SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE MAGNESIUM ZINC ALLOY REINFORCED WITH CARBON NANOFIBER FOR POTENTIAL MEDICAL IMPLANT APPLICATIONS

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DEDICATION

This thesis is dedicated to my beloved parents

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ABSTRACT

Magnesium (Mg) is becoming a potential material to replace conventional stainless-steel and titanium alloy medical implants. However, non-adequate mechanical stability of Mg could lead to premature failure and corrosion. Carbon nano fiber (CNF) has revolutionised the composite industries and continue to show a great promise in improving the mechanical properties and corrosion resistance of Mg. Thus, the primary purpose of this study is to develop Mg composites reinforced with CNF using a powder metallurgy method. The significant factors that influenced the process design were screened using two-level factorial design. Four factors; the percentage of CNF (0.1 - 2.0%), compaction pressure (100 – 400 MPa), sintering temperature (300 – 500°C), and sintering time (1 - 4 hrs), were analysed for three responses, namely elastic modulus, hardness, and weight loss. The significant factors were further subjected to the Box-Behnken design (BBD) of response surface methodology to obtain the optimum parameters. Selected specimens were subjected to X-ray diffraction (XRD), attenuated total reflection-Fourier transform infrared (ATR-FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM), hydrophobicity, thermogravimetric (TGA), X-ray photoelectron spectroscopy (XPS) and biocompatibility analyses. The results show that the mechanical properties and corrosion resistance of the composites were optimum at 2% CNF, 400 MPa of compaction pressure, and 500°C of sintering temperature with a significant effect at $P < 0.05$ for all variables except the sintering time ($P > 0.05$). The elastic modulus and hardness of the composites peaked at 4685 MPa and 60 Hv, respectively. The nanomechanical analysis also revealed that the highest elastic modulus (766 MPa), hardness (539 MPa), and stiffness (575 N/m) were achieved at the same condition. After three days of immersion in phosphate buffered saline, the minimum and maximum weight loss were recorded at 54% and 100%, respectively. The CNF significantly improved the surface morphology of Mg-Zn/2.0%CNF with average roughness (R_a) of 19.16 ± 3.4 nm, high hydrophobicity (> 100°) and good oxidation behaviour. Moreover, the controlled releases of Mg^{2+} and Zn^{2+} ions were achieved too. The XRD analysis verified the presence of Mg (35 – 80 θ), Mg-Zn alloy (35 – 40 θ) and CNF (53 θ) in the composite. The Raman spectroscopy analysis confirmed the presence of CNF in the Mg composites for all specimens. Besides, biocompatibility test confirmed the improvement of osteoblast cells viability and the composites were found non-toxic to the cells (> 70% viability). Further study on the optimisation using BBD showed that all factors significantly contributed towards high mechanical strength (5409.7 MPa of elastic modulus and 60.7 Hv of hardness) and corrosion resistance (up to 52%). The presence of Mg-Zn solid solution has improved the nanomechanical properties of the composites when 1.8% of CNF was compacted using 425 MPa at 500°C sintering temperature that resulted in the records of 832 MPa elastic modulus, 549.7 MPa hardness and 605 N/m stiffness. Hydrophobicity and R_a were the major contributing factors that produced high corrosion resistance and controlled ions release. The Mg-Zn/1.8%CNF has also successfully stimulated cell growth with non-toxic properties towards osteoblast cells. This work concludes that the optimum conditions and processing techniques for the fabrication Mg composite were found at 1.8% of CNF, 425 MPa of compaction pressure, and 500°C of sintering temperature.

