as the pre-adsorption sample, except both 600 and 631 cm\(^{-1}\) peak intensities are significantly smaller than those of the pre-adsorption sample as seen in Figure 3 insert. Both peaks are associated with the P–OH groups that act as sorption sites on the surface of the HAP powder [18, 19]. Collectively, their reduced intensities confirm the decrease in the number of free sites available due to \(\text{Cd}^{2+}\) ion attachment and collaborates the results of XRD analysis.

3.4. Effect of initial Cd\(^{2+}\) concentration and pH on adsorption
The influence of initial \(\text{Cd}^{2+}\) concentration and contact time on adsorption was carried out on initial cadmium concentrations ranging from 50 to 175 mg/L and over contact times ranging from 10 to 300 minutes. Solution temperature was maintained at 298 ± 1 K and solution pH was maintained at 7. During adsorption studies all solutions were magnetically stirred at 400 rpm and concentration measurements were taken at different interval times over a 300 minute test period (10, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min).
Figure 3: FT-IR Spectroscopy analysis of synthesized pure nanometre scale hydroxyapatite powder after drying and a cadmium loaded HAP residue. The study used 1 g/L HAP absorbent, a 100 mg/L Cd$^{2+}$ ion solution, with a pH of 7 and stirred at 400 rpm for a total contact time of 300 min.

Figure 4 (a) presents the adsorption results of a HAP absorber (1 g/L) placed in an initial Cd$^{2+}$ ion concentration of 100 mg/L maintained at 298 ± 1 K and stirred at 400 rpm for 300 min. Inspection of Figure 4 (a) reveals maximum Cd$^{2+}$ removal (75 %) occurred after 90 minutes and beyond this period no further adsorption was observed. The trend was typical of the absorber and further investigations were undertaken to quantify Cd$^{2+}$ ion uptake by increasing initial metal concentrations. The results of these investigations are presented in Figure 4 (b) and reveal increasing initial Cd$^{2+}$ ion concentration (50 to 175 mg/L) tended to produce a corresponding increase in capturing capacity of the absorber (45.75 to 110 mg/g). The increasing trend suggests the higher initial concentrations were able to overcome mass transfer related
resistances existing between the aqueous and solid absorber phase by effectively creating a driving force.

The effect of pH on adsorption was investigated by repeating batch equilibrium studies using an initial Cd\(^{2+}\) ion concentration of 100 mg/L (100 ppm) at various pH values ranging from 2 to 12. Figure 4 (c) presents the results of the pH study and illustrates the effect of pH on adsorption. It is apparent form Figure 4 (c) that Cd\(^{2+}\) adsorption steadily increases from pH 2 up to a value of 12 and pH influences the degree of Cd\(^{2+}\) removal from the solution. At lower pH values H\(^{+}\) ions compete with Cd\(^{2+}\) ions for binding sites on the absorber surface. However, as the pH increases there is a reduction in competition between H\(^{+}\), Cd\(^{2+}\) and Cd(OH)\(^{+}\) ions and as a result, metal uptake by the absorber increases [11]. The experimental results indicate Cd\(^{2+}\) removal was predominantly controlled by adsorption up to a pH of around 8. However, beyond pH 8 Cd\(^{2+}\) ion removal was significantly enhanced by cadmium hydroxide precipitation.

3.5. Adsorption kinetics

Kinetic analysis is essential for understanding any adsorption process. This is because sufficient residence time on the absorber surface is needed to complete the adsorption reaction. The amount of Cd\(^{2+}\) ions adsorbed at equilibrium time \((q_e)\) was calculated using equation 2 below:

\[
q_e = \frac{(C_o - C_e)}{m} \cdot \frac{V}{m}
\]  

(2)

where \(C_o\) and \(C_e\) are the initial and equilibrium concentrations (mg/L) of Cd\(^{2+}\) ions in solution, \(V\) is solution volume (L) and \(m\) is absorber mass (g) used during the experiments. The experimental adsorption data was analysed using three kinetic models: 1) Lagergren’s pseudo-first order law; 2) McKay and Ho’s pseudo-second-order law, and 3) the intra-particle diffusion model. In the first case, the Lagergren pseudo-first order law [36] is defined by equation 3 below:
where, \( q_t \) (mg/g) is adsorption at time \( t \) and \( k_1 \) (min\(^{-1}\)) is the pseudo-first order adsorption rate constant. In the second case, adsorption kinetics was examined using the McKay and Ho’s pseudo-second-order law [37], which is described by equation 4 below:

\[
\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t
\]  

where \( k_2 \) (g/min.mg) is the pseudo-second-order rate constant for adsorption. Figure 5 (a) presents kinetic data plotted using the Lagergren pseudo-first order equation (3), while Figure 5 (b) presents kinetic data plotted using the McKay and Ho’s pseudo-second-order equation (4). Inspection of the two plots reveals that they are both linear in nature and both models are reasonable representations of the kinetic data. In spite of the reasonable agreement, further analysis reveals that the second order model is superior and has a slightly higher correlation coefficient (\( R^2 \)) as seen in Table 1.
Figure 4: (a) Influence of initial Cd$^{2+}$ concentration and contact time; (b) capturing capacity of the HAP absorbent with increasing initial Cd$^{2+}$ ion concentration (50 to 175 mg/L); (c) the effect of pH on Cd$^{2+}$ adsorption, and (d) the effect of temperature % Cd$^{2+}$ removal.
**Figure 5:** Adsorption data modelled using three kinetic models: (a) Lagergren’s pseudo-first order law; (b) McKay and Ho’s pseudo-second-order law, and (c) the intra-particle diffusion model. [The study used 1 g/L HAP absorbent, a 100 mg/L Cd\(^{2+}\) ion solution, with a pH of 7 and stirred at 400 rpm for a total contact time of 300 min].

**Table 1:** A comparison between the pseudo kinetic (first and second order) rate constants and intra-particle kinetic diffusion constants and calculated \(q_e\) values (pH = 7, initial Cd\(^{2+}\) concentration = 100 mg/L, adsorbent dosage = 1 g/L, and agitation rate = 400 rpm).
However, the adsorption process is not a single step event, but instead involves a number of steps. The first step involves diffusion of sorbate from the aqueous solution to the absorber surface. This is followed by the much slower diffusion of sorbate into the internal porous voids of the absorber matrix. Weber and Morris [38] have described this multi-step process using an intra-particle diffusion model which is described by equation (5) below:

$$q_t = k_p t^{1/2} + C$$

(5)

where $C$ is the intercept that provides the ideal boundary layer thickness and $k_p$ is the intra-particle diffusion rate constant (mg/g.min$^{1/2}$ g). In the model, the resulting plot of $q_t$ versus $t^{1/2}$ will be linear if the intra-particle diffusion process is involved. In addition, if the linear line passes through the graphs origin, it indicates there is only one rate-controlling step. On the other hand, if the graph is composed of a series of linear plots, then each linear plot will represent a different stage in the adsorption process. The first plot is generally steeper and results from the rapid diffusion of sorbate from solution and its subsequent surface attachment to the absorber. The second linear portion of the graph has a smaller, more gradual gradient and indicates intra-
particle diffusion is the prevailing process. In the final equilibrium stage, the gradient levels off and indicates a significant reduction in diffusion due to the low solute concentration. When the adsorption data was plotted using the intra-particle diffusion model a typical multi-gradient line graph pattern was present and is presented in Figure 5 (c). For clarity, only the second and final equilibrium stages are plotted and the modelling parameters such as rate constants, intercepts and correlation coefficients are presented in Table 1.

3.6. Adsorption isotherms

Analysis of the experimental data was important in determining the distribution of Cd\(^{2+}\) ions between solution and HAP absorber when the adsorption process had reached its equilibrium state. There are a number of isotherm equations available to model the results of equilibrium data obtained from adsorption systems. The two most widely used equilibrium modelling equations are Freundlich and Langmuir. Freundlich is purely an empirical equation used to fit experimental data. It takes into account surface heterogeneity, the exponential distribution of active adsorption sites and their respective energies over a wide range of concentrations. Langmuir, unlike the Freundlich assumes maximum adsorption occurs when the surface is covered by a monolayer of adsorbate. The equilibrium data for Cd\(^{2+}\) ions in solution over a concentration range starting from 50 to 175 mg/L at constant temperature of 298 K, pH of 7, 1 g/L absorbent dose and a contact time of 300 minutes was analysed using Freundlich and Langmuir isotherms. The Freundlich isotherm equation used for modelling the equilibrium data is presented in its linear form below:

\[
\log q_e = \log k_F + \frac{1}{n} \log C_e
\]
where $k_F$ and $n$ are Freundlich parameters related to the extent of adsorption and the intensity of adsorption respectively. Both $k_F$ and $n$ parameters were determined via plotting log $q_e$ versus log $C_e$, the results of which are presented in Figure 6 (a).

The Langmuir isotherm used for modelling the equilibrium data is expressed mathematically in the linear form by equation (7) below:

$$\frac{C_e}{q_e} = \frac{1}{Q_m b} + \frac{C_e}{Q_m}$$

(7)

where, $Q_m$ (mg/g) is the monolayer adsorption capacity, $b$ (L/g) is the Langmuir constant that is related to the free energy of adsorption, $C_e$ (mg/L) and $q_e$ (mg/g) are the equilibrium concentrations of adsorbate in solution and on the surface of absorber respectively. Using the Langmuir isotherm, a linear plot of equilibrium data was obtained when $C_e/q_e$ was plotted against $C_e$ over the entire Cd$^{2+}$ ion concentration range as seen in Figure 6 (b). The adsorption isotherm is characterized by the two parameters $Q_m$ and $b$ that were determined from the plot. The parameters reflect the surface properties and affinity of the Cd$^{2+}$ ions for the HAP absorber. Both the Freundlich and Langmuir isotherm plots displayed good linear fits and were able to provide intercept and slope parameters which are listed in Table 2. For the Freundlich isotherm a value of 3.106 was obtained for $n$, which fell within the range of 1 to 10 and indicated adsorption was favourable. Furthermore, based on the coefficient of correlation ($R^2$) values, the Freundlich isotherm provided the best fit of the experimental data. However, unlike Langmuir, the Freundlich isotherm does not predict any maximum occupancy of Cd$^{2+}$ ions on the HAP absorber surface. Instead, it mathematically predicts infinite surface occupancy and the possibility of multi-layered adsorption. Nonetheless, the maximum monolayer adsorption capacity ($Q_m$) calculated from the Langmuir equation was found to be 123.45 mg/g. Analysis of isotherm data indicates adsorption capacity was the result of increasing equilibrium cadmium
concentrations in solution. The increased concentrations were able to increase the numbers of Cd$^{2+}$ ions at the absorber surface and enhance the probability of adsorption.

![Graphs showing linear fits of experimental data using (a) Freundlich and (b) Langmuir isotherms](image)

**Figure 6:** Linear fits of experimental data using (a) Freundlich and (b) Langmuir isotherms. [The study used 1 g/L HAP absorbent, a 100 mg/L Cd$^{2+}$ ion solution, with a pH of 7 and stirred at 400 rpm for a total contact time of 300 min].

In addition, a comparative evaluation of the Cd$^{2+}$ adsorption efficiency of the nanometre scale HAP absorber used in the present study and several other materials reported in the literature is made via Table 3. Inspection of Table 3 reveals the HAP absorber used in this study possesses a higher adsorption capacity to many other materials reported in the [11] listed in the table. But only activated carbon base materials such as Filtrasorb 400 have higher adsorption capacities [46].
3.7. The effect of temperature

An isothermal study was also carried out to investigate the effect of temperature on cadmium adsorption of HAP absorber. The five isotherms used were 283, 293, 303, 313 and 323 K, while the initial Cd²⁺ ion concentration (100 mg/L), contact time (300 min) and adsorbent dose (1 g/L) were kept constant during the study. The study revealed cadmium adsorption capacity steadily increased with increasing temperature [see Figure 4 (d)] and indicated the adsorption process was endothermic in nature.

To investigate the thermodynamics of the adsorption process, parameters such as free energy change ($\Delta G^0$), enthalpy change ($\Delta H^0$) and entropy change ($\Delta S^0$) were calculated using the following equations:

$$\Delta G^0 = -RT \ln K$$

(8)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

(9)

For the purpose of producing a linear graphical representation, equations (8) and (9) were combined and rearranged to give Van’t Hoff equation:

$$\ln K = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT}$$

(10)

where $R$ is the universal gas constant (8.314 J mol⁻¹ K⁻¹), $T$ is the temperature in Kelvin and $K$ is the thermodynamic equilibrium constant in units of L/g and is generally expressed by:

$$K = \frac{q_e}{C_e}$$

(11)
In order to ensure that $K$ in equation (11) is dimensionless we have adopted the method suggested by Milonjic in which the equilibrium constant $K$ is replaced with a new dimensionless equilibrium constant $K_d$ [39]:

$$K_d = \frac{\rho q_e}{C_e}$$

(12)

where $\rho$ is the density of water (~1000 g/L) and is assumed to remain constant over the entire temperature range studied and contains only a small absorber dose.

Table 2: Comparisons between Freundlich and Langmuir adsorption isotherm constants for Cd$^{2+}$ onto nanometre scale hydroxyapatite at 298 K.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$k_f$ (mg/g)</th>
<th>$n$</th>
<th>$R^2$</th>
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</thead>
<tbody>
<tr>
<td>298</td>
<td>28.47</td>
<td>3.106</td>
<td>0.9967</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$Q_{max}$ (mg/g)</th>
<th>$b$ (L/mg)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>123.45</td>
<td>0.091</td>
<td>0.9868</td>
</tr>
</tbody>
</table>

Table 3: Comparison between cadmium absorption capacities of various materials

<table>
<thead>
<tr>
<th>Absorbent</th>
<th>$q_m$ (mg/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice husk</td>
<td>2.00</td>
<td>[40]</td>
</tr>
</tbody>
</table>
Values of $K_d$ were calculated from the equilibrium concentrations over the temperature range. The values were found to increase with increasing temperature and revealed adsorption was endothermic in nature. The calculated changes in Gibbs free energy $\Delta G^0$ were plotted against temperature $T$ (K) and from the graph values of $\Delta H^0$ (slope) and $\Delta S^0$ (intercept) were determined (Graphs not shown). However, the thermodynamic parameters derived from this analysis are presented in Table 4. Inspection of Table 4 reveals that each of the $\Delta G^0$ values are negative for each isotherm. The result indicates cadmium adsorption is spontaneous in nature and the adsorption process is enhanced with increasing temperature. The positive value of $\Delta H^0$ (6872.12 J mol$^{-1}$) indicates the endothermic nature of the adsorption process and the positive value of $\Delta S^0$ (90.06 J mol$^{-1}$ K$^{-1}$) indicates the affinity of the nanometre scale HAP absorber for Cd$^{2+}$ ions. The positive values of both $\Delta H^0$ and $\Delta S^0$ are also responsible for making the reaction spontaneous, irreversible in nature and also suggest that there were some structural changes to the absorber. Throughout Cd$^{2+}$ ion removal from solution two distinct stages were clearly seen. During the first stage P-OH surface groups located on the absorber surface promoted surface complex’s to take place. The FT-IR spectroscopy analysis presented in Figure 3 confirms this.
stage. Since the 600 and 631 cm\(^{-1}\) peak intensities normally associated with P–OH groups were significantly reduced after Cd\(^{2+}\) ion adsorption had taken place. The reduction in peak intensity reflects the significant reduction in the number of free sites available for attachment to take place. During the second stage significant numbers of Cd\(^{2+}\) ions were substituted for Ca\(^{2+}\) ions in the HAP crystal lattice. The presence of cadmium in both the XRD and EDS analysis confirms the ion-exchange mechanism. The adsorption process can be described by the following equation:

\[
\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 + x\text{Cd}^{2+} \rightarrow \text{Ca}_{10-x} \text{Cd}_x (\text{PO}_4)_6 (\text{OH})_2 + x\text{Ca}^{2+} \quad (13)
\]

It is during the second stage when there is partial dissolution of calcium from the HAP lattice that results in a apatite precipitation described by \(\text{Ca}_{10-x} \text{Cd}_x (\text{PO}_4)_6 (\text{OH})_2\) and Ca\(^{2+}\) ions.

