EFFECTS OF SODIUM HYDROXIDE AND POLYDOPAMINE PRE-TREATMENT AND CALCIUM PHOSPHATE COATING ON THE PROPERTIES OF BIODEGRADABLE MAGNESIUM

MAHTAB ASSADIAN

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I would like to dedicate this thesis to my mother for her selflessness, my father for his encouragement, and my brother for his indulgence.

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ABSTRACT

Magnesium (Mg) has shown great promise as a potential biocompatible and biodegradable implant material. Some of its unique properties include high strength/weight ratio and closer elastic modulus to that of the human bone. However, in a pure state, its in-vivo corrosion is too rapid to be used for implants. Hydrogen evolution during degradation leads to the elevation of body fluid pH which causes infection or inflammation and delaying tissue healing process. Despite many studies to improve the corrosion resistance of pure Mg, reports on sodium hydroxide (NaOH) and polydopamine (PDA) pre-treated Mg followed by calcium-phosphate (Ca-P) coating using electrodeposition (ED) technique are hardly found in the literature especially fluoridated hydroxyapatite (FHA) coating. This research is aimed to improve the corrosion resistance of Mg by NaOH and PDA pre-treatment followed by different phases of Ca-P coatings. In the first stage, pure Mg was pre-treated with NaOH (1M, 30 minutes) and PDA (2 mg/ml in 10 mM Tris buffer, pH 8.5). In the second stage, different phases of Ca-P were coated on the pre-treated specimens using ED technique with two different electrolytes at current density equal to 1 mA/cm^2 for 60 minutes at room temperature. The pre-treated and coated specimens were analysed using X-ray photoelectron spectroscopy, grazing incidence X-ray diffraction, attenuated total reflectance-Fourier transform infrared spectroscopy, scanning electron microscopy, energy dispersive X-ray spectroscopy and optical microscopy. The specimens were also evaluated on their surface roughness, water contact angle and scratch hardness. In addition, corrosion behaviours of specimens were analysed using potentiodynamic polarization and in-vitro immersion tests. Results in the first stage showed that both pre-treatments decreased the corrosion rate of Mg, in particular coated substrates with PDA by almost 27 folds. PDA pre-treatment also improved surface properties by reducing water contact angle and increasing surface roughness by 2.3 and 4.5 folds respectively as compared to pure Mg. In the second stage, the results showed that dicalcium-phosphate dihydrate (DCPD) and FHA were formed on NaOH pre-treated specimens. It was found that DCPD coatings required post-treatment to convert DCPD to hydroxyapatite (HA). However, HA and FHA were able to be deposited directly on the PDA pre-treated specimens without requiring any post-treatment. In terms of adhesion strength between the Ca-P coatings and the substrates, PDA pre-treatment specimens were superior than NaOH pre-treatment. Comparing between FHA and HA coatings on both pre-treatments, FHA coated specimens demonstrated higher corrosion resistance and surface roughness. It is believed that by introducing fluorine into the coating, it stabilizes and increases the crystalline structure of FHA. The corrosion resistance of FHA-PDA coated Mg improved significantly (approximately 62 folds) as compared to uncoated pure Mg.