ABSTRAK

Magnesium (Mg) menjadi bahan berpotensi untuk menggantikan implan keluli-tahan karat dan aloi titanium. Walau bagaimanapun, kestabilan mekanikal Mg yang tidak mencukupi boleh menyebabkan kegagalan pramatang dan kakisan. Serat karbon nano (CNF) telah merevolusikan industri komposit dan menjanjikan penambahbaikkan dalam sifat mekanikal dan ketahanan kakisan. Oleh itu, tujuan utama kajian ini adalah untuk menghasilkan komposit Mg yang diteguhkan dengan CNF melalui kaedah metalurgi serbuk. Faktor penting yang mempengaruhi reka bentuk proses disaring dengan menggunakan reka bentuk pemfaktoran dua aras. Empat faktor; peratusan CNF (0.1 - 2.0%), tekanan pemadatan (100 - 400 MPa), suhu pembakaran (300 - 500°C), dan masa pembakaran (1 - 4 jam), dianalisis untuk tiga tindak balas, iaitu modulus elastik, kekerasan, dan penurunan berat. Faktor-faktor penting selanjutnya tertakluk pada reka bentuk *Box-Behnken* (BBD) bagi kaedah permukaan tindak balas untuk mendapatkan parameter optimum. Spesimen yang terpilih tertakluk pada analisis pembiasan sinar-X (XRD), pengurangan jumlah pantulan-Inframerah pengubah Fourier (ATR-FTIR), mikroskop pengimbasan elektron (SEM), mikroskopi daya atom (AFM), telap air, termogravimetrik (TGA), spektroskopi fotoelektron sinar-X (XPS) dan keserasian bio. Hasil kajian menunjukkan bahawa sifat mekanikal dan ketahanan kakisan komposit adalah optimum pada 2% CNF, 400 MPa tekanan pemadatan, dan suhu pembakaran 500°C dengan kesan yang ketara pada $P < 0.05$ untuk semua pemboleh ubah kecuali masa pembakaran ($P > 0.05$). Modulus elastik dan kekerasan komposit masing-masing mencapai nilai tertinggi pada 4685 MPa dan 60 Hv. Analisis nanomekanikal juga menunjukkan bahawa modulus elastik tertinggi (766 MPa), kekerasan (539 MPa), dan kekakuan (575 N/m) dicapai pada keadaan yang sama. Setelah tiga hari rendaman dalam larutan penimbal fosfat, penurunan berat yang minimum dan maksimum masing-masing dicatatkan pada 54% dan 100%. CNF telah menambahbaik morfologi permukaan Mg-Zn/2.0%CNF dengan purata kekasaran (R_a) 19.16 ± 3.4 nm, telap air tinggi (> 100°) dan sifat pengoksidaan yang baik. Lebih-lebih lagi, pelepasan ion Mg^{2+} dan Zn^{2+} yang terkawal juga diperhatikan. XRD mengesahkan adanya komposisi Mg (35 - 80 θ), aloi Mg-Zn (35 - 40 θ) dan CNF (53 θ) dalam komposit. Analisis spektroskopi Raman mengesahkan adanya CNF dalam komposit Mg untuk semua spesimen. Selain itu, ujian keserasian bio mengesahkan peningkatan pertumbuhan sel osteoblas dan komposit didapati tidak toksik pada sel (pertumbuhan sel > 70%). Kajian lebih lanjut mengenai pengoptimuman menggunakan BBD menunjukkan bahawa semua faktor menyumbang secara ketara terhadap kekuatan mekanik yang tinggi (modulus elastik 5409.7 MPa dan kekerasan 60.7 Hv) dan ketahanan kakisan (hingga 52%). Kehadiran larutan pepejal Mg-Zn telah meningkatkan sifat nanomekanik apabila 1.8% CNF dipadatkan menggunakan 425 MPa pada suhu pembakaran 500°C menghasilkan rekod modulus elastik 832 MPa, kekerasan 549.7 MPa dan kekakuan 605 N/m. Ketidak telapan air dan R_a adalah faktor penyumbang utama yang menyebabkan ketahanan kakisan yang tinggi dan pembebasan ion terkawal. Mg-Zn/1.8%CNF juga berjaya merangsang pertumbuhan sel dengan sifat tidak toksik terhadap sel osteoblas. Kajian ini menyimpulkan bahawa keadaan dan teknik pemprosesan yang dioptimum untuk pembuatan Mg komposit adalah pada 1.8% CNF, 425 MPa tekanan pemadatan, dan 500°C suhu pembakaran.