**Table 4:** Thermodynamic parameters for Cd\(^{2+}\) adsorption onto nanometre scale hydroxyapatite.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>(K_d)</th>
<th>(\Delta G^o) (J/mol)</th>
<th>(\Delta H^o) (J/mol)</th>
<th>(\Delta S^o) (J/mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>283</td>
<td>2773.58</td>
<td>-18653.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>293</td>
<td>3000.00</td>
<td>-19503.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303</td>
<td>3255.32</td>
<td>-20374.94</td>
<td>6872.12</td>
<td>90.06</td>
</tr>
<tr>
<td>313</td>
<td>3545.45</td>
<td>-21269.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>4000.00</td>
<td>-22273.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

The present study has shown a HAP powder synthesized via a combined ultrasound and microwave based technique has resulted in the development of a highly crystalline structure with a spherical mean particle size of 30 nm. The prepared nanometre scale HAP powder was found to be an effective adsorbent for the removal of Cd$^{2+}$ cations from aqueous solutions. The sorption performance was found to be a function of initial Cd$^{2+}$ concentration, temperature and solution pH. Furthermore, Cd$^{2+}$ removal was found to improve with increases in these parameters for specific contact times. The study also confirmed the sorption process was endothermic in nature and Cd$^{2+}$ sorption increased with temperature. Kinetic studies revealed the sorption process closely followed pseudo-second order kinetics. During sorption, the initial uptake rate of Cd$^{2+}$ was high, but this was followed by a much lower uptake rate. The ion-exchange mechanism (Cd$^{2+}$ → Ca$^{2+}$) was clearly identified as the major participant in the sorption process. The results of both XRD and EDS analysis confirmed ion-exchange was a major removal mechanism and sorption was heavily influenced by intra-particle diffusion. Isotherm studies indicated the Freundlich isotherm modelled experimental data better than the Langmuir isotherm. However, the Langmuir isotherm was used to determine maximum adsorption capacity of the HAP absorber and was found to be 123.45 mg/g. All calculated thermodynamic parameters ($\Delta G^\circ$, $\Delta H^\circ$ and $\Delta S^\circ$) clearly indicate sorption was thermodynamically favourable, endothermic and spontaneous in nature.

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Kinetic and Adsorption Behaviour of Aqueous Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ Using a 30 nm Hydroxyapatite Based Powder Synthesized via a Combined Ultrasound and Microwave Based Technique

Sridevi Brundavanam, Gérard Eddy Jai Poinern*, Derek Fawcett

Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Australia

Abstract The present study reports the kinetic and absorption performance of novel nanometre scale hydroxyapatite (HAP) absorber synthesised from a combined ultrasound and microwave based technique for the removal of metal ions (Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$) from aqueous solutions. After powder characterisation was carried out using XRD, SEM, EDS and FT-IR, batch adsorption studies were carried out. Kinetic studies established that Fe$^{2+}$ and Cu$^{2+}$ ion adsorption tended to follow a pseudo-second order model, while Zn$^{2+}$ ion adsorption tended to follow an intra-particle diffusion pattern. All three metal ion adsorption studies indicated an ion-exchange mechanism (metal ion $\rightarrow$ Ca$^{2+}$) was a primary participant in the sorption process and was influenced by intra-particle diffusion. The Isotherm studies indicated the Langmuir isotherm modelled Fe$^{2+}$ and Cu$^{2+}$ ion adsorption, while the Freundlich isotherm was the better model for Zn$^{2+}$ ion adsorption data. Maximum adsorption capacity of HAP determined via Langmuir isotherm was found to be 61.35 mg/g for Cu$^{2+}$ ions, 55.25 mg/g for Fe$^{2+}$ ions and 48.54 mg/g for Zn$^{2+}$ ions. The study established HAP as an effective absorbent material for the removal of Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ loaded aqueous solutions.

Keywords Hydroxyapatite, Adsorption, Metal ion, Ultrasound, Microwaves

1. Introduction

The presence of heavy metal contaminants in the environment is a well-recognised problem that threatens the world today. Even in small concentrations, their high solubility in aquatic environments makes them extremely hazardous to living organisms [1, 2]. Once heavy metals enter the food chain their toxicity together with their tendency to accumulate become acceptable concentrations in the human body can result in serious health disorders [3]. Metals are generally considered heavy metals when their density exceeds 5 g/cm$^3$ [4]. Metals and alloys with densities greater than 5 g cm$^{-3}$ are widely used as biomaterials due to their superior mechanical properties. The majority of these metallic biomaterials are used in load-bearing implants such as bone plates, fixation pins and screws, hip and knee replacements. Metallic biomaterials are grouped into three distinct categories: 1) stainless steels; 2) cobalt-based alloys, and 3) titanium alloys. Individually, each category consists of a primary metallic biomaterial that is composed of a number of secondary metallic constituents. For example, the main constituents of stainless steels are iron (Fe), chromium (Cr) and nickel (Ni). Most of the metal constituents used in the three metallic biomaterial groups like Cr, cobalt (Co), Fe, molybdenum (Mo), Ni, and tungsten (W) are used as alloying elements to improve the properties of the main constituent element [5]. For example, Fe based stainless steels have Cr (17-20%) added to improve corrosion resistance and Mo (2-4%) is added to improve resistance to pitting corrosion [6]. However, metallic constituents such as Cr and Mo are toxic and can only be tolerated in very small amounts in the body. Studies have shown that leaching of metallic surgical implants can result in the release of toxic metallic ions into surrounding cells, blood vessels and tissues [7-9]. The release of metallic ions can only be tolerated in very minute amounts, as in larger amounts they induce an unfavourable inflammatory response that significantly reduces the biocompatibility of the implant [8]. Importantly, exposure to heavy metals can lead to serious health problems such as cancer, nervous system and organ damage. For example, both Cr and Ni are known carcinogens and long-term exposure can lead to the development and growth of cancer.
Metallic biomaterials used in orthopaedic applications will generally experience load-induced stresses in the inner core of the implant. Whereas, the surface of the implant is exposed to the surrounding physiological environment in the body. The interaction between exposed surface and physiological environment is very important in soliciting a favourable biological response. Because of the importance that biocompatibility plays in delivering a successfully implantation procedure, there have numerous studies into improving interfacial properties between the implant surface and the physiological environment [9, 10]. To this end, bioactive coatings based on calcium phosphate ceramics have been extensively investigated and used to coat a variety of biomedical metallic implants. These coatings have been found to improve biocompatibility, promote implant attachment and restrain the release of metallic ions into the physiological environment [10, 12]. In particular, hydroxyapatite (HAP) is a thermodynamically stable mineral phase at physiological pH and has a hexagonal crystal structure composed of calcium phosphate groups. Both crystallographic and chemical studies reveal synthetic HAP is similar in chemical composition to the naturally occurring mineral phase found in human bone and teeth [13]. Synthetic HAP has the advantages of being biocompatible, non-toxic, and has enhanced bioactive properties towards bone cells and other body tissues [14].

Another interesting property of HAP results from its complex hexagonal structure. The structure provides effective high capacity absorbance for a variety of pharmaceutical products such as antibiotics, drugs, enzymes, hormones and steroids. The use of HAP as a slow and sustained release drug delivery platform has proven to be effective in the treatment of diseases such as osteomyelitis, osteoporosis and osseous cancer [15-18]. Likewise, HAP used in orthopaedic applications also has the potential to act as an adsorption matrix. In this case, adsorption results from a mass transfer process by which metallic particles are transferred from the physiological environment (liquid phase) to the HAP matrix, and become bound by physical and/or chemical interactions. HAP makes an attractive absorbent because of its advantageous surface properties such as its hydrophilic nature, surface charge, pH, porous structure and 2.6 P-OH surface groups per nm² which act as sorption sites [19, 20]. Generally speaking there are three steps involved in sorption of metallic particles onto the HAP absorber: (1) transfer of metallic ion particles from the physiological environment to the absorber surface; (2) adsorption on the metallic ion particles onto the surface, and (3) transport of the metallic ion particles within the absorber matrix.

In the present work the adsorption of copper (Cu), iron (Fe), and zinc (Zn) ions from aqueous solutions onto a solid nanometre scale HAP powder in agitated batch absorber vessels was studied. The nanometre scale HAP powder was synthesized via a combined ultrasound and microwave based technique to produce a spherical particle with a mean diameter of 30 nm. The main goal of this study was to examine the ability of the nanometre scale HAP powder to remove these ions from an aqueous solution and therefore evaluate its potential to store heavy metallic ions. The source of these metallic ions could arise from the corrosion and wear of surgical implants or by metabolic uptake via nutritional intake, drinking water and inhalation. The synthesized HAP powders were characterized using X-ray diffraction (XRD) spectroscopy, Scanning electron microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared Spectroscopy (FTIR). The metal ion adsorption capacity of the powders were investigated via the removal of Cu, Fe, and Zn ions from aqueous solutions using a batch equilibrium procedure. The kinetic behaviour of metal ion adsorption was investigated using Lagergren’s pseudo-first order, McKay & Ho’s pseudo-second order and intra-particle diffusion models. Furthermore, Langmuir and Freundlich adsorption isotherms were used to model the experimental data.

2. Materials and Methods

2.1. Materials

Adsorption experiments were conducted using three different metal salts. The first, FeCl₂.4H₂O was supplied by Chem-Supply (Australia). The second, CuCl₂.2H₂O was supplied by Sigma-Aldrich (United States) and the third, ZnCl₂ was supplied by Scharlau (Barcelona, Spain). Each respective metal salt was dissolved in an aqueous solution to make up a 1000 ppm stock solution and lower solution concentrations were produced by successive dilution of the stock solution. HAP powders were synthesized from high purity calcium nitrate tetra-hydrate [Ca(NO₃)₂·4H₂O] and potassium di-hydrogen phosphate [KH₂PO₄], while solution pH was controlled by the addition of ammonium hydroxide [NH₄OH]. All chemicals used to synthesize HAP were supplied by Chem-Supply (Australia). During HAP synthesis an Ultrasound Processor [UP50H: 50 W, 30 kHz, MS7 Sonotrode (7mm diameter, 80 mm length)] supplied by Hielscher Ultrasound Technology was used to deliver the ultrasound irradiation. All aqueous solutions were made using Milli-Q® water (18.3 MΩ cm⁻¹) produced by an ultrapure water system (Barnstead Ultrapure Water System D11931; Thermo Scientific, Dubuque, IA).

2.2. Synthesis of Nanometre Scale Hydroxyapatite

Ultrasonic and microwave processing are two techniques that significantly influence and enhance the properties of materials. Ultrasonic produces extremely rapid pressure and temperature variations that promote both physical effects and chemical reactions that directly influence particle size and morphology during synthesis. Microwave processing has the advantages of providing controlled volumetric heating, lower energy consumption, reduced reaction times, increased product yields, and improved material properties when compared to conventional heating methods [21]. A detailed synthesis procedure developed by
the authors is given elsewhere [21, 22]. But a brief description is give here in the interest of completeness and begins by adding a 40 mL solution of 0.32 M calcium nitrate tetra-hydrate into a small glass beaker. The pH of the solution is then adjusted to 9.0 by adding approximately 2.5 mL of ammonium hydroxide. The solution was then subjected to 50 W of ultrasound irradiation for 1 h set and maximum amplitude. The second hour of ultrasound treatment included slowly adding 60 mL of 0.19 M potassium di-hydrogen phosphate solution. During the addition of potassium di-hydrogen phosphate the solution pH was maintained at 9.0 and the Calcium/Phosphate [Ca/P] ratio was maintained at 1.67. After ultrasonic treatment, the solution was centrifuged (15,000 g) for 20 minutes at room temperature to produce a solid white precipitate. The precipitate was collected, washed and centrifuged for a further 10 minutes. At the end of the second centrifugation, the precipitate was placed into a fused silica crucible and then loaded into a domestic microwave oven for thermal treatment [Set at 100% power for 40 minutes: 1100W at 2450 MHz-LG® Australia] [21]. The resultant agglomerated powder was collected and then subjected to ball milling until an ultrafine nanometre scale HAP powder was produced.

2.3. Advanced Characterisation Techniques

2.3.1. X-ray Diffraction (XRD) Spectroscopy

XRD spectroscopy was used to study the synthesized powders, with the XRD patterns being used to identify the crystalline size and phases present. Spectroscopy data was recorded at room temperature, using a Siemens D500 series diffractometer [Cu Kα = 1.5406 Å radiation source] operating at 40 kV and 30 mA. The diffraction patterns were collected over a 2θ range of 20° to 60° with an incremental step size of 0.04° using flat plane geometry with 2 second acquisition time for each scan. The scan data was then used in conjunction with the Debye-Scherrer equation [Equation 1] to determine the crystalline size of each sample.

2.3.2. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The SEM technique was used to examine the size, shape and morphological features of absorbents before and after the batch adsorption studies. All micrographs were taken using a JCM-6000, NeoScopeTM with attached energy dispersive X-ray spectroscopy. Samples were mounted on individual substrate holders using carbon adhesive tape before being sputter coated with a 2 nm layer of gold to prevent charge build up using a Cressington 208HR High Resolution Sputter coater.

2.3.3. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was used to identify species and functional groups present in the HAP absorber before and after metal adsorption studies. Samples were examined using a Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment. Both FT-IR spectra were recorded in the range from 525 to 4000 cm⁻¹ in steps of 4 cm⁻¹.

2.4. Batch Adsorption Studies

All adsorption experiments were carried out using the batch equilibrium technique. During the experimental work the adsorption capacity of the HAP absorber for three metal ions, namely, Cu²⁺, Fe²⁺ and Zn²⁺ were investigated. The study also examined the influence of the initial metal ion concentration and contact time at 298°C. The influence of contact time on metal ion adsorption on the absorber was examined using aqueous solutions containing 300 mg/L of metal ions (300 ppm) prepared from the three metal chlorides. HAP samples (0.1 g) were taken from the stock solution (1g/L) were added to each of the 300 ppm metal chloride solutions. The magnetic stirring speed for each metal suspension were set to 400 rpm, while the temperature of each suspension was maintained at 298 ± 1 K. In addition, the initial and final pH of the suspensions were recorded. Sample volumes were taken from the suspension during the mixing process at pre-determined time intervals (10, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min) so that metal ion concentration in the respective solution could be measured. Once a sample volume was taken, an equivalent volume was added to maintain testing solution volume. Each solution volume was filtered using a Whatman® 0.22μm membrane syringe filter before being centrifuged at 15,000 g for 20 minutes. The concentration of Cu²⁺, Fe²⁺, Zn²⁺ and Ca²⁺ ions in the sample solutions were determined using atomic absorption spectroscopy (AAS). The instrument used was a Varian SpectraAA50 (Victoria, Australia) flame atomic absorption spectrometer operated in accordance with the manufacturer’s recommendations. Elemental analysis of Cu, Fe and Zn was carried out using an air-acetylene flame and Ca analysis was carried using a nitrous oxide (N₂O)–acetylene flame. Sample aspiration flow rate used was 5 mL min⁻¹. During analysis hollow cathode lamps of Fe, Cu, Zn and Ca (Varian) were used. While Fe, Cu, Zn and Ca concentration were measured at the wavelengths of 248.3, 324.8, 213.9 and 422.7 nm respectively. In addition, the influence of initial metal ion concentration was studied by first preparing a series of Schott reagent bottles containing 100 mL aqueous solutions consisting of varying concentrations of metal ions (100, 150, 200, 250 and 300 mg/L). All initial metal ion concentration experiments were carried out in triplicate. The data collected from the adsorption experiments were then used in the subsequent kinetic and adsorption isotherm modelling studies.

3. Results and Discussions

3.1. XRD Spectroscopy, SEM and EDS Analysis

XRD spectroscopy was carried out on all HAP powders
before and after adsorption studies. The resultant XRD patterns were used to determine crystalline size and phases present in the samples. An XRD pattern of a typical pure powder sample before adsorption testing is presented in Figure 1 and is indicated by the purple pattern. Examination of the pure HAP sample pattern reveals the presence of peaks that coincide with the known phases of pure HAP and is consistent with the phases listed in the ICDD database. The main (h k l) indices found in pure HAP, namely (002), (211), (112), (300), (202), (310), (222), (213) and (004) can be seen in the pattern for the synthesized HAP powder. In addition, the pattern showed no evidence of non-HAP phases. The crystalline size, \( t_{(hkl)} \), of each sample was calculated from the respective XRD patterns using the Debye-Scherrer equation [23-25]

\[
 t_{(hkl)} = \frac{0.9\lambda}{B\cos\theta_{(hkl)}}
\]

where, \( \lambda \) is the wavelength of the monochromatic X-ray beam, \( B \) is the Full Width at Half Maximum (FWHM) of the peak at the maximum intensity, \( \theta_{(hkl)} \) is the peak diffraction angle that satisfies Bragg’s law for the (h k l) plane and \( t_{(hkl)} \) is the crystallite size. The mean crystallite size calculated from the (002) reflection peak for pure HAP sample was found to be 30 nm.

After adsorption studies, XRD spectroscopy was carried out on the metal ion loaded samples and representative XRD patterns are presented in Figure 1. XRD analysis reveals that there was no major changes in peak locations and powder size resulting from metal ion adsorption, as seen in Figure 1. To investigate this result further, SEM and EDS analysis was carried out on all samples. SEM analysis revealed that all powder samples displayed the same agglomerated spherical and granular morphology.
Figure 3. (a) SEM micrograph of Cu\textsuperscript{2+} loaded sample; (b) EDS spectrum of Cu\textsuperscript{2+} loaded sample; (c) SEM micrograph of an Zn\textsuperscript{2+} loaded sample, and (d) EDS spectrum confirming the presence of Zn\textsuperscript{2+} ions.