ABSTRAK

Magnesium (Mg) telah menunjukkan potensi besar sebagai bahan implan bioserasi dan biodegradasi. Antara ciri-ciri unik magnesium termasuklah nisbah kekuatan/berat yang tinggi dan modulus elastik yang hampir sama dengan tulang manusia. Walaubagaimanapun, kakisan *in-vivo* Mg tulen terlalu pantas untuk digunakan sebagai implan. Pembebasan hidrogen semasa degradasi menjurus kepada peningkatan pH bendalir badan yang menyebabkan jangkitan atau inflamasi dan melambatkan proses penyembuhan tisu. Walaupun terdapat banyak kajian untuk meningkatkan ketahanan kakisan Mg tulen, namun laporan tentang pra-rawat Mg dengan sodium hidroksida (NaOH) dan polidopamin (PDA) diikuti salutan kalsium fosfat (Ca-P) menggunakan teknik elektroenapan (ED) sukar untuk dijumpai dalam literatur terutamanya salutan hidroksiapatit berflorida (FHA). Kajian ini bertujuan untuk meningkatkan rintangan kakisan Mg melalui pra-rawat NaOH dan PDA diikuti dengan salutan Ca-P dengan fasa berbeza. Dalam peringkat pertama, Mg tulen telah dipra-rawat dengan NaOH (1M, 30 minit) dan PDA (2 mg/ml dalam 10 mM Tris penampan, pH 8.5). Dalam peringkat kedua, Ca-P dengan fasa berbeza telah disalut pada spesimen pra-rawat menggunakan kaedah ED dengan dua elektrolit yang berbeza pada ketumpatan arus bersamaan dengan 1 mA/cm^2 selama 60 minit pada suhu bilik. Spesimen pra-rawatan dan yang disalut telah dianalisis dengan menggunakan spektroskop fotoelektron sinar-X, pembelauan sinar-X, pantulan lemah-spektroskop inframerah Fourier, mikroskop imbasan elektron, spektroskop tenaga serakan sinar-X dan mikroskop optik. Kekasaran permukaan, sudut sentuh dan kekerasan gores spesimen turut dinilai. Sebagai tambahan, tingkah laku kakisan spesimen telah dianalisis dengan menggunakan ujian polarisasi upayadinamik dan rendaman *in-vitro*. Keputusan peringkat pertama menunjukkan kedua-dua pra-rawatan mengurangkan kadar kakisan Mg, khususnya yang disalut dengan PDA, sehingga hampir 27 kali ganda. Pra-rawatan PDA juga menambah baik sifat permukaan dengan mengurangkan sudut sentuh sehingga 2.3 kali ganda dan meningkatkan kekasaran permukaan sehingga 4.5 kali ganda. Dalam peringkat kedua, keputusan menunjukkan dikalsium fosfat dihidrat (DCPD) dan FHA telah terbentuk pada spesimen pra-rawat natrium hidroksida. Didapati bahawa salutan DCPD memerlukan pasca-rawatan untuk menukar DCPD ke hidrokisapatit (HA). Walaubagaimanapun, HA dan FHA boleh dienap secara terus ke atas spesimen pra-rawat PDA tanpa memerlukan sebarang pasca-rawatan. Dari segi kekuatan lekatan antara salutan Ca-P dan substrat, spesimen pra-rawatan PDA lebih baik daripada pra-rawatan NaOH. Perbandingan antara salutan FHA dan HA terhadap kedua-dua pra-rawatan mendapati spesimen yang disalut dengan FHA menunjukkan ketahanan kakisan dan kekasaran permukaan yang tinggi. Adalah dipercayai bahawa dengan mencampurkan florin ke dalam salutan dapat menstabilkan dan meningkatkan struktur kristal FHA. Ketahanan kakisan Mg yang disalut dengan FHA-PDA telah meningkat dengan ketara (hampir 62 kali ganda) jika dibandingkan dengan Mg tulen yang tidak disalut.

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LIST OF ABBREVIATIONS

AAS - Atomic Absorption Spectroscopy
AC - Alternating Current
ACP - Amorphous Calcium Phosphate
Ag_2CrO_4 - Silver Chromate
aq - Aqueous
ASTM - American Society for Testing and Materials
ATR-FTIR - Attenuated Total Reflectance-Fourier Transform Infrared
Ca-P - Calcium Phosphate
CR - Corrosion Rate
CrO_3 - Chromium Trioxide
CS - Cefotaxime Sodium
DA-HCl - Dopamine Hydrochloride
DC - Direct Current
DCM - Dichloromethane
DCPD - Dicalcium Phosphate Dihydrate
DCPD-NaOH - Dicalcium Phosphate Dihydrate Which Was Coated on Naoh Pre-treated Specimen
ED - Electrodeposition
EDS - Energy Dispersive X-Ray Spectroscopy
EPD - Electrophoretic Deposition
ePTFE - Expanded Polytetraflouroethylene
FA - Fluorapatite
FHA - Fluoridated Hydroxyapatite
FHA-NaOH - Fluoridated Hydroxyapatite Which Was Coated on Naoh Pre-treated Specimen
FHA-PDA - Fluoridated Hydroxyapatite Which Was Coated on Polydopamine Pre-treated Specimen
GIXRD - Grazing Incidence X-Ray Diffraction
gr - Gram