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LIST OF ABBREVIATIONS

3D	-	3 dimensions
AFM	-	Atomic force microscopy
ANOVA	-	Analysis of variance
BBD	-	Box-Behnken design
BMI	-	Biodegradable metal implant
Ca	-	Calcium
Cd	-	Cadmium
CNF	-	Carbon nano fiber
CNT	-	Carbon nanotube
CO_2	-	Carbon dioxide
Cu	-	Copper
DMEM	-	Dulbecco's Modified Eagle Medium
DMSO	-	Dimethyl sulfoxide
DOE	-	Design of experiment
EDX	-	Energy dispersive X-ray
FBS	-	Fetal bovine serum
FTIR-ATR	-	Fourier transform infrared spectroscopy – attenuated total reflectance
hFOB	-	Human foetal osteoblast cell
ICP-OES	-	Inductively coupled plasma - optical emission spectrometry
ICP-OES	-	Inductively coupled plasma-optical emission spectroscopy
LOF	-	Lack of fit
Mg		Magnesium
MTT	-	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MWCNT	-	Multi-walled carbon nanotube
PBS	-	Phosphate-buffered saline
PM	-	Powder metallurgy
RSM	-	Response surface methodology
SEM	-	Scanning electron microscopy
SS	-	Stainless steel
TGA	-	Thermogravimetric analysis
Ti	-	Titanium
UV	-	Ultra-violet
XPS	-	X-ray photoelectron spectroscopy
XRD	-	X-ray diffraction
Zn	-	Zinc

LIST OF SYMBOLS

#	-	Number
%	-	Percent
°C	-	Degree Celsius
°C/min	-	Degree Celsius per minutes
±	-	Plus-minus
<	-	Less than
>	-	More than
a.u	-	Astronomical unit
Cell/mL	-	Cell/millilitre
cm^{-1}	-	1 per centimetre
cm^2/mL	-	Centimetre square per millilitre
eV	-	Electron volt
g	-	Gram
g	-	gas
GPa	-	Giga Pascal
hr	-	Hour
Hv	-	Vickers Pyramid Number
k	-	Number of independent factors
kg	-	Kilogram
kHz	-	Kilo Hertz
kV	-	Kilo volt
l	-	liquid
mg		Milligram
min	-	Minutes
mL	-	Millilitre
mL/min	-	Millilitre per minute
mm	-	Millimetre
mm^2	-	Millimetre square
MPa	-	Mega Pascal
N/m	-	Newton per meter
nm	-	Nanometre
RPM	-	Revolution per minute
s	-	solid
sec	-	Seconds
w/w	-	Weight/weight
α	-	Alpha
µL	-	Microlitre
µm	-	Micrometre

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Innovations in health sciences and biomaterial engineering are needed to discover effective methods. This includes design of new implants to address increase in orthopaedic fracture cases [1]. Much attention and research effort has been accorded to new technologies in biomedical implants such as knee, hip, retina and dental implants, degradable screws and plates, scaffolds and drug delivery devices. The biomedical implants need sufficient and speedy care for patients with bone fractured. The performance of the current titanium (Ti) and stainless steel (SS) that are used for external fixator screw has been thoroughly investigated and reported in many scientific fields for their biocompatibility. However, several problems raised as a result of using these materials [2, 3]. For instance, they interfere with imaging techniques such as computed tomography and magnetic resonance imaging, and the screw can block the radio-therapeutic beam and result in inadequate treatment. Another significant challenge is the extraction of the implant (implant screws) from human body after the material has fulfilled its function. The extraction of the screws needs additional surgical procedure. This takes time, cause discomfort, has cost implication and infection risk [4]. To overcome these, innovations in implant designs in biomedical engineering is still immensely needed as bone fracture is a part of human life.

Biodegradable metal implant (BMI) can be defined as the metal implant that decompose gradually in vivo, with an appropriate host response elicited by the released decomposition products. They dissolve completely upon fulfilling their function of assisting the tissue healing with no implant residues [5]. BMI is expected to replace non-degradable implants in the next era of biomedical engineering as they have no multiple cost, time and risk disadvantages associated with non-degradable implants and do not need secondary surgery as they degrade in human body with minimum side effect. Thus, biodegradable implants offer a considerable chance to cut cost, time and patient suffering, yet the most important benefit is by preventing second surgery.