Figure 2 (b) presents a typical EDS spectrum of an unloaded sample showing dominant peaks corresponding to Ca, P, and O, and confirms the chemical composition of HAP. Analysis of the EDS data revealed a Ca:P ratio of 1.66, which was very close to the ideal value of 1.67 normally associated with HAP. Also present was a minor peak that resulted from the carbon adhesive tape used to attach the sample to the SEM stub. Figure 2 (c) presents an SEM micrograph of a representative Fe\textsuperscript{2+} loaded sample that also displays the highly agglomerated nature of the synthesized powder. The accompanying EDS spectrum confirms the presence of Fe\textsuperscript{2+} ions present in the sample. The EDS also shows that there has been a reduction in the intensity of the Ca peak and suggests Fe\textsuperscript{2+} ions have replaced Ca\textsuperscript{2+} ions in the lattice. Studies have shown that divalent metal cations have a strong selectivity towards the Ca\textsuperscript{2+} in the HAP lattice via an ion-exchange mechanism [26, 27]. A similar trend can be seen for Cu\textsuperscript{2+} and Zn\textsuperscript{2+} ion load samples as seen in Figure 3. However, the reduction in the Ca peak for the Cu\textsuperscript{2+} and Zn\textsuperscript{2+} ion load samples is not as pronounced as the Fe\textsuperscript{2+} loaded sample seen in Figure 2 (d). The ion-exchange mechanism between the respective metal ion and Ca\textsuperscript{2+} can be represented by the equivalent molar exchange in the general HAP formula Ca\textsubscript{10−x}M\textsubscript{x}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}, where M represents the respective metal ion and x can vary from 0 to 10 depending on experimental parameters and reaction time. Studies reported in the literature have detected very small XRD shifts associated with the ion-exchange mechanism for divalent metal ions [26, 28]. Inspection of the XRD patterns of representative metal ion loaded samples presented in Figure 1 do not reveal any of these very small XRD shifts.

3.2. FT-IR Spectroscopy Studies

FT-IR spectroscopy was used to detect species and functional groups associated with peaks seen in sample spectra taken before and after adsorption studies. Figure 4 presents the results of a FT-IR spectroscopy study of HAP powder samples taken before and after metal ion adsorption studies. Starting from the right hand side of Figure 4 with a typical synthesized HAP powder sample (purple) prior to adsorption studies. The first three peaks encountered are 561 cm\textsuperscript{-1}, 600 and 631 cm\textsuperscript{-1} that are associated with \(v_4\) vibrations of the O-P-O modes. The peak located at 827 cm\textsuperscript{-1} indicates the presence of carbonates in the sample and is a consequence of atmospheric carbon dioxide interacting with HAP precursors during synthesis and has been reported in the literature by other researchers [29, 30]. The small peak located at 963 cm\textsuperscript{-1} is associated with \(v_1\) symmetric stretching vibrations associated with a P-O mode. While the much larger peak at 1026 cm\textsuperscript{-1} and the smaller peak located at 1090 cm\textsuperscript{-1} correspond to PO\textsubscript{4}\textsuperscript{3−} functional groups. While the peaks located at 1373 cm\textsuperscript{-1} and 1643 cm\textsuperscript{-1} correspond to CO\textsubscript{3}\textsuperscript{2−} functional groups. While the weak peak located at 3470 cm\textsuperscript{-1} corresponds to OH\textsuperscript{−} ion vibrations in the HAP crystal lattice. The three remaining spectra are
representative Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ loaded powder samples taken after adsorption testing. The results for the metal ion loaded samples are very much the same as the pre-adsorption sample, except that both 872 cm$^{-1}$ and 1373 cm$^{-1}$ peak intensities have vanished from all spectra. Both the missing peaks correspond to CO$_3^{2-}$ functional groups and suggest that the low pH of the batch adsorption technique resulted in the loss of the carbonates. For example, equilibrium pH of the Fe$^{2+}$ ion and HAP solution was 3.2, while Cu$^{2+}$ was 3.8 and Zn$^{2+}$ was 4.4.

**3.3. Adsorption Kinetics**

A proper understanding of adsorption kinetics is important since sufficient residence time on the absorber surface is needed to complete the adsorption reaction. This is reflected in the metal ion concentration profiles encountered during the batch adsorption studies and presented in Figure 5. Inspection of the profiles reveals that there is an initial rapid uptake of metal ions, but as time progresses the uptake slows and around the 150 minutes mark no further adsorption takes place. The quantity of metal ions adsorbed at equilibrium time ($q_e$) was determined by equation 2 below:

$$q_e = (C_0 - C_e) \frac{V}{m}$$  \hspace{1cm} (2)

where $C_0$ and $C_e$ are the initial and equilibrium concentrations (mg/L) of metal ions in solution, $V$ is solution volume (L) and $m$ is absorber mass (g) used during the experiments. Three kinetic models were used to examine the experimental data. The first was the Lagergren pseudo-first order law [31] that is defined by equation 3 below:

$$\log (q_e - q_t) = \log q_e - \frac{k_1}{2.303} t$$  \hspace{1cm} (3)

where $q_t$ (mg/g) is adsorption at time $t$ and $k_1$ (min$^{-1}$) is the pseudo-first order adsorption rate constant. The second model used was the McKay and Ho’s pseudo-second-order model given by equation 4 below:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \cdot t$$  \hspace{1cm} (4)

where $k_2$ (g/min.mg) is the pseudo-second-order rate constant for adsorption. The third model used was a multi-step intra-particle diffusion model proposed by Weber and Morris [33] and is defined by equation 5 below:

$$q_t = k_p t^{1/2} + C$$  \hspace{1cm} (5)

where $C$ is the intercept that provides the ideal boundary layer thickness and $k_p$ is the intra-particle diffusion rate constant (mg/g.min$^{1/2}$).

**Figure 6 (a) presents kinetic data plotted using the Lagergren pseudo-first order equation (3), Figure 6 (b) displays the kinetic data plotted using the McKay and Ho’s pseudo-second-order equation (4) and Figure 6 (c) represents the multi-gradient line data of intra-particle diffusion model (5). Inspection of Figures 6 (a) and (b) reveals that both the pseudo-first order and pseudo-second-order models gave reasonable representation of the data obtained. In spite of the good initial agreement, further analysis reveals the pseudo-second order model gives a better representation of the data for Fe$^{2+}$ and Cu$^{2+}$ ions with a slightly higher correlation coefficient ($R^2$) as seen in Table 1. However, in the case of Zn$^{2+}$ ions, the intra-particle diffusion model gave a better representation of the data as seen in Figure 6 (c) and Table 1.

**3.4. Adsorption Isotherms**

There are two widely used equilibrium equations for modelling equilibrium data obtained from adsorption systems. The first model is the Freundlich and is purely an empirical equation that takes into account surface heterogeneity, the exponential distribution of active adsorption sites and their respective energies over a wide range of concentrations. The second model is the Langmuir equation and unlike the Freundlich, it assumes maximum adsorption occurs when the entire surface of the absorber is
covered by a monolayer of adsorbate. The equilibrium data for metal ions in solution for initial concentrations consisting of 100, 150, 200, 250 and 300 mg/L at constant temperature of 298 K, 1 g/L absorbent dose and a contact time of 300 minutes were analysed using Freundlich and Langmuir isotherms. The linear form of the Freundlich isotherm used for modelling the data is expressed by in equation (6):

\[
\log q_e = \log k_F + \frac{1}{n} \log C_e
\]  (6)

Table 1. A comparison between the pseudo kinetic (first and second order) rate constants and intra-particle kinetic diffusion constants

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Temperature (K)</th>
<th>k_1 (min^{-1})</th>
<th>q_e (mg/g)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe^{2+}</td>
<td>298</td>
<td>33.92</td>
<td>55.24</td>
<td>0.9904</td>
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<tr>
<td>Cu^{2+}</td>
<td>298</td>
<td>19.99</td>
<td>62.50</td>
<td>0.9805</td>
</tr>
<tr>
<td>Zn^{2+}</td>
<td>298</td>
<td>115.53</td>
<td>57.14</td>
<td>0.9875</td>
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</table>

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Temperature (K)</th>
<th>k_2 (g/mg.min)</th>
<th>q_e (mg/g)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe^{2+}</td>
<td>298</td>
<td>6.84 x 10^{-4}</td>
<td>52.91</td>
<td>0.9951</td>
</tr>
<tr>
<td>Cu^{2+}</td>
<td>298</td>
<td>12.15 x 10^{-4}</td>
<td>59.17</td>
<td>0.9980</td>
</tr>
<tr>
<td>Zn^{2+}</td>
<td>298</td>
<td>3.85 x 10^{-4}</td>
<td>42.01</td>
<td>0.9734</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Temperature (K)</th>
<th>k_p (g/mg.min)</th>
<th>C_e (mg/g)</th>
<th>R^2</th>
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</thead>
<tbody>
<tr>
<td>Fe^{2+}</td>
<td>298</td>
<td>4.29</td>
<td>1.12</td>
<td>0.9934</td>
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<tr>
<td>Cu^{2+}</td>
<td>298</td>
<td>4.17</td>
<td>13.17</td>
<td>0.9080</td>
</tr>
<tr>
<td>Zn^{2+}</td>
<td>298</td>
<td>3.77</td>
<td>-7.98</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

where \(k_F\) and \(n\) are Freundlich parameters related to the extent of adsorption and the intensity of adsorption respectively. Both \(k_F\) and \(n\) parameters were determined via plotting \(\log q_e\) versus \(\log C_e\), the results of which are presented in Figure 7 (a). The linear form of the Langmuir isotherm used for modelling the data is expressed by equation (7):

\[
\frac{C_e}{q_e} = \frac{1}{Q_m b} + \frac{C_e}{Q_m}
\]  (7)

where, \(Q_m\) (mg/g) is the monolayer adsorption capacity, \(b\) (L/g) is the Langmuir constant that is related to the free energy of adsorption, while \(C_e\) (mg/L) is the equilibrium concentration of adsorbate in solution and \(q_e\) (mg/g) is the concentration of adsorbate on the surface of absorber.

Both \(Q_m\) and \(b\) parameters were determined via plotting \(C_e/q_e\) versus \(C_e\) over the entire metal ion concentration range as seen in Figure 7 (b). The parameters reflect the surface properties and affinity of the metal ions for the nanometre scale HAP absorber. Both Langmuir and Freundlich plots exhibited good linear fits and were able to provide slope parameters and intercepts. The results of the respective isotherm analysis are listed in Table 2. For the Freundlich isotherm, if \(n\) falls within the range of 1 to 10 it indicates the adsorption process is favourable. In this study, \(n\) for all metal ions fell within this range and confirmed that adsorption was favourable. Furthermore, based on the coefficient of correlation (R^2) values, the Langmuir isotherm provided the best fit for Fe^{2+} and Cu^{2+} experimental data. While the Freundlich isothermal provided the best fit for Zn^{2+} experimental data. The maximum monolayer adsorption capacity (\(Q_m\)) for respective metal ions was calculated using the Langmuir equation and revealed the maximum adsorption capacity occurred for Cu^{2+} ions (61.35 mg/g). This was followed by Fe^{2+} ions (55.25 mg/g) and then Zn^{2+} ions (48.54 mg/g). The study also found that adsorption capacity was the result of increasing equilibrium metal ion concentrations in solution. The increased concentrations were able to increase the numbers of metal ions at the absorber surface and enhance the probability of adsorption.

**Figure 6.** Metal ion adsorption data modelled using three kinetic models: (a) Lagrergen’s pseudo-first order law; (b) McKay and Ho’s pseudo-second-order law, and (c) the intra-particle diffusion model
Table 2. Comparisons between Freundlich and Langmuir adsorption isotherm constants for metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+}) onto nanometre scale hydroxyapatite at 298 K

<table>
<thead>
<tr>
<th>Metal</th>
<th>Temperature (K)</th>
<th>k_f (mg/g)</th>
<th>n</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
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<td>Fe^{2+}</td>
<td>298</td>
<td>19.02</td>
<td>5.685</td>
<td>0.9986</td>
</tr>
<tr>
<td>Cu^{2+}</td>
<td>298</td>
<td>21.17</td>
<td>5.640</td>
<td>0.9975</td>
</tr>
<tr>
<td>Zn^{2+}</td>
<td>298</td>
<td>3.53</td>
<td>2.448</td>
<td>0.9919</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metal</th>
<th>Temperature (K)</th>
<th>Q_{max} (mg/g)</th>
<th>b (L/mg)</th>
<th>R^2</th>
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</thead>
<tbody>
<tr>
<td>Fe^{2+}</td>
<td>298</td>
<td>55.25</td>
<td>0.036</td>
<td>0.9992</td>
</tr>
<tr>
<td>Cu^{2+}</td>
<td>298</td>
<td>61.35</td>
<td>0.038</td>
<td>0.9993</td>
</tr>
<tr>
<td>Zn^{2+}</td>
<td>298</td>
<td>48.54</td>
<td>0.009</td>
<td>0.9817</td>
</tr>
</tbody>
</table>

Figure 7. Linear fits of experimental data using (a) Freundlich and (b) Langmuir isotherms

Table 3 presents a comparison between the results of this study and with those reported in the literature for selected adsorbents. Comparing the adsorption capacity of HAP powder used in this study with the different adsorbents listed in Table 3 reveals the powder is an effective adsorbent for the removal of Fe^{2+}, Cu^{2+} and Zn^{2+} from aqueous solutions.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Q (mg/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated carbon derived from Cicer arietinum</td>
<td>-</td>
<td>17.77</td>
</tr>
<tr>
<td>Bentonite</td>
<td>-</td>
<td>14.10</td>
</tr>
<tr>
<td>Bone Charcoal</td>
<td>-</td>
<td>45.80</td>
</tr>
<tr>
<td>Cow Bone Charcoal</td>
<td>31.43</td>
<td>35.44</td>
</tr>
<tr>
<td>Egg Shell</td>
<td>-</td>
<td>34.48</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>-</td>
<td>36.9</td>
</tr>
<tr>
<td>Hydroxyapatite (30 nm)</td>
<td>55.25</td>
<td>61.35</td>
</tr>
</tbody>
</table>

4. Conclusions

The results of the present study have revealed that a nanometre scale HAP powder synthesized using a combined ultrasonic and microwave based technique was capable of producing a highly crystalline powder consisting of spherical particle morphology with a mean particle size of 30 nm. The powder was found to be an effective adsorbent for the removal of metal ions such as Fe^{2+}, Cu^{2+} and Zn^{2+} from aqueous solutions. Kinetic studies revealed the sorption process for Fe^{2+} and Cu^{2+} ions closely followed pseudo-second order kinetics. While the slightly higher correlation coefficient (R^2) of 0.9995 for Zn^{2+} ions indicated that the Intra-particle kinetic diffusion kinetics was more suited to modelling the experimental data. Overall, the sorption performance was found to be a function of initial metal ion concentration. With initial uptake rate of metal ions being high compared to the much lower uptake rates occurring in the later part of the absorption period. The ion-exchange mechanism (metal ion → Ca^{2+}) was seen to be a participant in the sorption process which was influenced by intra-particle diffusion. Isotherm studies indicated the Langmuir isotherm modelled Fe^{2+} and Cu^{2+} ion adsorption data better than the Freundlich isotherm. However, the Freundlich isotherm modelled the Zn^{2+} ion adsorption data. The Langmuir isotherm was used to determine maximum adsorption capacity of the HAP absorber for each metal ion. The study found the metal ion with the largest maximum adsorption capacity was Cu^{2+} ions (61.35 mg/g). This was followed by Fe^{2+} ions (55.25 mg/g) and then Zn^{2+} ions (48.54 mg/g).

ACKNOWLEDGEMENTS

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REFERENCES


FTIR, XRD, SEM and solid state NMR investigations of carbonate-containing hydroxyapatite nano-particles synthesised by hydroxide-gel technique, J. Physics and Chemistry of Solids, 64(2), 193-199.


4.3. Chapter Summary

This chapter consisted of two case studies that examined the chemical adsorption capabilities of HAP and its ability to function like the mineral phase of bone tissue. The prepared nanometre scale HAP powders were found to be an effective adsorbent for the removal of metal ions (Cd$^{2+}$, Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$) from aqueous solutions. The sorption performance of the metal ions was found to be a function of initial metal concentration, temperature and solution pH. Furthermore, metal ion removal was found to improve with increases in these parameters for specific contact times. Kinetic studies were also carried out and revealed that during sorption, the initial uptake rate of metal ions was high, but this was followed by a much lower uptake rate. The ion-exchange mechanism (Metal ion $\rightarrow$ M$^{2+}$) was clearly identified as a participant in the sorption process.
Chapter 5 - Growth of flower-like brushite structures on magnesium substrates and their subsequent low temperature transformation to hydroxyapatite.