HA	-	Hydroxyapatite
HA-NaOH	-	Hydroxyapatite Which Was Coated on Naoh Pre-treated Specimen
HA-PDA	-	Hydroxyapatite Which Was Coated on Polydopamine Pre-treated Specimen
Hs	-	Scratch Hardness
H_2O	-	Water (Dihydrogen Monoxide)
JCPDS-ICDD	-	The Joint Committee on Powder Diffraction Standards-International Centre for Diffraction Data
MAO	-	Micro Arc Oxidation
Mg	-	Magnesium
mN	-	Mill Newton
NaOH	-	Sodium Hydroxide
OCP	-	Octacalcium Phosphate
Pa	-	Pascal
PDA	-	Polydopamine
PDO	-	Poly (P-Dioxanone)
PET	-	Polyethylene Terephthalate
PGA	-	Polyglycolide
PLA	-	Polylactide
PLGA	-	Poly-Lactic-Co-Glycolic Acid
PCL	-	Poly-Caprolactone
PLLA	-	Poly-L-Lactic Acid
PTMC	-	Polytrimethylenecarbonate
PUR	-	Polyurethane
QDs	-	Quantum Dots
Ra	-	Arithmetic Average of Absolute Values of The Roughness
SAMs	-	Self-Assembled Monolayers
SBF	-	Simulated Body Fluids
$S\ cm^{-1}$	-	Siemens Per Centimetre
Si	-	Silicon
SS	-	Stainless Steel
TCP	-	Tricalcium Phosphate
UHMWPE	-	Ultrahigh Molecular Weight Polyethylene

WHO	-	World Health Organization
WE43	-	Mg Based Alloy With Y 4%, Nd 2.25%, 0.15% Zr
XPS	-	X-Ray Photoelectron Spectroscopy

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Replacing body parts is not new knowledge. The Etruscans, inhabitant of Etruria, replaced body parts like missing teeth. They used oxen bone as carved artificial teeth (Taba *et al.*, 2005). The use of biomaterials as a new science is about 100 years old, when different type of metal depends on applications and properties used for bone fracture fixation in 1895 (Hermawan *et al.*, 2011). One definition of biomaterials is any materials that interact with biological system or biomedical device which performs, replaces or restore a natural function to a body tissue (Xin *et al.*, 2011). Generally biocompatibility, cytotoxicity, and basic structure-properties are the important concern for biomaterials field.

A wide range of materials, like metals, ceramics, polymers or natural material is used as biomedical materials or biomaterials (Muhonen, 2008). Metals and their alloys have been used widely as orthopaedic implants and bone graft substitutes, due to their high strength (elastic modulus larger than 100 GPa), fatigue and ductility; reasonable corrosion resistance and biocompatibility. Although, permanent metallic materials such as titanium alloys, stainless steel and cobalt-based alloys have important role in hard tissue implants, particularly in load-bearing applications (Wang *et al.*, 2012a), they have several limitations, which include proportionately higher elastic modulus compared to natural bone that has effect on new bone growth and potential of releasing corrosion products and even metallic ions into the body from these materials. Moreover, these kinds of implants (permanent implants) do not degrade spontaneously into the human body (Chiu *et al.*, 2007).

In this sense, biometals with sufficient degradation rate and adequate tensile strength are attractive candidates as hard tissue (bone, dentine, and dental enamel) repairing implants. Biodegradable implants support tissue and after revival and healing of the tissue, degrade and replace by healed tissues (Witte *et al.*, 2008) . Metals like magnesium (Mg) show potential for this purpose, especially as compared to biodegradable polymers such as polylactic acid and polyglycolic acid due to the poor mechanical properties of the latter (Tschon *et al.*, 2009; Witte *et al.*, 2006).

Mg alloys have received a lot of interest in recent years. First of all by resorbing spontaneously, they reduce the cost and patient morbidity and infection by avoiding an implant removal surgery. Except admirable mechanical properties like high tensile and compressive strength, an elastic modulus that is closest to the human bone, Mg is a natural component of the body with noteworthy functions in human metabolism (Vormann, 2003). 1 mol (24g) of Mg can be found in human body. Mg is cofactor for many enzymatic reactions and metabolism processes like protein synthesis and stabilization of DNA and RNA (Hartwig, 2001; Staiger *et al.*, 2006). Biocompatibility of Mg has been reported by several researchers (Henderson *et al.*, 2013; Willbold *et al.*, 2013; Witte *et al.*, 2006; Witte *et al.*, 2005; Witte *et al.*, 2007b). Without any toxicity, irritation and allergy, releasing Mg during degradation even have beneficial effect like enhance new bone formation (Saris *et al.*, 2000; Xu *et al.*, 2007).