Magnesium (Mg) alloys are the most studied biodegradable metals due to its usage as materials in temporary medical implants like coronary stents and bone fracture fixation screws [6]. When a Mg alloy is immersed in a physiological medium, the contact between the fresh surface and an electrolyte-containing aqueous medium lead to higher initial corrosion rates. This process involves the release of hydrogen and the alkalinization of the environment [7]. Moreover, it is an essential element needed for bone function, and the alloys are characterised by its low elastic modulus (40-45 GPa), which is the closest to that of human bone compared to other metallic biomaterials. Recently, bone screws and pins made of Mg-Zn-Ca-Zn and Mg-RE alloys have been approved for clinical use in Korea and Germany [8, 9]. However, many found that the mechanical properties and degradation resistance of Mg alloys are yet to be ideal and needs further improvement. The combination of limited strength and rapid degradation rate may lead to a premature loss of mechanical integrity of an implant before a fractured bone is entirely healed [10]. The characteristics of strength and degradation should be balanced to make sure the bone segments could receive adequate stabilisation during healing [11]. One of the promising methods to improve both strength and degradation resistance of Mg alloys is composite reinforcement by carbonaceous particles [12].

Different forms of carbonaceous particles had successfully been used to reinforce different types of metal alloys, including carbon nanofiber (CNF) [13], graphene nanoplate [14], carbon nanotube (CNT) [15], and carbon fiber [16]. CNF reinforcement enhance the mechanical strength of CNF-A7XXX composites as high as 89.83 GPa while being chemically stable [17, 18]. The limited strength and rapid degradation of Mg alloys used in bone implants can be increased and improved through the addition of CNF. The dispersion of CNF and the high interface of CNF guarantee the strengthening effect and increases the degradation resistance of the composite. However, the use of CNF particles for reinforcing biodegradable Mg alloys is still limited. Literature search showed that the highest average yield strength of 74 MPa can be recorded for 1.5% wt CNF in porous Mg, which was enhanced by 54%

compared to porous Mg alone. Fibers are regarded as a potent reinforcing category that influences directional strength and stability of the composites [19]. They add strength to the matrix, which influenced and enhanced its desired property [20-22]. A CNF-Mg alloy composite can be fabricated through powder metallurgy (PM) process however attention must be paid to at least four parameters; percentage of reinforcement particles, compaction pressure, sintering temperature, and sintering time [23]. According to Orowan strengthening effect, increasing the CNF percentage up to 2% in a metal matrix could increase the mechanical aspect of a composite [20]. The usage of less than 1% of CNF as reinforcement improved the ultimate compressive force to 114 MPa, which represents about 14% increase to that of pure Mg, as well as hardness improvement of 37% [22]. An increment in compaction pressure will lead to increased contact area between powder particles and further decreases porosity thereby improving the strength and stiffness of the composite [24, 25]. Similarly, increasing the compaction pressure will decreases the composite pore size form a more compact or dense material [26]. Finally, both sintering temperature and time will transform the compacted powders into sintered metals and determines the composite final structure and property of the composite [27].