5.1. Overview and author contributions

Chapter five addresses two components outlined in aims 3 and 4 of this research project. The first component, listed in aim 3 was to develop and use a direct chemical immersion technique to form calcium phosphate coating on magnesium substrates. The second component was to conduct corrosion studies to investigate the degradation behaviour of various coating types in phosphate buffer saline (PBS) solution and Ringer’s solution at 37 ºC and at a pH of 7.4 to simulate the body’s physiological fluid environment. Case study 4 is composed of two parts. The first part developed a straightforward and cost effective chemical immersion technique that deposited di-calcium phosphate dihydrate or brushite [DCPD; CaHPO$_4$.2H$_2$O] coatings on Mg substrates. In the second half of the study the DCPD coatings were converted into hydroxyapatite [(HAP); Ca$_{10}$(OH)$_2$(PO$_4$)$_6$] via immersion in a sodium hydroxide solution bath at 80 ºC for 2h. The size, morphology and crystallinity of both DCPD and HAP coating were examined using X-ray diffraction (XRD) spectroscopy, Field emission scanning electron microscopy (FESEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared spectroscopy (FT-IR). The second part of the research was to investigate the degradation behaviour of pure Mg substrates and the various substrate coatings. The degradation behaviour was evaluated in two electrolytes, the first was phosphate buffer saline (PBS) solution and the second was Ringer’s solution. All degradation studies were carried out at human body temperature (37 ºC).
S. Brundavanam conducted all chemical immersion studies, low temperature transformation procedures and performed advanced characterisation techniques [(XRD), (SEM), (EDS) and (FT-IR)] to fully investigate the physical and chemical properties of pure Mg substrates and substrate coatings. S. Brundavanam also carried out all electrochemical measurements associated with substrate degradation studies with the assistance of Associate Professor Gamini Senanayake. S. Brundavanam actively participated in the subsequent analysis of the data from chemical immersion studies and advanced characterisation studies. S. Brundavanam carried out all studies forming Case Study 4 under the supervision of G.E.J. Poinern. During the studies D. Fawcett (MANRG - Research Fellow) assisted S. Brundavanam in addressing the various technical aspects of the research. S. Brundavanam also contributed to writing the research article and assisted with editorial changes recommended by reviewers for the peer-reviewed research article that formed Case Studies 4. All authors provided feedback during the preparation of the paper.

5.2. Published Research Article:

Case Study 4

Growth of Flower-Like Brushite Structures on Magnesium Substrates and Their Subsequent Low Temperature Transformation to Hydroxyapatite

Sridevi Brundavanam, Gérard Eddy Jai Poinern*, Derek Fawcett

Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Australia

Abstract Dicalcium phosphate dihydrate (DCPD) Brushite coatings composed of flower-like structures were formed on magnesium substrates via a straightforward chemical immersion technique in order to slow down the corrosion rate of the metallic substrates. Moreover, the synthesised DCPD coatings were also converted to hydroxyapatite (HAP) coating using a low-temperature hydrothermal process to further investigate their ability to reduce the corrosion rate of the substrates in phosphate buffer saline (PBS) and Ringer’s solutions. Degradation studies found DCPD coatings were capable of providing the most significant reduction in the corrosion rate of around 0.100 mm/yr compared to 3.828 mm/yr for the uncoated substrates soaked in Ringer’s solution at 37ºC.

Keywords Magnesium, DCPD, Hydroxyapatite, Coating, Simulated Biodegradation

1. Introduction

Ideally, orthopaedic and dental devices must have a surface chemistry and physical structure that is biological compatible. In addition, they must also induce a positive osteogenic response and avoid any unfavourable immune responses [1]. Biodegradable implant materials currently in use and under development offer many attractive features for a number of clinical applications such as bone fixation, controlled release of pharmaceuticals, endovascular stents and orthopaedic implants [2-4]. The use of biodegradable materials in orthopaedic surgical procedures is of particular interest, since the function of many implants usually comes to an end when tissue regeneration and healing has taken place [3, 5]. The majority of these implants are biologically inert and engineered from metallic materials such as cobalt-chromium based alloys, stainless steels and titanium alloys. Metallic materials are very appealing for load bearing applications due to their ductility, high strength, fracture toughness and anticorrosion properties [6-8]. Typically, many of these implants are only needed for a short period of time to provide the necessary structural and mechanical support during tissue regeneration. However, to remove the implant after tissue regeneration requires a second surgical procedure. The additional surgical procedure is both costly and significantly increases the risk of infection and scarring of the patient [9]. Alternatively, the implant can be left in situ and as a result a number of potentially detrimental effects can take place such as corrosion of the implant material itself, inflammatory responses, stress-shielding and subsequent weakening of surrounding tissues. For example, there can be a significant release of toxic metallic ions such as chromium, cobalt and nickel during biological corrosion and mechanical wear. The production of these toxic metallic ions immediately solicits an unfavourable inflammatory response from the body’s immune system. The unfavourable immune response significantly reduces the biocompatibility of the implant and often leads to secondary revision surgery [10-12]. The other major disadvantage of metallic implants is their superior mechanical properties that are often many times greater than those of natural bone tissues. For instance, cobalt-chromium based alloys can have an elastic modulus that is ten times greater than that of bone and titanium based alloys are typically five times greater [6, 13, 14]. The significant difference between the mechanical properties of the implant and the surrounding bone creates a stress-shielding effect that causes bone resorption and subsequent implant failure. Furthermore, metallic implants are biologically inert and do not biologically or chemically interact with the surrounding tissues. The lack of interaction results in very little interfacial bonding or osteointegration taking place [15]. One currently used technique to improve the osteointegration of metallic implants involves coating them with a bioactive material such as calcium phosphate [16].

An attractive alternative to conventional metallic
implants is to develop a biologically degradable implant that achieves complete dissolution by the end of the tissue regeneration period [17]. Some successful outcomes have been achieved using biodegradable polymers in applications such as sutures, bone and dental cements, bone grafting materials, plates, screws, pins and fixation devices [18-24]. However, their low mechanical strength means they are only suitable for low-load bearing applications and soft tissue reconstruction. On the other hand magnesium (Mg) is a metallic material with the potential to overcome the limitations of conventional metallic implant materials and degradable polymeric materials. Magnesium offers a number of attractive features such as biocompatibility, biodegradability and mechanical properties similar to bone [25]. The density of Mg is 1.74 g/cm$^3$ at 20°C and is slightly less than bone which ranges from 1.8 to 2.1 g/cm$^3$. There is also a close similarity between the elastic modulus of Mg (45 GPa) and bone which varies from 40 to 57 GPa [26, 27]. The close similarity of its mechanical properties and its favourable biocompatibility makes Mg a promising material for the development of biodegradable orthopaedic implants [25, 28].

Despite Mg many advantages, but its poor corrosion resistance in chloride rich body fluids (pH ranges between 7.4 and 7.6) has severely limited its use in medical applications. The rapid corrosion rate results in two fundamental problems. The first involves the rapid formation of subcutaneous hydrogen gas bubbles that appear during the first week after surgery [29, 30]. However, the bubbles can be drawn off using a subcutaneous needle [31]. The second problem results in the loss of mechanical integrity of the implant and surrounding bone tissue that prevents effective tissue regeneration. However, this vulnerability to corrosion can be considered as an advantage when designing a biodegradable implant. For example, controlled degradation of the implant will allow natural bone tissues to regenerate and replace the implant [32]. Generally, Mg corrodes in an aqueous environment according to the following equations. The anodic reaction can be explained by the partial reaction expressed in equation (1). While the partial reaction occurring at the cathode can be expressed by equation (2).

\begin{align*}
\text{Anodic reaction: } & \quad \text{Mg} \rightarrow \text{Mg}^{2+} + 2e^- \quad (1) \\
\text{Cathodic reaction: } & \quad 2\text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{H}_2 \quad (2)
\end{align*}

The complete corrosion process is presented in equation (3). However, corrosion occurring in the body environment is not straightforward and is complicated by the influence of factors such as: 1) the pH of body fluids; 2) variations in the pH value; 3) concentration of ions; 4) the presence of proteins and protein adsorption on the surface of the implant material; and 5) the influence of surrounding tissues [25, 33, 34].

\begin{equation}
\text{Mg}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{Mg(OH)}_2 + \text{H}_2\text{(g)} \quad (3)
\end{equation}

The most significant by-products produced during degradation are hydrogen gas and Mg ions. Studies by Song have suggested that hydrogen levels of around 0.01 ml/cm$^2$/day does not constitute a serious threat to body tissues [35]. While other studies have shown the release of Mg ions can promote cellular adhesion and cellular differentiation of bone cells [36, 37]. Another positive effect resulting from the release of Mg ions is their antibacterial properties that have the potential to prevent biological film formation on implant surfaces [38]. The results of the studies clearly indicate reducing the corrosion rate will reduce the formation levels of Mg ions and hydrogen to acceptable levels and make Mg an ideal biodegradable implant material. One effective method of reducing the corrosion rate is to coat it with a non-corrosive protective layer. Therefore, developing an effective biocompatible coating capable of regulating the corrosion rate is essential for the development of a biodegradable Mg implants. 

Calcium phosphates (CaP) have been widely used to coat metal implants because of their excellent biocompatibility, non-toxicity, bioactivity and bone inductivity properties. Several techniques have been used to deposit CaP coating on Mg and other metal substrate materials such as anodization [39], biomimetic methods [40], electro-less deposition [41], electro-deposition [42, 43], ion-beam-assist ed deposition [44], chemical [45, 46] and hydrothermal [47]. Many of these techniques require complex equipment, multiple processing steps and high temperature treatments to produce an effective substrate coating.

In this study a straightforward and cost effective chemical immersion technique was used to deposit di-calcium phosphate dehydrate or brushite [DCPD; CaHPO$_4$.2H$_2$O] coatings on Mg substrates. The DCPD coatings were subsequently converted into hydroxyapatite [(HAP); Ca$_{10}$(OH)$_2$(PO$_4$)$_6$] via immersion in a sodium hydroxide solution at 80 ºC for 2h. The size, morphology and crystallinity of both DCPD and HAP coating were examined using X-ray diffraction (XRD) spectroscopy, Field emission scanning electron microscopy (FESEM), Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infrared spectroscopy (FT-IR). The degradation behaviour of Mg and Mg coated substrates were evaluated in phosphate buffer saline (PBS) solution and Ringer’s solution at body temperature (37°C).

2. Materials and Methods

2.1. Materials

All chemicals used in this work were supplied by Chem-Supply (Australia) and all aqueous solutions were made using Milli-Q® water (18.3 MΩ cm$^{-1}$) produced by an ultrapure water system (Barnstead Ultrapure Water System D11931; Thermo Scientific, Dubuque, IA).

2.2. Mg Substrate Pre-treatment

Mg (99.9% pure) sheets were cut into rectangular strips 40 mm in length, 3 mm in width and 0.15 mm in thickness.
The strips were polished using 1200 grit silicon carbide (SiC) paper to remove all surface oxides and contaminants. After polishing, the strips were cleaned in a 5 wt% nitric acid (HNO₃) solution followed by ultrasonically rinsing in acetone for 10 min. The acetone was then lightly rinsed from the substrates using Milli-Q® water before being allowed to air dry. After drying the weight of each substrate was recorded using an Ohaus PA214C microbalance.

2.3. Substrate Surface Treatment

The electrolyte used to form the DCPD coatings was prepared by adding 0.32 M of Ca(NO₃)₂ and 0.19 M of KH₂PO₄ to 100 mL of Milli-Q® water. The mixture was then thoroughly stirred at 400 rpm for 10 min. The electrolyte was prepared at 25 ± 1°C and the resulting pH was 4. DCPD coating were prepared by immersing in the prepared electrolyte. Individual substrates were removed from the electrolyte at pre-determined time intervals (3, 15, 30, 60, and 180) so that the size and morphology of the forming coating could be quantified using advanced characterisation techniques. At the end of each immersion period samples were removed from the electrolytic solution, washed in Milli-Q® water, and then allowed to air dry for 24 h. Conversion of DCPD coatings to hydroxyapatite (HAP) was achieved by immersing the DCPD coated substrates into a 1M solution of sodium hydroxide (NaOH) at 80°C for 2 h. At the end of this period substrates were removed from the electrolyte solution, washed in Milli-Q® water, and then allowed to air dry for at least 24 h. The HAP coatings were then examined using the advanced characterisation techniques discussed below.

2.4. Corrosion Testing

The corrosion resistance of coated and non-coated Mg substrates were tested in freshly prepared phosphate buffer saline (PBS) solution and Ringer’s solution. The composition of the PBS solution (in g/L) consisted of 8.006 NaCl, 0.201 KCl, 1.420 Na₂HPO₄ and 0.240 KH₂PO₄. The Ringer’s solution composition (in g/L) consisted of 8.6 NaCl, 0.6 KCl and 0.66 CaCl₂. 2H₂O. The pH of the respective solutions were adjusted to 7.4 and maintained at 37°C during the corrosion studies to match body fluid pH and temperature. Polarization curves were generated using an EG&G Princeton Potentiostat/galvanostat (Model 273A, supplied EG&G Princeton applied research) configured for a three-electrode experimental set-up. The working electrode in all corrosion tests consisted of a test substrate with a surface area of 1 cm². A saturated calomel electrode (SCE) was used as the reference electrode and a platinum wire (Pt) was used as the counter electrode. A Tafel test procedure was performed over a voltage range from -2.5 V up to 1.0 V, with a step size of 10 mV and a 1s time interval for the 10 mV scan rate. From the resulting experimentally derived polarization curves parameters such as corrosion potential (E_corr), corrosion current density (I_corr), anodic/cathodic Tafel slopes (β_a and β_c) and corrosion rate were derived. The polarization resistance ($R_p$) was calculated at the near open circuit potential (OCP) using the Stern–Geary equation:

$$R_p = \frac{(β_aβ_c)}{2.303I_{corr}(β_a+β_c)}$$

2.5. Advanced Characterisation Techniques

2.5.1. X-ray Diffraction (XRD) Spectroscopy

XRD spectroscopy technique was used to examine and identify crystalline size and phases present in the surface coating. Spectroscopy data was recorded at room temperature, using a GBC® eMMA X-ray Powder Diffractometer [Cu Kα = 1.5406 Å radiation source] operating at 35 kV and 28 mA. The diffraction patterns were collected over a 2θ range of 20° to 60° with an incremental step size of 0.02° using flat plane geometry with 2 second acquisition time for each scan.

2.5.2. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The SEM technique was used to study the size, shape and morphological features of the surface coating formed on the substrates during the immersion process. All micrographs were taken using a JCM-6000, NeoScope™ operating at 80kV. The samples were then allowed to slowly dry over a 24-hour period. After sample preparation a bright field TEM study was carried out using a Phillips CM-100 electron microscope (Phillips Corporation Eindhoven, The Netherlands) operating at 80kV.

2.5.3. Transmission Electron Microscopy (TEM)

The size, morphology and topography of DCPD particles formed were investigated using TEM. Sample preparation consisted removing a portion of the coating from the surface of the substrate. The samples were then placed into small tubes containing Milli-Q® water. The tubes were then sealed and placed into an ultrasonic bath for 10 minutes. The suspensions were then filtered 2 times before a single drop from each sample was deposited onto its respective carbon-coated copper TEM grid using a micropipette. The samples were then allowed to slowly dry over a 24-hour period. After sample preparation a bright field TEM study was carried out using a Phillips CM-100 electron microscope (Phillips Corporation Eindhoven, The Netherlands) operating at 80kV.

2.5.4. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was used to investigate CaP powders synthesized during the immersion process using a Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment. FT-IR spectra were recorded in the range from 525 to 4000cm⁻¹ in steps of 4 cm⁻¹.
3. Results and Discussions

3.1. XRD Analysis of Calcium Phosphate Formation on Substrates

Before chemical immersion a representative Mg substrate was examined using the XRD technique [49]. The resulting Mg XRD pattern is presented in Figure 1 (c) and confirms the substrate purity. The XRD pattern also revealed that there was no contamination present on the substrate surface. XRD analysis was also carried out on chemically treated substrates. Figure 1 (b) presents a typical XRD pattern of a substrate after an immersion period of 180 min. Analysis of pattern (b) reveals the presence of a crystalline calcium phosphate phase identified as di-calcium phosphate di-hydrate (DCPD) or Brushite (JCPDS 11-293). No other calcium phosphate phases were found in the samples. However, the XRD analysis did confirm the presence of Mg peaks (002), (101) and (102) produced by the underlying substrate. The coating produced during immersion was formed via the following reaction [9, 48]:

\[
\text{HPO}_4^{2-} + \text{Ca}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{CaHPO}_4 \cdot 2\text{H}_2\text{O} \quad (5)
\]

Typically, the mass of DCPD formed during a 180 min immersion period was collected, dried and weighed. The mass was found to be 3.5 mg [Figure 3 (d)] and equated to a coating thickness of around 10 µm. In the next stage of the study, the DCPD coatings were converted into HAP by immersing in a 1M solution of sodium hydroxide at 80 ºC for 2 h. A representative XRD pattern for the converted coating is presented in Figure 1 (a). Analysis of the pattern reveals the DCPD coating was fully converted, and the HAP phases present were consistent with phases listed in the ICDD database. Also present are the Mg peaks seen in the earlier DCPD coatings. The consistent presence of Mg peaks suggests that the coating was unable to fully mask the underlining substrate due to regions of poor surface coverage. The surface coverage was further investigated using SEM analysis and discussed in the following section.

3.2. SEM and EDS Analysis of Coating Formation

The size, morphology and topographical features of the deposited DCPD coatings were examined using SEM and the composition of the coatings were investigated using EDS spectroscopy. Figure 2 presents a series of SEM micrographs taken during the formation of a typical DCPD coating at immersion periods of 3, 15, 30, 60, and 180 min. Figure 2 (a) is a micrograph of a representative Mg substrate prior to chemical immersion. Micrograph 2 (b) was taken after a 3 min immersion period. The image reveals the presence of scattered individual small DCPD plate assemblies that are typically around 50 µm in size. After 15 minutes the scattered plate-like assemblies are beginning to grow into flower-like features. At this point in time the features are around 150 µm in size as seen in Figure 2 (c). For an immersion period of 30 min, the flower-like features have grown in size and are starting to merge with nearby neighbours as seen in Figure 2 (d). Also clearly visible at this stage were large areas of exposed underlining substrate. Following a further 30 minutes of immersion, the flower-like features completely cover the substrate surface as seen in Figures 2 (e) and 3 (a). An enlarged view of the flower-like petals is presented in Figure 3 (b), while Figure 3 (c) presents a detailed end view of the petals showing their plate-like structure. Typically, at the end of a 180 min immersion period substrates were completely covered with large flower-like features typically around 500 to 700 µm in size as seen in Figure 2 (f).