The main challenge for Mg is corrosion kinetics in vivo that is faster than bone healing. Rapid release of degradation products, hydrogen gas production and gas bubble formation postpone tissue healing (Staiger *et al.*, 2006). Inasmuch, various methods including purification, alloying, anodising, and surface coating are utilized for improving degradation resistance of Mg implants (Dorozhkin, 2014). Except the mechanical properties, biocompatibility may be affected by alloying due to toxicity potential of elements (Witte, 2010; Wong *et al.*, 2010).

Surface treatment and coating can improve corrosion resistance (Abdal-hay *et al.*, 2013; Jamesh *et al.*, 2012). Various methods like electroless coating (Ambat and Zhou, 2004), biomimetic coating (Yanovska *et al.*, 2012a), laser surface melting (Guan *et al.*, 2009), laser surface cladding (Jun *et al.*, 2006) plasma spraying (Yang *et al.*, 2010b), pulse laser deposition (Khandelwal *et al.*, 2013), sol–gel (Jafari *et al.*, 2013), electroplating (Zhu *et al.*, 2006) and electrophoretic deposition (Jamesh *et al.*, 2012) are used for coating different materials on Mg. Electrodeposition (ED) with benefits like capability of coating complex-shaped implants and simplicity in instrumentation is a capable technique to enhance the corrosion resistance as well as biocompatibility. This method has been used for coating ceramics such as HA onto metal like titanium or stainless steel, but there is lack of study for coating ceramics on Mg by ED (Tian and Liu, 2014).

In orthopaedic area, calcium phosphate (Ca-P) bioceramics have been applied because of osseointegration and biocompatibility. Among different types of Ca-P phases as dicalcium phosphate dihydrate (brushite, DCPD, $CaHPO_4 . 2H_2O$), Anhydrous dicalcium phosphate (monetite, ADCP, $CaHPO_4$) octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6 . 5H_2O$) and tricalcium phosphate (whitlockite, TCP, $Ca_3(PO_4)_2$). However, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and fluoridated hydroxyapatite (FHA, $Ca_{10}(PO_4)_6F_x(OH)_{2-x}$) are of greater interest because of their stability and bioactivity (Metoki *et al.*, 2014; Shadanbaz and Dias, 2012). Moreover, FHA has higher stability and lower solubility compared to HA (Bianco *et al.*, 2010; Roche and Stanton, 2014). In fact, fluoride (F^-) is an essential element in human body and trace amount of fluoride can help mineralisation and crystallization of Ca-P to forming new bone and regeneration of osteoblastic cells (Chen and Miao, 2005; Kim *et al.*, 2004; Meng *et al.*, 2011).

However, bonding between Ca-P coating and metallic substrate is poor and ready to crack and also Ca-P ceramic layer cannot sufficiently postpone Mg degradation in aggressive media (Bai *et al.*, 2010; Iqbal *et al.*, 2012). Therefore, other layers like polymer binders could be applied to improve adhesion and corrosion resistance. (Zhitomirsky *et al.*, 2009). Biocompatible polymers are used to enhance the adhesion and corrosion resistance of Mg. Recently, surface properties are modified based on mussel adhesive mechanism (Zhang *et al.*, 2013). Polydopamine with catechol and amine groups is appropriate candidate for conjugation of Ca to substrate (Lee *et al.*, 2007).

Owing to the potentiality of dopamine in biomedical application, it is rarely reported about coating of dopamine film on metal implant surface. Therefore, the combination of dopamine film and Ca-P phases becomes an innovative coating on Mg surface.

1.2 Statement of the Problem

Mg has desirable properties as a biodegradable metal. However the problem of using Mg as implant is its high corrosion rate. Rapid degradation of Mg implants would emit high volume of hydrogen leading to swelling of tissue surrounding implant (Hiromoto *et al.*, 2015). In addition, it would alkalinise the physiological environment adjacent implantation spot that causes inflammation (Wu *et al.*, 2013). Most importantly, it would descend the integrity of the implant and weaken its mechanical strength in long term usage (Dorozhkin, 2014). As such, effort to increase the corrosion resistance of Mg for maintaining the mechanical properties during bone healing is essential. It has been reported that the healing time for untreated implants is higher than that for implants with treated surfaces (Shadanbaz and Dias, 2012).