The combination of the above listed parameters can be determined effectively through the use of design of experiment (DOE); a method that is suitable for characterisation, optimisation, and modelling process involving materials and composition preparation [28]. The process involves planning and designing of experiments base on specific parameters such as temperature, composition, and time, which should be predetermined before conducting the DOE. Fitting data from previous studies should be captured and analysed for the right interpretation of post-experimental works in order to validate the results, objectives, and conclusion [29]. There are many designs in the DOE, including two-level factorial, Plackett Burman, Taguchi methods and Box-Behnken but the factorial design which studies the effect of two or more factors are commonly used to design experiments for developing new materials, processes and screening factors [30]. It helps to determine the most influential variables in the process of responses in material properties by identifying the vital factors affects both process and material properties. This in turn reduces the number of experiments to save time and cost [30]. A two-level factorial DOE allows an analysis of multiple factors simultaneously while maintaining data collection quality, as shown in the work of Gou *et al.* [31]. These authors analysed the substantial effect of suspension concentration, sonication time, and vacuum pressure on the pore size of single-walled nanotube using a half-normal plot and regression model without the second-order effect. The most popular designs for fitting a second-order polynomial are the central composite designs [32] and the designs of Box and Behnken design (BBD) [33]. In most cases, for three factors, BBD gives some benefit in having fewer runs. BBD an independent quadratic design with no embedded factorial or fractional factorial design. In BBD, the treatment combinations are at the midpoints of process space edges and centre [33]. The BBD, that is a part of the Response Surface Methodology (RSM), can be used to identify the optimum manufacturing parameter of composites by evaluating the effect and interactions of factors found on the two-level factorial design [34].

In the present study, we investigate an optimised material design and process of biodegradable CNF-reinforced Mg-Zn alloy composites fabricated through the powder metallurgy (PM) process using the two-level factorial and BBD methods. Moreover, it is aimed to improve mechanical properties, corrosion resistance and biological activity of the composites for targeted orthopaedic implants.

1.2 Problem Statement

After bone fracture healing has taken place, external fixator screws (Ti and SS) no longer have any function and implant extraction procedure become an obligation. However, 48% of medical doctors believed it is more risky to extract the non-biodegradable implant out than leaving it inside human body [35]. This was supported by Kovar *et al.* [36] where post-extraction of non-biodegradable implant increased the rate of complications to 28%. A statistical analysis made by University of Mississippi Medical Center and University of Alabama at Birmingham showed that 17% of patients are faced with acute bacterial infections after extraction of implant [37]. From the same research [37], 7 out of 17 patients faced bone re-fracture and wound drainage. These postoperative complications are very serious as it affects not only health condition, but also the social and economic status of the patient(s) [35]. Even though almost 52% medical doctor believed the implant should not be removed after bone heal due to risks and complications associated with the removal procedures [35], a clinical analysis conducted by Niznick [38] found that 400 out of 4235 patients faced implant fracture in bone after a few years of healing period. Indirectly, it urged doctors to extract it and that implies secondary surgery. Besides, the implant will also interfere with the image interpretation and interference of the radio-therapeutic treatment which results to an inadequate treatment [39].

Biodegradable metal such as Mg-Zn alloy is a promising implant that can replace the conventional screw in external fixator because both elements are essential trace elements in human body [40, 41]. This includes bone screws and pins made of Mg-Zn-Ca-Zn and Mg-RE alloys that clinical use in Korea and Germany [8, 9]. However, research findings that Mg and its alloy degrade rapidly and easily lost its mechanical integrity over the corrosion period [42]. Moreover, the degradation of Mg can release hydrogen gas which affects the cell growth. The tolerable rate of hydrogen release was reported to be only 0.01 ml/cm^2 per day in the human body [41, 42]. Thus, controlling of the hydrogen release rate from the biodegradation of Mg is imperative. In addition, a low mechanical stability of Mg-Zn alloy (60.62 MPa) is seen as serious challenge to their biodegradability advantage especially when used in load bearing areas of the body such as mandible [43]. Due to the low mechanical stability, most of the manufacturer of biodegradable implants have increased the dimension of these fixative devices but the enlarged dimension may cause difficulties in wound closure.

In addition to enhanced mechanical properties of biodegradable material, CNF demonstrates a great potential for promoting bone regeneration [39]. Many biomedical studies to date, have demonstrated wide and successful incorporation of CNF in tissue engineering polymers including poly(lactic-co-glycolic acid) [44], poly(L-lactide) [45] and metal matrix composite such as CNF-Mg [46]. However, the incorporation of CNF with Mg up to 10% could be interpreted as a failure as it reduces the mechanical and degradation properties of the composite [22]. Such scenario can be linked to the poor interfacial bonding between the CNFs clusters and Mg matrix, which can weakens the crack bridging effect of the CNF [22]. According to other studies, there are several factors that affect the performance of the composite such as the ratio of reinforcement element to the Mg, production method, hydrophobicity of the reinforcement and porosity content [47-49].