![Figure 1. XRD patterns for uncoated and coated substrates: (a) hydroxyapatite coating converted from a DCPD coating; (b) DCPD coating, and (c) pure magnesium substrate](image1)

![Figure 2. (a) Cleaned magnesium substrate and subsequent formation of DCPD coating after electrolyte immersion periods of (b) 3; (c) 15; (d) 30; (e) 60, and (f) 180 min](image2)
it was significantly reduced as reflected in the levelling off seen in Figure 3 (d). Also seen during the later part of the immersion period was the significant reduction in the evolution of gas bubbles. The significant reduction in formation rate and gas evolution suggests the coating was able to impede the flow of electrolyte towards the substrate surface. Thus, the levelling off seen in Figure 3 (d) after 180 minutes indicates that the coatings were providing some degree of protection to the underlining substrate.

Analysis of DCPD coatings using EDS spectroscopy revealed that they contained elements such as Ca, O and P but no Mg. The analysis indicates that Mg ions were not substituted for Ca ions during the formation of the DCPD coating.

Figure 3. (a) DCPD (brushite) coating decorated with ornate flowers covering the entire substrate surface; (b) enlarged view of flower petals; (c) detailed end view of DCPD crystal plates forming the petal structure, and (d) mass of DCPD coating formed with increasing immersion periods.

Figure 4. (a) a representative SEM micrograph of a DCPD flower decorating the coating; (b) EDS analysis of the flower showing its elemental composition; (c) a typical SEM micrograph taken after the conversion to form HAP, and (d) EDS analysis of the coating after conversion.
Moreover, the EDS analysis confirms that the Mg peaks seen in the XRD patterns were the result of the underlying substrate and not Mg in the coating. Figure 4 (a) presents a representative SEM micrograph of a DCPD flower-like structure forming part of the coating, while Figure 4 (b) presents the results of the EDS analysis and the elemental breakdown of components present in the coating.

Figure 4 (c) and (d) present a representative set of results confirming that the conversion process had converted the DCPD coatings into HAP. Figure 4 (d) presents the results of the EDS analysis and the elemental breakdown of the components present in the converted HAP coating. The analysis revealed that calcium to phosphate ratio (Ca:P) ranged from 1.5 to 2.0, with a mean value of around 1.71. The ideal Ca:P ratio for HAP is 1.67 [50], which is approximately 2.4% smaller than the 1.71 determine using the present conversion process.

3.3. FT-IR and TEM Analysis of DCPD Formation

Analysis of the FT-IR spectroscopy data was carried out to identify species, functional groups and vibration modes associated with each peak of the DCPD and HAP coatings. Figure 5 presents the results of the FT-IR analysis. Conversion of DCPD to HAP was confirmed by the appearance of a peak located at 601 cm$^{-1}$ that corresponds to PO$_4^{3-}$ functional groups normally associated with HAP and not DCPD. TEM analysis of the DCPD coatings revealed that they were composed of plate-like structures that were configured in a flower-like feature. A TEM image of a single DCPD plate taken from a representative coating is presented in Figure 6 (a). Its appearance is solid, angular and typical of DCPD structures seen in the coatings. However, after chemical conversion the appearance and structure of the coating dramatically changes as seen in Figure 6 (b). The solid plate-like structure are transformed into a completely different morphology composed of HAP.

3.4. Corrosion Resistance of Coatings

To be an effective biodegradable material an Mg based implant must slowly degrade and allow regenerating bone tissues to progressively take over from the load carrying function of the implant. To achieve this objective the corrosion rate of the Mg implant must be effectively controlled and allow sufficient time for successful tissue regeneration to take place. Furthermore, by effectively controlling the corrosion rate of a biodegradable implant means that it is possible to avoid the long-term complications normally associated with conventional metal implants. This study has examined the performance of two CaP coatings in reducing the effects of corrosion on Mg substrates in PBS and Ringer’s solutions at 37°C. Representative potentiodynamic polarization curves produced by the corrosion tests for Mg substrates with and without CaP coatings are presented in Figure 7.

![Figure 6. TEM images taken before and after conversion: (a) a typical plate-like structure associated with DCPD coating, and (b) a typical HAP structure seen after the conversion process](image)

![Figure 5. Results of FT-IR analysis showing the presence of PO$_4^{3-}$ functional groups at peak position 601 cm$^{-1}$ characteristic of HAP formation](image)

![Table 1. Corrosion rates of DCPD and HAP coated Mg substrates determined from polarization curves](table)

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Sample</th>
<th>Corrosion Rate (mm/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humid air [51]</td>
<td>Mg substrate</td>
<td>$1.0 \times 10^{-5}$</td>
</tr>
<tr>
<td>Distilled water [51]</td>
<td>Mg substrate</td>
<td>$1.5 \times 10^{-2}$</td>
</tr>
<tr>
<td>Seawater [51]</td>
<td>Mg substrate</td>
<td>0.25</td>
</tr>
<tr>
<td>Phosphate buffer saline solution (This study)</td>
<td>DCPD coated Mg substrate</td>
<td>1.829</td>
</tr>
<tr>
<td></td>
<td>HAP coated Mg substrate</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>Mg substrate</td>
<td>0.279</td>
</tr>
<tr>
<td>Ringer’s solution (This study)</td>
<td>Mg substrate</td>
<td>3.828</td>
</tr>
<tr>
<td></td>
<td>DCPD coated Mg substrate</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>HAP coated Mg substrate</td>
<td>0.264</td>
</tr>
</tbody>
</table>
The second electrolyte used in examining corrosion behaviour of substrates was Ringer’s solution. The Ringer’s solution test results are presented in Figure 7 (b). Like the PBS solution results, corrosion testing in Ringer’s solution also confirmed a significant improvement in corrosion resistance of coated substrates. The results also confirmed DCPD coatings were superior to HAP coating. In Ringer’s solution, uncoated Mg substrates had the highest corrosion rate of 3.828 mm/yr. And again DCPD coated substrate’s had a corrosion rate (0.1 mm/yr.) that was slightly less than half of an equivalent HAP coating (0.264 mm/yr.) as seen in Table 1. Corrosion studies confirmed that uncoated substrates found both PBS and Ringer’s solutions highly aggressive. Similarly, body fluids are similar in nature to the test solutions and are as equally aggressive towards uncoated Mg. However, the corrosion studies have shown that both DCPD and HAP coatings have the potential to significantly reduce the degradation rate of Mg substrates in PBS and Ringer’s solutions. It is expected that there would be a similar reduction in corrosion rates for DCPD and HAP coated substrates in vivo. However, further studies are needed to investigate and quantify degradation behaviour in vivo.

4. Conclusions

The corrosion rate of Mg substrates in PBS and Ringer’s solutions at 37°C was significantly reduced by the presence of a DCPD or HAP coating. The initial DCPD coatings were formed via a straightforward chemical immersion technique. The coatings were subsequently converted to HAP via a low-temperature hydrothermal process in order to examine which coating had the most effective corrosion resistance. FT-IR analysis was used to confirm DCPD conversion to HAP. Corrosion studies revealed that DCPD coated substrates had the lowest corrosion rate in both PBS (0.126 mm/yr) and Ringer’s solution (0.1 mm/yr) compared to HAP coated and uncoated Mg substrates. Both DCPD and HAP coatings have the capacity to significantly reduce the corrosive effects of PBS solution and Ringer’s solution. Since both these solutions are similar in nature to body fluids, the corrosion studies suggest that both coatings have the potential to reduce similar corrosive effects found in the body environment. However, further in vivo studies are needed to fully study and quantify the corrosive effects of actual body fluids on DCPD and HAP coatings formed in this study. This is of particular importance since Mg based degradable implants with DCPD and HAP coatings offer the potential to significantly improve biocompatibility and promote bone formation during tissue regeneration in hard tissue trauma treatments.

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REFERENCES


5.3. Chapter Summary

This chapter examined the degradation behaviour of DCPD and HAP coatings formed on Mg substrates. Degradation behavioural studies were carried out in PBS and Ringer’s solutions at 37 ºC. The initial DCPD flower-like coatings were formed via a straightforward chemical immersion technique. A selection of DCPD coatings was subsequently converted to HAP coatings via a low-temperature hydrothermal process. FT-IR analysis was used to confirm the conversion of DCPD coatings to the more mechanically stable HAP coatings. A range of uncoated Mg substrates, DCPD and HAP coated substrates underwent degradation studies. The studies revealed DCPD coated substrates had the lowest corrosion rate in both PBS (0.13 mm/yr) and Ringer’s solution (0.1 mm/yr) compared to HAP coated substrates [PBS (0.28 mm/yr) and Ringer’s solution (0.26 mm/yr)] and uncoated Mg substrates [PBS (1.83 mm/yr) and Ringer’s solution (3.83 mm/yr)]. The research has clearly shown that both DCPD and HAP coatings have the capacity to reduce the effects of corrosion in both PBS and Ringer’s solution. The results also suggest that the coatings have the potential to reduce similar corrosive effects found in the physiological environment. However, this needs to be further investigated via in vivo studies to fully appraise the corrosive effects on the respective coatings in the physiological environment. This is of particular importance since extending the operational life of an Mg based implant is dependent on the performance of the respective coating in the physiological environment. Furthermore, any potential enhancement in biocompatibility and bone formation resulting from the respective coating material needs to be fully investigated in future studies.
Chapter 6 – Case Studies 5 and 6: Electrochemical synthesis of amorphous calcium phosphate coating on magnesium substrates in aqueous solutions.

6.1. Overview and author contributions

The sixth chapter addresses two components outlined in aims 3 and 4 of the present research. The first component, listed in aim 3 was to develop and use an electrochemical technique to produce amorphous calcium phosphate (ACP) coatings on Mg substrates. In addition, ACP coatings were subsequently converted into HAP coating via immersion in a sodium hydroxide solution bath at 80 ºC for 2h. The second was to undertake corrosion studies to investigate the degradation behaviour of the respective coatings in phosphate buffer saline (PBS) solution and Ringer’s solution at 37 ºC and a pH of 7.4 to simulate body fluid conditions. Importantly, corrosion studies are needed to quantify the degradation rate of the coatings, which will determine their suitability in providing sufficient protection to the underlining substrate during tissue regeneration. In the ideal case, the coating should slowly degrade during the healing process and at the same time permit the slow controlled degradation of the underlining Mg substrate. Case study 5 examines the formation of tube-like structures produced during the electrochemical process and uses microscopy to assist in developing a formation mechanism to explain their growth. Case study 6 examines coating formation, coverage and appraises the degree of protection each coating type was able to provide to the underlining Mg substrate during the corrosion studies.

S. Brundavanam carried out all electrochemical/corrosion studies and conducted all advanced characterisation techniques [(XRD), (TEM), (SEM), (EDS) and (FT-IR)] to
investigate the structure and surface properties of the ACP and HAP coatings. G.E.J. Poinern acting as principal supervisor assisted S. Brundavanam in designing the research program for Chapter 6. S. Brundavanam was also actively involved with the assistance of D. Fawcett (MANRG - Research Fellow) in analysing the experimental results and interpreting the advanced characterisation data. S. Brundavanam was heavily involved in compiling the experimental data and writing the subsequent research articles. In addition, S. Brundavanam assisted both G.E.J. Poinern and D. Fawcett with editorial changes recommended by reviewers for the respective peer-reviewed research articles that form Case Studies 5 and 6. All authors provided feedback during the preparation of the respective research articles.

6.2. Published Research Articles

Case Study 5


Case Study 6

Electrochemical Synthesis of Micrometre Amorphous Calcium Phosphate Tubes and their Transformation to Hydroxyapatite Tubes

Sridevi Brundavanam¹, Gérrard Eddy Jai Poinern², Derek Fawcett³

¹Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Western Australia 6150, Australia Fax: +61 8 9360-6183

Abstract: Amorphous calcium phosphate tube-like structures were formed on magnesium substrates using a straightforward electrochemical process. The effect of voltage, time and the resulting hydrogen gas evolution were found to be responsible for sculpturing the tube-like structures covering the entire substrate surface. Scanning electron microscopy and Energy Dispersive Spectroscopy was used to quantify the size, shape, structure and composition of the tube-like structures. Microscopy analysis was used to develop a model to describe the mechanism behind the formation of the tube-like structures. Also investigated was a hydrothermal technique used to convert the amorphous calcium phosphate structures into hydroxyapatite. Both X-ray diffraction spectroscopy and Fourier Transform Infrared spectroscopy were used to confirm the effectiveness of the conversion process.

Keywords: Electrochemical synthesis, amorphous calcium phosphate, microstructures

1. Introduction

Amorphous calcium phosphate (ACP) is the first mineral phase that precipitates from highly saturated aqueous solutions containing calcium and phosphate ions due to its lower surface energy compared to other calcium phosphates such as octacalcium phosphate (OCP) and hydroxyapatite (HAP) [1]. Unlike other calcium phosphates, ACP is metastable, has a non-crystalline character with short-range order and lacks long-range periodic uniformity normally associated with crystalline structures [2]. Analysis of X-ray diffraction measurements have revealed that ACP has only two very broad and diffuse peaks, with a maximum occurring at a 20 angle of 25°. No other features, normally associated with crystalline materials are observed [3]. This pattern is typical for non-crystalline or amorphous materials and confirms the lack of long-range periodic regularity of ACP. The spherical structural unit of synthetic ACP is composed of randomly assembled clusters of ions approximately 9.5Å in diameter and with the chemical composition of Ca₁₀ (PO₄)₆ [4]. Also contained within the interstices of a typical ACP particle is tightly bound water that can be as much as 20% by weight of the particle [5]. In aqueous solutions ACP particles will readily agglomerate, but are thermodynamically unstable. Therefore, ACP can be considered a transient or metastable phase that will easily transform to more thermodynamically stable crystalline calcium phosphate phases such as HAP via a process of dissolution, nucleation, and crystal growth [6]. The amorphous structure of ACP being unstable is also highly reactive and rapidly hydrolyses into more stable calcium phosphate phases in body fluids. It is due to the metastable properties of ACP that makes it more osteoconductive than hydroxyapatite and also gives it superior biodegradability when compared to tricalcium phosphate when used in vivo [7- 9]. The existence of ACP in aqueous solutions is governed by a number of factors such as ionic strength, pH value, temperature and the presence of contaminants or additives [10]. Stabilising metastable ACP in aqueous solutions can be achieved by the introduction of additive ions or molecules to prevent transformation to more stable crystalline calcium phosphate phases. And under in vivo conditions, the presence of protein based molecules and similar substances can delay the transformation of ACP due to the effects of kinetic stabilization [11]. The role of calcium binding proteins and ionic species involved in the exact mechanism of biological mineralization and transformation of ACP to HAP in vivo are not fully understood [12-14]. However, because of its
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biological compatibility and physiochemical properties ACP will continue to be used in the rapidly developing field of tissue engineering, dentistry and orthopaedics.

This study examines the formation ACP micrometre scale tubular structures formed on magnesium substrates during a straightforward electrochemical synthesis process. The electrodes consist of a platinum wire mesh (anode) and an Mg substrate (cathode) immersed in an electrolyte consisting of calcium nitrate and potassium di-hydrogen phosphate. Advanced characterisation techniques such as X-ray diffraction (XRD) spectroscopy, Transmission electron microscopy (TEM), Fourier Transform Infrared spectroscopy (FT-IR).

Scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) were used to determine size, morphology, composition and architecture of the formed ACP structures. Furthermore, a proposed formation model is presented to explain the growth of the ACP micrometre scale structures.

2. Materials and methods

2.1. Materials
All chemicals used in this work were supplied by Chem-Supply (Australia) and all aqueous solutions were made using Milli-Q® water (18.3 MΩ cm⁻¹) produced by an ultrapure water system (Barnstead Ultrapure Water System D11931; Thermo Scientific, Dubuque, IA).

2.2. Mg substrate surface pre-treatment
Magnesium with a purity of 99.9% was used throughout this study. The sheet was cut into rectangular strips that were 40 mm in length, 3 mm in width and 0.15 mm in thickness. The strips were polishing using 1200 grit silicon carbide (SiC) paper to remove all surface oxides and contaminants. After polishing, the strips were cleaned in 5 wt% nitric acid (HNO₃) solution followed by ultrasonically rinsing in acetone for 10 min. The acetone was then lightly rinsed from the substrates using Milli-Q® water before being allowed to air dry. After drying the weight of each substrate was recorded using an Ohaus PA214C microbalance.