Coating of calcium-phosphate has been widely applied on metallic implants for reducing corrosion rate as well as improving osseointegration of bone. Prior to coating, magnesium implants have been pre-treated with various methods, i.e. 1) acid solution such as hydrofluoric acid (Meng *et al.*, 2011), 2) alkaline solution such as sodium hydroxide at different temperatures (25 – 100 ˚C) (Grubač *et al.*, 2013) and concentrations (1-10 M) (Weng and Webster, 2012) and 3) polymer solution such as polydopamine (Chen *et al.*, 2015). These pre-treatments has demonstrated some success with improvement in corrosion resistance, good homogeneity and adhesion of apatite coating. Various coating techniques have been reported for coating calcium-phosphate on magnesium such as sol-gel (Rojaee *et al.*, 2013a), dip coating (Abdal-hay *et al.*, 2012) and electrodeposition (Guan *et al.*, 2012). Compared to other coating techniques, electrodeposition has several advantages which includes controlled coating thickness and homogeneity, cheaper technique and able to be conducted at low temperature (Yang *et al.*, 2010a). Despite many studies, reports on sodium hydroxide and polydopamine pre-treated magnesium followed by calcium-phosphate coating using electrodeposition technique are hardly found in the literature especially fluoridated hydroxyapatite coating. As such, the wettability properties, roughness, corrosion behaviours, and scratch hardness are unknown.

1.3 Research Objectives

The objectives of this research are:

1. To investigate the effects of pre-treatments on the corrosion behaviour of Mg.

2. To investigate the effects of electrodeposited fluoridated hydroxyapatite and hydroxyapatite coatings on corrosion behaviour of Mg.

3. To characterise pure Mg pre-treated with NaOH and PDA and coated with fluoridated hydroxyapatite and hydroxyapatite.

1.4 Research Scopes

The research was conducted within the following scopes:

i. Pure Mg was used as the substrate and it was pre-treated with sodium hydroxide (NaOH) and polydopamine (PDA). The concentration for NaOH was limited to 1M and the immersion time was fixed to 30 minutes. Dopamine solution was prepared using 2 mg/ml in 10 mM Tris-HCl and the pH was fixed at 8.5 in 24 hours immersion. The pre-treatment was conducted at room temperature.

ii. The surface morphology, wettability properties, roughness, corrosion behaviours were investigated before and after pre-treatments.

iii. Electrodeposition (ED) method was used to coat DCPD, HA, FHA on pre-treated Mg. The deposition was carried out using current density of 1 mA/cm^2 for 60 minutes at room temperature.

iv. The coated specimens were analysed under X-Ray photoelectron spectroscopy, grazing incidence X-ray diffraction, attenuated total reflectance-Fourier transformed infrared spectroscopy, scanning electron microscopy, and energy dispersive X-ray spectroscopy, optical microscopy.

v. Ca-P coated specimen properties were examined in terms of scratch hardness, roughness and wettability.

vi. The corrosion behaviour of the uncoated and coated specimens were investigated using potentiodynamic polarization and immersion tests. Immersion test was conducted in simulated body fluids (SBF) for 2 weeks to evaluate the pH value, weight loss and ion concentration of Mg.

1.5 Importance of Research

Recently, Mg is regarded as a biodegradable material, has attracted much attentions in biomedical applications due to its potential in eliminating revision surgery after implantation. It would also be able to avoid inflammation between the tissue and implant. However, high degradation rate of this biodegradable material still a major concern that restricts its applications. Improvements in surface treatment and coating technique are expected to reduce corrosion rate as well as expedites the healing time and promoting new bone growth. In addition, the overall cost of implant will reduce with decreasing unnecessary infection and patient morbidity. In general, the outcome of this study indirectly increase the sustainability aspect of biodegradable implant.

1.6 Organization of the Thesis

This thesis consists of five chapters which explain the related concepts to the topic as well as the achievements. Chapter one gives an overview of the current research like the statement of the problem, objectives, and scopes of the study as well as the research importance. Chapter two reviews the literature on biomaterials, classification of them and their properties. This is followed by focus in biodegradable magnesium and surface coatings. Chapter three presents the methodology of research and explains sample preparations and tests. It begins with experimental methodology and is followed by the instruments which are used. Chapter four demonstrates and discusses findings of the experimental works. The relevant information regarding the specimens and analysis are provided. In chapter five the findings of the research are concluded. Moreover, the suggested studies are recommended for further investigations.

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