To date, there is no previous biomedical study that focuses on the optimum factors for incorporation of CNF with Mg-Zn alloy. This composite will be able to possess excellent mechanical properties and corrosion resistance when the factors that affect the performance of the composite is optimized. Thus, this study was conducted to formulate the optimum biodegradable Mg-Zn alloy reinforced CNF through PM process using design of experiment.

1.3 Research Questions

Based on the above elaboration in section 1.2, the research questions of this study can be summarised as follows:

1. How can Mg-Zn alloy be reinforced with CNF to be fabricated?
2. What is the effect of the optimized Mg-Zn alloy reinforced CNF on physico-chemical and mechanical properties?
3. How does the characteristics of the optimized Mg-Zn alloy reinforced CNF affect the mechanical and corrosion properties?
4. How does the optimized Mg-Zn reinforced with CNF affect the cell viability?

1.4 Objective of Study

The major aim of this study is optimisation of Mg-Zn alloy reinforced with CNF considering important parameters such as composition of CNF, sintering time, sintering temperature and compaction pressure. Through this study, the desire biomaterial properties were fabricated to achieve the following objectives:

5. To optimize the synthesis condition of Mg-Zn alloy reinforced with CNF using powder metallurgy method.

6. To investigate the effect of the optimized Mg-Zn alloy reinforced with CNF by characterizing its effect on physico-chemical, mechanical and corrosion properties.

7. To determine cell compatibility of the optimized specimens with osteoblast cell through cell viability test.

1.5 Scope of Study

The main scope of this study covers fabrication and optimization of biodegradable Mg-Zn alloy reinforced CNF through PM process. Two-level factorial design was adopted to screen the most influential factors for 36 specimens such as composition of CNF (0.1 – 2.0%), compaction pressure (100 – 400 MPa), sintering time (1 – 3 hrs) and sintering temperature (300 – 500°C) that affect mechanical and corrosion behaviour of Mg composites. The BBD model, which constitutes a subset of the classic RSM was applied to efficiently investigate the screened factors. There were three significant factors for 17 specimens such as percentage of CNF (1.8 – 2.2%), compaction pressure (375 – 425 MPa) and sintering temperature (475 – 525°C).

Using Design Expert Version 12, regression statistics, graphical structure and statistical analysis were determined, and the optimum parameters of material were investigated based on elastic modulus, hardness, and percentage of weight loss for three days. The characterisation of the optimized material was conducted using atomic force microscopy (AFM), scanning electron microscopy (SEM) attached with energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The physico-chemical properties of the material were further characterized using contact angle measurement, thermogravimetric analysis (TGA), Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy, and Raman spectroscopy. The mechanical properties of the optimized material were also determined using Instron (compression test) and Vicker hardness machine (hardness test). The study further investigated nanomechanical properties (elasticity, stiffness, and hardness) of the material using AFM. The corrosion behaviour of the material was characterized using static immersion test for three days. The percentage of cell viability of osteoblast cell was measured using indirect MTT assay.

1.6 Significance of Study

This study was strategically planned to develop a new metal composite for biomedical implant application. This will be beneficial to the biomaterial research as well the biomedical manufacturing industry, specifically the orthopaedic field. The incorporation of CNF in Mg-Zn alloy is crucial to providing a new characteristic of Mg based composite with a high mechanical property and optimum corrosion resistance. The ability of Mg-Zn-CNF to degrade in human body with no side effect can overcome the problem of implant removal that normally bring about the secondary surgery. As well, it will help to avoid the risk and complications of surgical procedure after the fracture bone heal. This research is a pre-liminary result for the development of a prototype of external fixator screw.

Design of experiment (DOE) was used as a tool to meet a specific aim. In this study, two-level factorial design was used to identify the most significant factors that contribute to the enhancement of elastic modulus, hardness and corrosion. This step reduced the number of experiments before optimization process was done. BBD was used to optimize the significant factors that give the maximum elastic modulus, hardness and corrosion resistance.

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