2.3. Electrochemical formation of ACP surface features
The formation of ACP surface features on Mg substrates was accomplished using an electrochemical cell consisting of two electrodes and an electrolyte. The electrolyte consisted of an aqueous solution containing 0.32 M of Ca(NO₃)₂, 4H₂O and 0.19 M of KH₂PO₄. The electrodes used in this experimental setup consisted of a platinum wire mesh (anode) and an Mg substrate that was used as the cathode. A standard laboratory DC power supply [GW INSTEK GPS-2303] was used to supply the required voltage range with a maximum current density of 60 mA/cm² in the dynamic mode setting. Studies were carried out over a voltage range between 2 and 7 volts and over periods ranging from 30 s to 5 min at a room temperature of 25 ± 1 °C. Two voltage/time procedures were followed: 1) fixed voltage (6 V) and variable time (30 s to 5 min) and, 2) fixed time 3 min and variable voltage (2 to 7 V). Both procedures were found to give sufficient experiment data for analysis purposes. After each experimental run the substrates were removed from the electrolyte, rinsed using Milli-Q® water and then air-dried before being stored ready for advanced characterisation.

2.4. Conversion of ACP structures to hydroxyapatite
Conversion of the ACP structures and coatings to hydroxyapatite (HAP) was achieved by immersing the ACP coated substrates into a 1M solution of sodium hydroxide (NaOH) at 80 °C for 2 h. At the end of this period the substrates were removed from the electrolyte solution, washed in Milli-Q® water, and then allowed to slowly dry in air for at least 24 h. Finally, coated Mg substrates were heat treated in an electric furnace at 250 ºC for 1 hour and then allowed to cool to room temperature in air. The HAP coatings were then examined using the advanced characterisation techniques discussed below.

2.5. Advanced characterisation techniques

2.5.1. X-ray diffraction (XRD) spectroscopy
XRD spectroscopy was used to study and to identify the crystalline phases present in the surface structures and coating formed during electrochemical synthesis. Spectroscopy data was recorded at room temperature, using a GBC® eMMa X-ray Powder Diffractometer [Cu Kα = 1.5406 Å radiation source] operating at 35 kV and 28 mA. The diffraction patterns were collected over a 20° range of 2° to 60° with an incremental step size of 0.02° using flat plane geometry with 2 second acquisition time for each scan.

2.5.2. Transmission electron microscopy (TEM)
The size and morphology of ACP and HAP particles synthesized were investigated using TEM. Sample preparation consisted removing a portion of the synthesized material from the surface of the magnesium substrate. The material was then placed into small tubes containing Milli-Q® water. The tubes were then sealed and placed into an ultrasonic bath for 10 minutes. The suspensions were then filtered 2 times, and then a single drop from each sample was deposited onto its respective carbon-coated copper TEM grid using a micropipette and then allowed to slowly dry over a 24-hour period. After sample preparation a bright field TEM study was carried out.
2.5.3. Fourier Transform Infrared spectroscopy (FT-IR)
FT-IR spectroscopy investigations of the synthesized ACP and HAP were carried out using a Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment. Spectra were recorded in the range from 525 to 4000cm\(^{-1}\) in steps of 4 cm\(^{-1}\).

2.5.4. Scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)
SEM was used to study the size and morphological features formed on the substrates during the creation of the surface coating. All micrographs were taken using a JCM-6000, NeoScope\(^*\) with attached energy dispersive X-ray spectroscopy. Samples were mounted on individual substrate holders using carbon adhesive tape before being sputter coated with a 2 nm layer of gold to prevent charge build up using a Cressington 208HR High Resolution Sputter coater.

3. Results and Discussions
3.1. XRD spectroscopy identification of calcium phosphate phases
XRD spectroscopy was used to identify the calcium phosphate phases formed on Mg substrates during the electrochemical synthesis process. Selections of typical XRD patterns are presented in Figure 1 (a). In Figure 1 (a) a representative XRD pattern for an Mg substrate prior to the electrochemical surface treatment is presented. Inspection of the pattern reveals three dominant peaks [(002), (101) and (102)] and two minor peaks [(100) and (110)] associated with crystalline Mg and one small peak (101) associated with Mg(OH)\(_2\). Apart from Mg(OH)\(_2\), no other surface contaminants were found on the substrate surfaces. During electrochemical treatment, a calcium phosphate layer formed over the surface of the substrates. Subsequent XRD investigation revealed a characteristic pattern of an amorphous material as seen in Figure 1 (a). Also present in the XRD pattern are three peaks [(002), (101) and (102)] that were identified as Mg from the underlining substrate and Mg present in the ACP structures. No other calcium phosphate crystalline phases were detected in the structured surface coatings. Subsequent conversion of structured ACP surface coatings to HAP was also confirmed by XRD analysis. HAP was confirmed by the presence and identification of spectra peaks with Miller indices (002), (211) and (202) that were consistent with phases incorporated in the ICDD (International Centre for Diffraction Data) databases. Also present is the HAP pattern was the presence of (002) related peak indicating that Mg had been detected. The strong signal suggests that not only was Mg present in the substrate coating, but the underlining Mg substrate was also detected. The extensive and large crack formations in both the coating and substrate surface are believed to be responsible for the release of Mg ions and their subsequent incorporation into the forming surface structures. The incorporation of Mg ions into the coatings was also found in the EDS spectra as seen in Figure 5.

3.2. Microscopy analysis of ACP and ACP based structural features found in coatings
TEM microscopy was used to examine the structure of the initial ACP coatings found on the substrates. XRD analysis identified the formation before conversion as ACP and TEM images confirm the spherical structural nature normally associated with ACP particles as seen in Figures 2 (a) and (b). While the SEM micrograph presented in Figure 2 (c) shows the highly agglomerated nature of the ACP particles. The particles range in size from 60 nm to 250 nm, with a mean particle size of 120 nm. And because ACP is thermodynamically unstable, it tends to transform into more thermodynamically stable crystalline calcium phosphate phases and this can be seen in Figures 2 (a) and (b) with the presence of partially formed plate-like features. The partially formed plate-like features, which are indicated by blue arrows, were not detected in the XRD patterns of the initial ACP coatings. Partially formed plate-like structures were also seen in SEM images of the tubular formations as seen in Figure 2 (d), but again were not seen in the XRD spectra.

3.3. Formation of ACP structures
The electrochemical formation of ACP surface features on Mg substrates was investigated using two voltage/time procedures. The first used a fixed time period (3 min) with a variable voltage range and the second used a variable time period (30 s to 5 min) with a fixed voltage of 6 V. The first procedure investigated the effect of voltage in forming ACP surface features. The results of this study revealed that there was a voltage dependence effect and it was responsible in initiating tubular structure formations above 4 V. Below 4 V, only small numbers of hydrogen bubbles were produced and almost no calcium phosphate materials could be seen forming except for the occasional small outcrops as seen in Figures 3 (a) and (b). At this stage the surface of the Mg substrate was relatively smooth and didn’t show any signs of surface cracking or significant corrosion. However, from 4 V onwards, gas evolution was clearly visible across the entire surface of the substrate. With the dissolution of the Mg substrate deposits of spherical, granular shaped ACP particles and small tubules could be seen close to small cracks that had formed in the surface of the substrate as seen in Figure 3 (c). The surface cracks seen in the
substrate are the result of hydroxyl ions accumulating at the solid/liquid interface [15]. The accumulation of hydroxyl ions causes a localized region of high pH that subsequently produces the precipitation of ACP at the substrate surface. As the voltage was increased, precipitation of ACP increased and two dominant features could be seen forming. The first was surface cracking of the coating that could be seen extending down to the underlying Mg substrate. And the second feature was widespread tube formation across the surface of the substrate as revealed in Figures 3 (d) to (f). An interesting feature of the tubes was the variation in diameters that ranged from 30 µm up to a maximum of around 100 µm.

During the second voltage/time procedure the voltage was maintained at 6 V while the time period ranged from 30 s to 5 min. After 30 s in the electrochemical cell, tubes could be seen forming over the entire substrate surface as seen in Figure 4 (a). In addition to the tubular features was the presence of numerous ACP plate-like particles. The plate-like features had mean diameters of around 5 µm and a thickness of around 1.5 µm and were covering the surface between the tubes as seen in Figure 4 (b). Also present was surface cracks, which were seen earlier in the variable voltage procedure for voltages above 4 V. One minute into the electrochemical procedure saw a significant increase in hydrogen evolution and an increase in size and number of tubules being formed as seen Figure 4 (c). Three minutes into the procedure saw a dramatic increase in the size and depth of cracks in the surface coating, which exposed more of the underlying Mg substrate to the electrolyte, Figure (d). Also noticeable during this period was the reduced rate in new tubules being formed as indicated graphically in Figure 4 (f). At this point in time it is believed that most of the gas being formed was exiting the coating via the extensive network of large cracks covering the surface.

Figure 5 presents a SEM micrograph of a representative tubular structure formed after 3 minutes under constant voltage. EDS analysis was carried out on two locations along the length of the tube. Location (a) was taken at a position three quarters the length of the tube from the coating surface and location (b) was taken a quarter of the length from the coating surface. The spectra resulting from EDS analysis of both locations is presented in Figure 5. Both spectra are characteristic of ACP, but of particular interest is the presence of Mg in the tubular structure. The presence of Mg in the coating structures confirms the results of the XRD, which indicated the presence of Mg. Also present in both spectra was the presence of oxygen, which suggests that the presence of Mg in the coating structures could be in the form of Mg(OH)₂. The presence of Mg(OH)₂ in tubular structures could explain the existence of plate-like features seen in Figure 2 (d).

3.4. Tube Formation Model
Because of the tube-like structures present in the formation of the deposition layer, it is suspected that the release of hydrogen bubbles at the substrate surface (cathode) is one of the key factors responsible for sculpturing the deposition layer. This assumption is based on the circular geometry and consistent wall structure of the tube-like structures that appear to have been built up around an initial gas bubble residing on the metal/electrolyte interface. During the electrochemical process, the Mg substrate acting as cathode, reduces hydrogen and increases local pH. With the increase in pH, the stability of HPO₄⁻² significantly increases compared to that of H₂PO₄⁻ and as a result the concentration of HPO₄⁻² increases until maximum solubility is reached. Simultaneously, migration and coalescence of hydrogen at the surface of the cathode results in the formation of gaseous macroscopic bubbles as graphically presented in Figure 6 (a). It is believed that if the bubbles are small enough they will not be displaced by buoyancy. At this point, ACP begins to coat the substrate surface not occupied by bubbles and as a result the coating acts as an insulator that prevents electron flow from the substrate. However, the cathodic substrate continues to supply a current via the bubble/ACP interface that maintains hydrogen reduction, ion migration and diffusion. ACP continues being precipitated from solution and deposited around surface bubbles. At the same time hydrogen gas continues to migrate into the forming tube structure feeding the resident bubble. However, when the bubble gains sufficient volume, buoyancy forces dislodge the bubble from the substrate surface. If there is sufficient hydrogen migration a second entrapped bubble forms and so one until a steady stream of small gas bubbles leave the tube formation site. As the bubble forming process continues, the length of the tube steadily increases with the self-assembly of the ACP particles forming the tube wall structure. If electrochemical conditions are suitable and sustained then ACP deposition and growth of tube-like structures continue. However, when gas migrates into the tube bases ceases there is a rapid in flow of electrolyte resulting in rapid deposition of ACP within the tube-like structures.

3.5. Conversion of ACP coatings to HAP coatings
ACP coating structures were converted to hydroxyapatite (HAP) using a process that involved immersing substrates into sodium hydroxide at 80 °C for 2 h followed by a 1 h thermal treatment in a furnace at 250 °C. XRD analysis mentioned above confirmed that HAP was produced by the conversion process. Further confirmation of the effectiveness of the conversion process came from analysis of the FT-
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IR spectroscopy data as presented in Figure 1 (b). Conversion of ACP to HAP was confirmed by the appearance of the peak located at 602.9 cm\(^{-1}\), which is characteristic of PO\(_4^{3-}\) functional groups normally associated with HAP and not seen in ACP. In addition, SEM analysis of the converted coatings revealed that the tube-like structures had been transformed into urchin-like structures as seen in Figures 7 (a) and (b). TEM images of particles making up the coatings prior to conversion were spherical and ranged in size from 30 µm to 100 µm in diameter. After conversion the urchin-like structures were predominantly composed of needle-like and plate-like HAP particles. The particle width ranged from 30 nm to 90 nm, width varied between 80 nm and 200 nm and the length ranged from 450 nm up to 800 nm. Also present were a small number of granular particles ranged in size from 30 nm to 100 nm. Figure 7 (c) presents an SEM image of HAP particles found deposited over the surface of the substrate and Figure 7 (d) presents a representative TEM image of HAP particles forming in the urchin-like structures.

4. Conclusion
A straightforward electrochemical technique was used to synthesize ACP coatings with micrometre scale tube-like structures on Mg substrates. The size, structure and composition of the tube-like structures were investigated and the subsequent results were used to propose a formation model. The model explained the formation mechanism and the importance of hydrogen evolution in creating tube-like structures. Also investigated was a hydrothermal process for transforming the ACP coatings into HAP. Both XRD and FT-IR confirmed the conversion of ACP into HAP, with the ACP tube-like structures being transformed into urchin-like features. Furthermore, the spherical geometry characteristic of ACP particles was the prevailing morphology of the particles prior to the conversion process. After conversion two HAP particle morphologies were present. The first consisted of a small number of granular particles and the second consisted of plate-like particles. The nanometre scale particles composed the micrometre scale urchin-like features that dominated the substrate surface. However, further studies are needed to fully investigate the physical and chemical properties of coatings and structures formed in this preliminary study.

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Disclosure
The authors report no conflict of interest in this work.

References
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Figure 1 (a) Representative XRD patterns of Mg substrates, ACP and HAP coatings and (b) Results of a comparative FT-IR study confirming the conversion of ACP to HAP

Figure 2 (a) and (b) TEM images of ACP particles, (c) SEM image of agglomerated ACP particles and (d) ACP tubular structure composed of ACP particles and partially formed plate-like particles.
Figure 3 Growth of ACP tubules over a period of 3 min at voltages of (a) 2, (b) 3, (c) 4, (d) 5, (e) 6 and (f) 7 volts.
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Figure 4 SEM micrographs recording the growth of ACP tubules using the constant voltage procedure (6Volts): (a) and (b) 30 s; (c) 1 min; (d) 3 min; (e) 5 min, and (f) number of tubular structures formed as a function of deposition time.

Figure 5 EDS analysis showing the chemical composition of two separated locations along the length of a typical tube-like structure formed during the electrochemical process.
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Figure 6 Tubule formation mechanism: (a) a typical gas bubble residing on the metal/electrolyte interface, (b) buildup of deposited ACP around the bubble, (c) bubble stream formation, and (d) wall structure formation.

Figure 7 (a) a representative ACP tube-like structure after HAP conversion; (b) enlarged view of urchin-like formation after conversion; (c) enlarged SEM image of substrate surface showing HAP particles, and (d) TEM image of HAP particles formed after conversion.
Growth and Corrosion Behaviour of Amorphous Micrometre Scale Calcium Phosphate Coatings on Magnesium Substrates

Derek Fawcett, Sridevi Brundavanam, Gérard Eddy Jai Poinern*

Murdoch Applied Nanotechnology Research Group, Department of Physics, Energy Studies and Nanotechnology, School of Engineering and Energy, Murdoch University, Murdoch, Western Australia, Australia

Abstract  Amorphous calcium phosphate (ACP) coatings were formed on magnesium substrates via a straightforward electrochemical technique in order to improve the corrosion resistance of the substrates. X-ray diffraction spectroscopy and microscopy techniques were used to investigate the size, morphology, composition and structure of the ACP coatings. Analysis of the ACP coatings revealed the presence of micrometre scale fissures and tubular structures. Despite the presence of these features, the coatings were still capable of significantly reducing the corrosion rate in both PBS and Ringer’s solutions. Ringer’s solution was found to be the most aggressive towards Mg substrates with a corrosion rate of 3.828 mm/yr. However, after electrochemical treatment, the corrosion rate of substrates coated with ACP was reduced to 0.557 mm/yr. The significant improvement in corrosion resistance is a first step in controlling the corrosion rate of biodegradable Mg substrates for potential use in hard tissue applications.

Keywords  Biodegradability, Electrochemical synthesis, Magnesium, Calcium phosphates

1. Introduction

Magnesium (Mg) is a silvery coloured metal that is currently being investigated as a potential biologically degradable implant material for bone tissue engineering. Mg is a lightweight metal with a high strength to weight ratio that also has a number of advantageous properties such as good biocompatibility and biodegradable in the aqueous physiological environment of the body [1]. In terms of mechanical properties, Mg is similar in nature to bone in terms of density and elastic modulus. The density of Mg is around 1.74 g/cm³ at 20 °C, while bone tissue ranges from 1.8 to 2.1 g/cm³. In terms of elastic modulus, bone tissue varies from 40 to 57 GPa and pure Mg around 45 GPa [2, 3]. Interestingly, it is the close similarity between the elastic modules that makes Mg a potential biocompatible material suitable for reducing stress shielding and preventing bone resorption. Stress shielding and the resulting bone resorption are significant problems that can be encountered with conventional metallic implant materials [4]. In addition, unlike Mg, which has been shown to be physiologically beneficial in the body [5, 6], traditional metallic implants can release toxic metallic ions such as chromium, cobalt and nickel. The release of these toxic ions during biological corrosion and mechanical wear often leads to an unfavourable immune response that significantly reduces the biocompatibility of the implant and often leads to secondary revision surgery [7, 8].

However, before Mg can be used in the manufacture of biodegradable orthopaedic implants two fundamental problems need to be addressed and both stem from the metal’s rapid corrosion in chloride rich aqueous body fluids [9]. The first involves the rapid formation of hydrogen gas, which overwhelms surrounding tissues and results in the formation of subcutaneous bubbles [10, 11]. The second problem results from the rapid loss of mechanical strength and structural support between the Mg implant and the surrounding bone tissue that impedes the healing process. Therefore, effectively reducing the corrosion rate would significantly improve the mechanical and structural integrity of the implant for longer periods of time and also reduce the levels of subcutaneous gas formation. One interesting method of improving the corrosion resistance of a susceptible implant material is to coat it with a non-corrosive protective layer that is both biocompatible and bioactive. Bioactive coatings based on calcium phosphates have been successfully applied to coat variety of conventional metal implants in order to improve their biocompatibility and reduce the release of corrosion products into the human body [12].

In this study an amorphous calcium phosphate (ACP)
[Ca$_9$(PO$_4$)$_6$] coatings were formed on magnesium substrates during a straightforward electrochemical synthesis process. The electrochemical cell consisted of two electrodes. The first electrode was a platinum wire mesh (anode) and the second was an Mg substrate that acted as the cathode. Both electrodes were immersed in an electrolyte consisting of calcium nitrate and potassium di-hydrogen phosphate. ACP has a non-crystalline character that lacks long-range periodic uniformity normally associated with crystalline structures and readily precipitates from highly saturated aqueous solutions containing calcium and phosphate ions due to its lower surface energy [13, 14]. It has a spherical structural unit composed of randomly assembled clusters of ions approximately 9.5Å in diameter and also contained within the interstices is tightly bound water, which can be as much as 20% by weight of the particle [15]. It is the metastable properties of ACP in body fluids that makes it more osteoconductive than hydroxyapatite and also gives it superior biodegradability when compared to tri-calcium phosphate [16-18]. And because of its metastable properties, ACP will transform to a more thermodynamically stable crystalline calcium phosphate phase such as hydroxyapatite via a process of dissolution, nucleation, and crystal growth [19].

Advanced characterisation techniques such as X-ray diffraction (XRD) spectroscopy, Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) were used to determine size, morphology, crystalline structure and composition of the ACP coatings formed by the electrochemical process. The corrosion resistance of the coatings was then evaluated in phosphate buffer saline (PBS) solution and Ringer’s solution at body temperature (37°C).

2. Materials and Methods

2.1. Materials

All aqueous solutions were made using Milli-Q® water (18.3 MΩ cm$^{-1}$) produced by an ultrapure water system (Barnstead Ultrapure Water System D11931; Thermo Scientific, Dubuque, IA). All chemicals and reagents used in this study were supplied by Chem-Supply (Australia).

2.2. Surface Pre-treatment of Mg Substrates

High purity (99.9%) Mg foil 0.15 mm in thickness was cut into rectangular strips 40 mm in length and 3 mm in width. Silicon carbide (Si C) paper (1200 grit) was used to remove all surface oxides and contaminants. After initial surface cleaning, the strips were chemically cleaned in 5% wt. nitric acid (HNO$_3$) solution followed by ultrasonically rinsing in acetone for 10 min. This was followed by rinsing with Milli-Q® water to remove acetone and then allowing the substrates to air dry. After drying the weight of each substrate was recorded using an Ohaus PA214C microbalance.

2.3. Electrochemical Synthesis of ACP Coatings

The electrochemical cell was composed of two electrodes and an electrolyte. The electrolyte consisted of an aqueous solution containing 0.32 M of Ca(NO$_3$)$_2$·4H$_2$O and 0.19 M of KH$_2$PO$_4$. The electrodes consisted of a platinum wire mesh (anode) and an Mg substrate, which was used as the cathode. A standard laboratory DC power supply [GW INSTEK GPS-2303] was used to supply the required voltage range with a maximum current density of 60mA/cm$^2$ in the dynamic mode setting with a fixed voltage (6 V) and variable immersion times ranging from 30 s to 5 min.

2.4. Advanced Characterisation of Coatings

2.4.1. X-ray Diffraction (XRD) Spectroscopy

XRD spectroscopy was used to identify the presence of any crystalline phases present in the surface coating formed during electrochemical synthesis. Spectroscopy data was recorded at room temperature, using a GBC® eMMA X-ray Powder Diffractometer [Cu K$\alpha$ = 1.5406 Å radiation source] operating at 35 kV and 28 mA. The diffraction patterns were collected over a 20° range of 20° to 60° with an incremental step size of 0.02° using flat plane geometry with 2 second acquisition time for each scan.

2.4.2. Transmission Electron Microscopy (TEM)

The size and morphology of ACP particles formed during the synthesis process were investigated using TEM. Sample material taken from substrates was placed into small tubes containing Milli-Q® water, sealed and placed into an ultrasonic bath for 10 minutes. Suspensions were then filtered 2 times before a single drop from each sample was placed onto a carbon-coated copper TEM grid using a micropipette. After a 24-hour period drying period, samples were investigated using a Philips CM-100 electron microscope (Phillips Corporation Eindhoven, The Netherlands) operating at 80kV.

2.4.3. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The size and morphology of coating features formed on Mg substrates were examined using SEM, while the elemental composition of the coatings was investigated using EDS. All micrographs were taken using a JCM-6000, NeoScopeTM with attached energy dispersive X-ray spectroscopy. Samples were mounted on individual substrate holders using carbon adhesive tape before being sputter coated with a 2 nm layer of gold to prevent charge build up using a Cressington 208HR High Resolution Sputter coater.

2.5. Corrosion Resistance of ACP Coating

The corrosion resistance of pure Mg substrates and ACP coating substrates was investigated using freshly prepared...
phosphate buffer saline (PBS) solution and Ringer’s solution. The composition of the PBS solution (in g/L) consisted of 8.006 NaCl, 0.201 KCl, 1.420 Na$_2$HPO$_4$ and 0.240 KH$_2$PO$_4$. The Ringer’s solution composition (in g/L) consisted of 8.6 NaCl, 0.6 KCl and 0.66 CaCl$_2$.2H$_2$O. The pH and temperature of both test solutions was maintained at 7.4 and 37ºC respectively so that body fluid pH and temperature could be replicated. An EG&G Princeton Potentiostat/galvanostat (Model 273A supplied EG&G Princeton applied research) using a three-electrode arrangement was used to generate polarization curves. The working electrode was a test substrate with a surface area of 1 cm$^2$. The remaining two electrodes consisted of a saturated calomel electrode (SCE) that was used as the reference electrode, while the counter electrode was a platinum wire (Pt). A Tafel test procedure was performed using a voltage range between -2.5 V and 1.0 V, with a step size of 10 mV and a 1s time interval for the 10 mV scan rate. From the resulting experimental polarization curves the corrosion rate (mm/yr) was calculated.

3. Results and Discussions

3.1. XRD Spectroscopy and TEM Analysis

The XRD technique was used to examine Mg substrates before and after the electrochemical process. Figure 1 presents a representative set of XRD patterns for untreated and treated substrates. Examination of the untreated substrate reveals the presence of six peaks over the 2θ range from 20º to 60º. The pattern contains three major peaks [(002), (101) and (102)] and two minor peaks [(100) and (110)] that correlate to the crystalline structure of the Mg substrate. Also present in the pattern was a minor peak (101) that indicated the presence of Mg(OH)$_2$ on the substrate surface. No other surface contaminants were found on the substrate. Subsequent electrochemical processing of substrates produced a thin white calcium phosphate coating over the surface. The coating was identified as amorphous calcium phosphate due to the presence of a very large broad and diffuse peak centred on the 2θ angle of 25°, characteristic of non-crystalline calcium phosphate materials [20]. However, three peaks could be seen in the XRD pattern [(002), (101) and (102)] and were identified as the underlining Mg substrate.

The inserted TEM image presented in Figure 1 is a representative of the spherical morphology and highly agglomerated nature of the ACP particles that make up the surface coatings of the treated substrates. Particle size analysis revealed that the particles ranged in size from 60 nm up to 250 nm, with a mean particle size of 120 nm.

3.2. SEM and EDS Analysis of Coating Growth

The SEM technique was used to study the growth of the ACP coating thickness during the electrochemical process. The technique was also used to investigate the surface features and structure of the coatings. Figures 2 (b), (c) and (d) present micrographs showing the growth and increasing coating thickness with treatment time. Figure 2 (a) graphically presents the growth in coating thickness and reveals that the growth rate varies during the treatment. At the end of the 5 minute treatment period, a typical coating reached on average a thickness of around 70 µm. Analysis of SEM micrographs of the coatings revealed a granular structure which can be seen throughout the cross-sections. In addition, with treatment time the coating tends to detach from the underlining substrate. Also noticeable were the very rough surfaces of the respective coatings that are exposed to the electrolyte and the presence of tubular surface features.

Further SEM investigation of the coating surface revealed a landscape covered by tubular structures and large fissures. Figure 3 (a) presents a representative landscape...
showing numerous micrometre scale tubular structures formed during the electrochemical treatment. Also seen in Figure 3 (a) are the surface fissures which can be as large as 30 μm in width and zigzag across the surface for many 100’s of μm’s. Many of these fissures, like the tubular structures provide the electrolyte access to the underling Mg substrate. Both the fissures and tubular structures have been formed as a result of hydrogen gas evolution occurring at the metal-coating interface. Figure 3 (b) presents an enlarged view of a representative tubular formation seen in the coating. The central channel diameter of the tube was estimated to be around 45 μm and extended down through the coating to the underlining Mg substrate.

Figure 3 (c) presents the results of the EDS analysis and gives a breakdown of the elemental components present in the ACP coating. The analysis reveals the presence of significant amounts of Ca, P and K and traces of O and Mg. The mean calcium to phosphate ionic ratio (Ca:P) of the coatings was estimated to be 2.1, which is within the 1.2 to 2.2 range specified by Dorozhkin [13] for ACP. Initially, the production of ACP was fairly rapid, however, after 3 minutes the deposition rate significantly reduced as seen in Figure 3 (d). The build-up of ACP after 3 minutes, which equated to 7.6 mg in mass and a thickness of 55 μm was significantly reducing the amount of electrolyte in contact with the underlining substrate and was providing some degree of isolation from the electrolyte. And by the end of the 5 minute treatment period the mass of a representative coating was around 10 mg with a typical thickness of 70 μm. During the 3 to 5 minute treatment period the coating formation rate was around 45% lower than the 0 to 3 minute period. The EDS analysis also found the presence of small amounts of Mg in the coatings that indicate the possibility of Mg$^{2+}$ ions being substituted for Ca$^{2+}$ ions during the formation of the coatings. The small amounts of Mg seen in the EDS analysis may have contributed to, but was not fully responsible for the intense Mg peaks seen in the XRD. These strong Mg peaks were primarily the result of the underlining Mg substrate.

3.3. Corrosion Resistance

The corrosion behaviour of pure Mg substrates and ACP coated Mg substrates were studied by immersing substrates into two types of aqueous mediums and then generate polarization curves using a three-electrode based a Potentiostat/galvanostat device. Both the PBS solution and the Ringers solution were maintained at 37 °C and a pH of 7.4 during the test procedure. Representative polarization curves produced during the corrosion tests for pure Mg substrates and ACP coated substrates are presented in Figure 4. Inspection of the polarization curves of both PBS and Ringer solutions reveals that both solutions are highly corrosive to uncoated substrates. For example in seawater, the corrosion rate is typically around 0.25 mm/yr. [21], the PBS solution was calculated from the Potentiostat/galvanostat device to be 1.829 mm/yr. and Ringers solution was calculated to be 3.828 mm/yr. Clearly from these measurements it is clear that uncoated Mg substrates will rapidly corrode in these fluids without protection and explains why previous attempts of using Mg as an implant have failed to deliver satisfactory clinical outcomes. Ringer’s solution was found to be the most aggressive environment for Mg substrates and this was still the case for the ACP coated substrates. The ACP coating used in the corrosion studies was able to reduce the corrosion rate from 3.828 mm/yr. down to 0.557 mm/yr. A similar trend was also seen for the tests carried out in PBS solution. In this case the original corrosion rate of 1.829 mm/yr. was reduced to 0.142 mm/yr. as listed in Table 1.

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**Figure 3.** (a) SEM micrograph of a typical coating surface landscape; (b) enlarged view of a surface tubular structure; (c) EDS elemental analysis, and (d) deposition rate of ACP coating with time
Table 1. Corrosion rates of uncoated and ACP coated Mg substrates determined from polarization curves produced from corrosion studies carried out in PBS and Ringer’s solution.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Sample</th>
<th>Corrosion Rate (mm/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humid air [21]</td>
<td>Mg substrate</td>
<td>1.0 x 10^{-7}</td>
</tr>
<tr>
<td>Distilled water [21]</td>
<td>Mg substrate</td>
<td>1.5 x 10^{-2}</td>
</tr>
<tr>
<td>Seawater [21]</td>
<td>Mg substrate</td>
<td>0.25</td>
</tr>
<tr>
<td>Phosphate buffer saline solution</td>
<td>Mg substrate</td>
<td>1.829</td>
</tr>
<tr>
<td>(This study)</td>
<td>ACP coated Mg substrate</td>
<td>0.142</td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>Mg substrate</td>
<td>3.828</td>
</tr>
<tr>
<td>(This study)</td>
<td>ACP coated Mg substrate</td>
<td>0.557</td>
</tr>
</tbody>
</table>

The results of the corrosion studies are interesting when you consider the structure of the ACP coating. There is clear evidence that the coating integrity is far from satisfactory, with many micrometre scale surface fissures and tubular structures present as seen in Figure 3 (a). Both of which clearly provide access to the underlining Mg substrate for the respective corrosive solutions. Also seen in SEM micrographs of coating cross-sections is clear evidence that the coating is not securely anchored to the substrate’s surface. Figures 2 (c) and (d) clearly show significant detachment of the coating, with the coating lifting as much as 10 µm from the substrate surface.

The most important property of a biodegradable medical implant material is that it must slowly degrade and allow regenerating bone tissues to progressively take over from the load carrying function of the implant. In the case of Mg, the corrosion rate must be effectively controlled and thus allowing sufficient time for successful tissue regeneration to take place.

However, the rapid corrosion rates for uncoated Mg substrates recorded in this study clearly demonstrate that the mechanical integrity needed during the healing process could not be sustained for any significant length of time. Moreover, the rapid corrosion would produce the evolution of significant amounts of hydrogen gas, which surrounding tissues would find difficult to deal with. In spite of this the corrosion studies of the coated Mg substrates has shown a significant reduction in the corrosion rates for both PBS and Ringer’s solutions. The studies have shown that the ACP coatings formed on the substrate surfaces during the electrochemical treatment have been able to provide some degree of protection from aggressive aqueous environments and reduce the corrosion rate. This result is important since controlling the corrosion rate of biodegradable Mg substrates is the first step in developing Mg based implants for hard tissue applications. It also provides the opportunity to develop biodegradable Mg based implants that have the potential to circumvent the long-term complications normally associated with conventional metal implants currently used in clinical applications. However, further research is needed to improve the quality and mechanical stability of the ACP coatings. In addition, further studies are needed to study the behaviour of the ACP coating in vivo since ACP is a metastable calcium phosphate phase. And as such will easily transform into more thermodynamically stable crystalline calcium phosphate phases such as hydroxyapatite via a process of dissolution, nucleation, and crystal growth [22].

4. Conclusions

A straightforward electrochemical process was used to form ACP coatings on Mg substrates with the objective of improving the corrosion resistance of the substrate. XRD spectroscopy confirmed the formation ACP on the
substrates while TEM image analysis revealed the characteristic spherical morphology normally associated with ACP particles. The image analysis also confirmed the highly agglomerative nature of the particles. SEM micrographs taken during the synthesis process reveal the formation and subsequent growth of micrometre scale tubular structures and surface fissures in the respective coatings. Despite the presence of both features, the coatings were still capable of significantly reducing the corrosion rate in both PBS and Ringer’s solutions. Ringer’s solution was found to be the most aggressive with a substrate corrosion rate of 3.828 mm/yr. However, the substrate corrosion rate was significantly reduced (0.557 mm/yr.) by the presence of a 70 µm ACP coating. The significant improvement in corrosion resistance of ACP coated substrates is an important result and the present study demonstrates the viability of using this electrochemical process to control the corrosion rate. Importantly, this improvement was achieved in spite of numerous tubular structures and surface fissures being produced during coating formation. The disadvantage of the ACP coating technique is unquestionably the presence of surface fissures and tubular structures. Conversely, the coatings advantages include biocompatibility, osteoconductivity, resorption potential and corrosion resistance. However, further studies are needed to build on these advantages and perhaps modifying the current electrochemical process to reduce the coating imperfections. Thus, being able to control the corrosion rate of ACP coated Mg substrates is an important first step in developing a programmable biodegradable Mg based implant for hard tissue applications.

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REFERENCES


6.3. Chapter Summary

The chapter consisted of two cases studies (5 and 6) that examined the formation of ACP coating on Mg substrates via an electrochemical technique. The size, structure and composition of the respective coatings and tube-like surface structures were studied. Analysis of the data was used to propose a possible tube formation mechanism. The proposed mechanism explained the importance of hydrogen evolution in forming the tubes during synthesis. The research also discussed the use of a low temperature hydrothermal process for transforming ACP coating into HAP. Furthermore, the degradation behaviour of pure Mg substrates, ACP and HAP coated substrates were studied in aqueous mediums using a Potentiostat/galvanostat device. The aqueous solutions used were PBS solution and the Ringers solution. The solutions were maintained at 37 °C and a pH of 7.4 during the test procedure. ACP coatings were found to reduce the corrosion rate from 3.83 mm/yr. (pure Mg substrate) down to 0.56 mm/yr. in Ringers solution. And a similar trend was also seen in PBS solutions that reduced significantly the original corrosion rate of 1.83 mm/yr. (pure Mg substrate) down to 0.14 mm/yr.
Chapter 7 General discussions, conclusions and future work

Current metallic biomaterials such as stainless steel, cobalt chromium and titanium alloys are regularly used to replace damaged or diseased bone tissues in clinical load-bearing applications [1]. At the same time, biologically compatible polymers and ceramics with lower mechanical strength and lower fracture toughness are routinely used in low load-bearing applications [2, 3]. Despite the many benefits provided by each of these biomaterials there still exists problems such as unfavourable inflammatory responses, non-uniform tissue integration, unfavourable degradation rates and achieving the appropriate mechanical compliance for long term use. In particular, the release of detrimental metallic ions and the subsequent adverse inflammatory response can significantly reduce the biocompatibility of these materials. Also problems related to stress shielding and bone resorption can also lead to implant failure [4]. And if a metallic implant is needed for short-term temporary support it often needs to be removed by a second surgical procedure that significantly increases health costs. The above mentioned clinical issues clearly demonstrate the need for new biologically compatible materials that are on one hand capable of providing short-term mechanical compliance and structural support during the healing process. And on the other hand are capable of safely degrading with time and hence avoid the issues normally associated with current long term in situ metallic implants. Research results of the present work have successfully shown that calcium phosphate (ACP, DCPD and HAP) coatings can prolong the life of Mg substrates in PBS and Ringers solutions. The results also suggest that calcium phosphate coated Mg substrates have the potential to be used as biodegradable implants. Section 7.1 discusses the research outcomes and achievements
attained during this thesis. The chapter concludes with recommendations for future work.

7.1. Research results and achievements

Research undertaken in the present work was designed to develop viable biodegradable and biocompatible calcium phosphate coatings capable of extending the operational life of Mg substrates. This thesis is at the forefront of developing a practical and effective biodegradable Mg implants for potential bone tissue regeneration and bone tissue engineering applications. The research objectives of the thesis were stated in the four aims presented in section 1.3 and the subsequent research outcomes were presented as series of Case Studies.

Chapter 2 presented a comprehensive literature review composed of two articles that present the current state of research into using Mg based materials for potential biomedical applications. The first article presented a wide-ranging review of material properties, biological corrosion mechanisms, alloying elements, surface modifications and treatments to control the biodegradability of Mg substrates. The second review article focused on using chemical immersion techniques to produce calcium phosphate coatings with the potential to reduce biological degradation of Mg based substrates. The article was of particular importance since it demonstrated how a straightforward wet chemical based technique could be used to form calcium phosphate coatings on Mg substrates. The article also presented some preliminary results that showed a significant improvement in corrosion resistance of DCPD coated Mg substrates when immersed in a PBS solution with a pH of 7.4 and a solution temperature of 37 ºC.
Chapter 3 focused on chemically synthesizing HAP in sufficient quantities to undertake a number of metal ion adsorption studies. Therefore, Aim 1 of the present work was to optimise the existing MANRG procedure to produce the necessary quantities of HAP needed for adsorption studies. Also presented in Chapter 3 was a new synthesis technique for producing nanometre scale hydroxyapatite powders directly from calcium hydroxide and phosphoric acid. The new process (Case Study 1) was able to produce particle sizes ranging from 12.2 nm to 15.4 nm depending on the ultrasonic power used. The synthesis process was capable of producing significant quantities of HAP and has the potential to be scaled up to produce larger quantities.

The main thrust of Chapter 4 was to address the objective of Aim 2, which was to investigate the chemical adsorption capabilities of a highly desirable biocompatible HAP coating material. The results of these studies form the basis of Case Studies 2 & 3. The adsorption studies were undertaken to investigate how effective HAP’s complex hexagonal structure could accumulate metallic ions like the mineral phase found in bone tissue. The adsorption studies were designed to model the adsorption behaviour of HAP towards a number of metal ions (Cd$^{2+}$, Fe$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$) from aqueous solutions and give an indication of metal ion adsorption capacity of a typical HAP coating. This is of particular importance because the coating must permit the transfer of metal ions during substrate degradation. In the case of Mg, besides being biologically degradable, it is also biologically absorbable and its corrosion products are considered physiologically beneficial. For example, the body uses Mg in a number of metabolic processes and to form apatite in the bone matrix [5, 6]. Other beneficial metal ions, in small quantities include Cu$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$. However, not all metal ions are beneficial. For example, Cd$^{2+}$ ions readily exchange with Ca$^{2+}$ ions in bone and results in a number of bone
tissue related diseases as discussed in Case Study 2. The results discussed in Case Study 2 revealed that HAP was indeed an effective adsorbent for the removal of Cd\(^{2+}\) ions from aqueous solutions. The sorption process was endothermic and spontaneous in nature and followed pseudo-second order kinetics and increased with temperature. Importantly, the study identified intra-particle diffusion and ion-exchange (Cd\(^{2+}\) → Ca\(^{2+}\)) as the major participants in the sorption process. While the maximum adsorption capacity for Cd\(^{2+}\) ions was found to be 123.45 mg/g. Case Study 3 also confirmed intra-particle diffusion and ion-exchange (metal ion → Ca\(^{2+}\)) were major participants in the sorption process for Cu\(^{2+}\), Fe\(^{2+}\) and Zn\(^{2+}\) ions. Fe\(^{2+}\) and Cu\(^{2+}\) ions closely followed pseudo-second order kinetics and Zn\(^{2+}\) ions tended to follow intra-particle kinetic diffusion. The maximum adsorption capacities were found to be 61.35 mg/g for Cu\(^{2+}\) ions, 55.25 mg/g for Fe\(^{2+}\) ions and 48.54 mg/g for Zn\(^{2+}\) ions. Both case studies have confirmed that HAP’s complex hexagonal structure tends to behave in a similar way to the mineral component found in bone. The results suggest that when HAP is used as a coating for Mg substrates it will tend to behave in a similar manner as the mineral component found in the bone matrix. However, future _in vivo_ studies in the appropriate physiological environment would need to be carried out to fully quantity coating adsorption, diffusion and desorption processes _in situ_. This aspect of future research will be discussed at length in section 7.2.

Chapter 5 presented Case Study 4, which discussed a straightforward chemical immersion technique and subsequent low temperature thermal treatment to produce calcium phosphate coatings on Mg substrates. The study also examined the corrosion resistance of the respective coatings (DCPD and HAP) in PBS and Ringer’s solutions. The case study addresses two components detailed in aims 3 and 4 of the present work.
The first component, listed in aim 3 was to develop and use a chemical immersion technique to produce calcium phosphate coatings on Mg substrates. The second, listed in aim 4 was to study the degradation behaviour of each coating type in PBS and Ringer’s solutions. The chemical immersion technique successfully produced DCPD coatings that significantly improved the corrosion resistance of Mg substrates. The degradation studies revealed that the corrosion rate was reduced from 1.83 mm/yr. for pure Mg down to 0.13 mm/yr. for DCPD coated substrates in PBS solutions. Whereas degradation studies carried out in Ringer’s solution reduced the corrosion rate from 3.83 mm/yr. for pure Mg down to 0.1 mm/yr. for DCPD coated substrates. HAP coatings produced via the low-temperature hydrothermal process from DCPD were also found to significantly reduce the corrosion rate. In PBS solutions, the corrosion rate was reduced from 1.83 mm/yr. for pure Mg down to 0.28 mm/yr. for HAP coated substrates. While in Ringer’s solution, the corrosion rate was reduced from 3.83 mm/yr. for pure Mg down to 0.26 mm/yr. for HAP coated substrates. These results have clearly demonstrated that both DCPD and HAP coatings are capable of significantly slowing down the degradation rate of Mg. It is also evident from these studies that both DCPD and HAP coatings have the ability to prolong the survive-ability of Mg substrates. However, further in vivo studies are needed to fully investigate degradation processes operating in the physiological environment and their long-term effect on DCPD and HAP coated Mg implants. This is of particular importance since the Mg implant is not intended for long-term placement. Its goal is to slowly degrade and be replaced by regenerating bone tissue as discussed in future research section 7.2.

Chapter 6 investigated an electrochemical technique that was used to produce ACP and HAP coatings on Mg substrates. The chapter addresses two components outlined in
The first component discussed in aim 3 was to develop new and straightforward techniques to synthesize calcium phosphate coatings. In this case an electrochemical technique was used to produce ACP coating on Mg substrates. In addition, the ACP coatings were subsequently converted into HAP coating via immersion in a sodium hydroxide solution bath at 80 °C for 2h. The second component that formed part of aim 4 was to undertake degradation studies to investigate the corrosion resistance of the ACP and HAP coating in PBS solution and Ringer’s solution set at 37 °C and pH 7.4 to simulate body fluid conditions. Each respective component forms one of the two cases studies. Both case studies examine the various aspects of ACP formation and deposition on Mg substrates via the electrochemical technique. Case Study 5 studied the size, structure and composition of the coating and the interesting tube-like surface structures via TEM, SEM, EDS and XRD. The results of the advanced characterisation techniques were used to propose a possible formation mechanism that was responsible for the formation of the tube-like surface structures during the electrochemical process. Importantly, the proposed formation mechanism explained the significance of hydrogen evolution in modelling the architecture of the tube-like structures as seen in Figure 7.1. Case Study 5 also investigated a low temperature hydrothermal process for transforming ACP coating structures into HAP. The two-step process consisted of first immersing ACP coated substrates into a 1M solution of sodium hydroxide at 80 °C for 2 h. In the second step the substrates were thermal treated for 1h in a standard furnace at 250 °C. Subsequent XRD analysis confirmed the conversion process and also revealed the presence Mg. The strong Mg signal in the XRD spectrum suggests that not only was Mg present in the coating, but the underlining Mg substrate was also detected. SEM and EDS studies also revealed the presence of extensive cracking in both the surface coatings and underlining substrate
surfaces. The extensive cracking is believed to be responsible for the release of Mg ions and their subsequent incorporation into the forming surface structures.

![Diagram](image)

Figure 7.1 (a) a typical gas bubble residing on the metal/electrolyte interface, (b) build-up of deposited ACP around the bubble, (c) bubble stream formation and (d) wall structure formation of ACP.

Case study 6 specifically focused on the coating thickness, mass deposition, coverage, and appraised the degree of protection or corrosion resistance given by ACP coatings to the underlining Mg substrate. The corrosion/degradation behaviour was studied in aqueous based mediums using a Potentiostat/galvanostat device. The two aqueous based medium used during the degradation studies were PBS solution and Ringers solution. Both solutions were maintained at 37 °C and a pH of 7.4 during the test procedure. Despite the presence of numerous tube-like structures covering the Mg substrate, a 70 µm thick ACP layer could reduce the corrosion rate of 3.83 mm/yr. for pure Mg
substrates down to 0.56 mm/yr. in Ringers solution. A similar trend was also seen for tests carried out in PBS solution. In a PBS solution the corrosion rate for a pure Mg substrate was found to be 1.83 mm/yr. However, the presence of a 70 µm thick ACP layer was able to reduce the corrosion rate down to 0.14 mm/yr. In spite of the promising reduction in the corrosion rate provided by the ACP coatings, SEM cross-sectional images of the coatings revealed regions were the coating was detached from the underlining substrate. The presence of coating detachment is of concern and suggests that further studies are needed to determine coating attachment integrity. The electrochemical process is capable of producing coatings with the potential to increase corrosion resistance and reduce Mg degradation. Nevertheless, the presence of coating detachment over the substrate and the existence of tube-like structures, which extend down to the underlining substrate are of concern. Both Case Study 5 and 6 have shown that the electrochemical process in its present form was unsuitable for producing impervious surface coatings. The presence of tube-like structures that give direct access to the underlining substrate weakens the mechanical integrity of the deposited coating and makes this technique unsuitable for producing a stable effective chemical barrier between the substrate and a surrounding fluid environment. Case Study 4 discussed earlier, using chemical immersion and a subsequent low temperature thermal treatment produced a superior coating. Both DCPD and HAP coatings both provided an effective barrier between the underlining Mg substrate and the surrounding fluid environment during degradation studies. Both coatings also had a much higher degree of surface attachment integrity than the ACP coatings seen in Case Studies 5 and 6. Further studies are of course needed, but the results of the present work have clearly indicates that chemical immersion was capable of producing a superior coating. Therefore, optimising the chemical immersion technique and the subsequent low temperature thermal
treatment developed in Case Study 4 offers the best method of producing a high quality, controllable and mechanically stable surface coating on Mg substrates. However, we still need to investigate better techniques to improve the degradation protection of HAP coated Mg substrate.

7.2 Recommendations for future work

Research undertaken in this thesis was highly successful in developing viable DCPD and HAP coatings capable of significantly slowing down the degradation rate of Mg substrates in PBS and Ringer’s solutions. The research has clearly demonstrated that a chemical immersion technique and subsequent low temperature thermal treatment could produce structurally stable coatings capable of prolonging the survive-ability of Mg substrates in both test solutions. Evaluation and subsequent analysis of the various physical and chemical properties of each respective coating has revealed that they were mechanically robust, structurally stable and similar in composition to the mineral component found in bone tissue. Therefore, the next logical step in developing a viable biodegradable Mg implant using the coatings produced in the present work would be to conduct a detailed mechanical characterisation to study the adhesion strength between coatings and the metal substrate and to evaluate the biological compatibility of the respective coatings via a series of in vitro cell line studies. This is of particular importance since it would establish the biocompatibility of the coatings towards cell types commonly found in the physiological environment. Initially, the in vitro studies would need to focus on cells directly associated with bone tissues such as osteoblasts and osteoclasts. A second series of in vitro studies would also need to be carried out to establish the wider biological compatibility of the coatings towards other cell types.
found in body tissues. This will be very important, since the coatings should be non-toxic and non-immunogenic towards all cell types.

In due course, if the *in vitro* studies were positive, the next step would be to undertake *in vivo* studies in an appropriate animal model. The subsequent *in vivo* studies would be able to give definitive data regarding biological compatibility and coated substrate degradation rates in a physiological environment. The advantage of being able to balance the degradation rate is that it will allow bone cells to steadily replace the coated substrate and at the same time promote the formation of bone tissues and blood vessels necessary for the delivery of nutrients.

Another avenue for future research would be to investigate using the coatings for the slow release of pharmaceutical products. The adsorptions studies carried out in the present work have shown that the HAP coatings were an effective absorber of metallic ions. Reports in the literature reveal that HAPs structural matrix can store and deliver a variety of pharmaceutical products such as antibiotics, drugs, enzymes, hormones and steroids. For example, both HAP-antibiotic and HAP-drug based delivery platforms have been successfully used in slow release of pharmaceutical products [7-9]. In particular, the slow and sustained release of pharmaceuticals has been shown to be effective in the treatment of diseases such as osteomyelitis, osteoporosis and osseous cancer [10]. Therefore, future studies investigating the use of the substrate coating for the slow and sustained release of pharmaceuticals during the healing process has therapeutic benefits.
An additional route for future research would be to further investigate the electrochemical method developed in Chapter 6. Despite the technique being unable to produce a structurally stable and impervious chemical barrier, the technique did produce coatings that were able to improve the corrosion resistance of the underlying Mg substrates. At present the electrochemical technique used only a low voltage DC power supply. However, an AC or pulsed DC power supply could be investigated in the future and may be able to deliver an impervious and stable coating. Also, it would be interesting to compare the metal adsorption data of the synthesised powdered nano HAP with nano HAP deposited on Mg substrate.

The results of the present research have successfully shown that calcium phosphate (DCPD and HAP) coatings have the capacity to slow down the degradation rate of Mg substrates. These coating materials have demonstrated that it is possible for a coated Mg substrate to safely degrade over extended periods of time and hence avoid the issues normally associated with current long term in situ metallic implants. Importantly, the coated substrates have the potential to biologically degrade at a controlled rate and allow regenerating bone tissues to progressively replace the substrate. Therefore, the next step in developing a viable Mg based biodegradable metallic implant using the calcium phosphate coatings developed in the present work would be a series of in vitro studies to establish material compatibility with a wide range of cell lines. This would be followed by in vivo studies that would establish the biocompatibility and degradation behaviour of the coated Mg substrates in a suitable animal model. The results of the future studies could ultimately lead to clinical application of Mg based biodegradable metallic implants.
References


