GINSENG ENCAPSULATED POLY(LACTIC-*co*-GLYCOLIC ACID)/ POLYANILINE MICROCAPSULES COATED ON STAINLESS STEEL 316L USING ELECTRODEPOSITION TECHNIQUE FOR DRUG-ELUTING STENT APPLICATION

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DEDICATION

This thesis is dedicated to my father, Lukman bin Abdullah and my late mother, Lezam binti Yunos who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my husband, Muhammad Faris bin Yahya who taught me that even the largest task can be accomplished if it is done one step at a time and to my family and family-in-laws for your endless supports and motivations.

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ABSTRACT

Drug-eluting stent (DES) has successfully minimised the occurrence of restenosis and in-stent neointimal formation. However, its drawback of polymer hypersensitivity is often led to late-stent thrombosis. Besides, incomplete coating and bridging on stent surfaces as well as rapid release of anti-proliferative drugs to the site of implantation have contributed to late endotheliasation. The incorporation of ginseng within biodegradable polymer coating will address these issues due to its specific therapeutic values. Therefore, 30 mg ginseng was encapsulated in poly (lactic-*co*-glycolic acid) (PLGA) microcapsules to be electrodeposited as a coating on stainless steel 316L (SS316L). A proficient technique of electrodeposition was performed at different currents (1 - 3 mA) and deposition times (20 - 60 seconds) while different polyaniline (PANI) compositions (0.5 – 2.0 mg) were also adopted in the electrodeposition process to drive the formation of microcapsules coating. Based on different currents and deposition times, it was found that electrodeposition with addition of PANI conducted at 2 mA current and 40 seconds deposition time has formed low wettability and uniform microcapsules coating through the analyses of ATR-FTIR, SEM and contact angle. Reduction in current or deposition time caused less attachment of microcapsules coating with high wettability records. Increasing current or prolonging deposition time has led to debris formation and melted microcapsules with non-uniform wettability measurements. The colour of electrolytes was also changed from milky white to dark yellow when the current and the deposition time increased. Based on different composition of PANI, the utilisation of 1.5 mg PANI has assisted the formation of stable, uniform and rounded microcapsules coating with appropriate wettability and surface roughness through the ATR-FTIR, XPS, SEM, AFM and contact angle analyses. Low PANI content (0.5 mg) was not enough to drive the formation of microcapsules coating while higher content of PANI (2.0 mg) caused the deposition of melted microcapsules. A month coating stability analysis showed that the coating stability was improved at the utilisation of 1.5 mg PANI with moderate PLGA degradation and less appearance of melted microcapsules. The similar coating also has promoted greater endothelial cell proliferation and attachment compared to other coating variation through MTT assay and VP-SEM analyses. The capabilities of ginseng encapsulated PLGA/PANI microcapsules coating in delivering its therapeutic values would be beneficial in addressing the complication of current DES.

ABSTRAK

Sten pembebasan ubat (DES) berjaya meminimumkan kejadian restenosis dan pembentukan neointima dalam sten. Walau bagaimanapun, kehiperpekaan polimer sering menyebabkan kelewatan trombosis pada sten. Selain itu, penyalutan dan penitian permukaan sten yang tidak lengkap serta pelepasan ubat anti-proliferatif secara pantas ke lokasi penempelan turut menyumbang pada kelewatan endotelisasi. Masalah ini dapat ditangani dengan menggabungkan ginseng dalam lapisan polimer terbiodegradasikan berikutan nilai terapeutiknya. Oleh itu, ginseng sebanyak 30 mg dikapsul dalam mikrokapsul poli(asid laktik-ko-glikolik) (PLGA) melalui elektropemendapan dengan menyalut keluli tahan karat 316L (SS316L). Tempoh pemendapan (20–60 saat) dan arus (1–3 mA) serta komposisi polianilin (PANI) (0.5-2.0 mg) yang berbeza digunakan semasa proses elektromendapan supaya menggalakkan pembentukan salutan mikrokapsul dapat dibentuk dengan cekap. Analisis ATR-FTIR, SEM dan sudut sentuhan menunjukkan arus 2 mA dan tempoh pemendapan selama 40 saat mendorong penambahan PANI yang membentuk salutan kebolehbasahan yang rendah dan salutan mikrokapsul yang seragam. Pengurangan arus atau tempoh pemendapan mengurangkan pelekatan salutan mikrokapsul dengan rekod kebolehbasahan yang tinggi. Peningkatan arus atau pemanjangan tempoh pemendapan menyebabkan puing terbentuk dan mikrokapsul melebur pada kebolehbasahan yang tidak seragam. Warna elektrolit juga berubah daripada putih susu kepada kuning gelap apabila arus dan tempoh pemendapan ditingkatkan. Berdasarkan komposisi PANI, analisis ATR-FTIR, XPS, SEM, AFM dan sudut sentuhan menunjukkan penggunaan PANI sebanyak 1.5 mg dapat membantu pembentukan salutan mikrokapsul yang stabil, seragam dan bulat dengan kebolehbasahan dan kekasaran permukaan yang sepatutnya. Komposisi PANI yang rendah (0.5 mg) tidak mencukupi untuk mendorong pembentukan salutan mikrokapsul manakala komposisi PANI yang lebih besar (2.0 mg) menyebabkan pemendapan mikrokapsul lebur. Analisis kestabilan salutan yang dijalankan selama sebulan menunjukkan kestabilan salutan meningkat apabila 1.5 mg PANI digunakan, dengan degradasi PLGA yang sederhana serta kurang pembentukan mikrokapsul yang lebur. Jenis salutan ini meningkatkan percambahan dan pelekatan sel endotelial dengan lebih mantap berbanding dengan variasi salutan lain melalui ujian MTT dan analisis VP-SEM. Salutan mikrokapsul ginseng beserta dengan PLGA/PANI dapat dimanfaatkan untuk memberikan nilai terapeutik dalam menangani komplikasi sten pembebasan ubat yang sedia ada.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscope
ANOVA	-	Analysis of Variance
ATP	-	Adenosine Triphosphate
ATR-FTIR	-	Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy
BMS	-	Bare Metal Stent
$C_3H_8O_3$	-	Glycerol
CAGR	-	Compound Annual Growth Rate
CNCs	-	Cellulose Nanocrystals
CVD	-	Cardiovascular Disease
DC	-	Direct Current
DCM	-	Dichloromethane
DES	-	Drug-Eluting Stent
DI	-	Deionized
DMSO	-	dimethyl Sulfoxide
DNA	-	Deoxyribonucleic Acid
EC	-	Endothelial Cells
FDA	-	Food and Drug Administration
GP	-	Ginseng Pharmacopuncture
H_2O_2	-	Hydrogen Peroxide
H_3PO_4	-	Phosphoric Acid
Hcy	-	Homocysteine
hDPSCs	-	Human Dental Pulp Stem Cells
HF	-	Hydrofluoric Acid
HNO_3	-	Nitric Acid
HUVEC	-	Human Umbilical Vein Endothelial Cells
ISR	-	In-Stent Restenosis
JNK	-	c-Jun N-terminal kinases
LDL	-	Low-Density Lipoprotein
MAPK	-	Mitogen-Activated Protein Kinases

MI	-	Myocardial Infarction
MOE	-	Magnolia Cortex
MTT	-	(3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium)
NaCl	-	Sodium Chloride
NF-κB	-	Nuclear Factor Kappa B
NHA	-	Nano-Hydroxyapatite
NICE	-	National Institute for Health and Care Excellence
PANI	-	Polyaniline
PBS	-	Phosphate Buffer Saline
PCI	-	Percutaneous Coronary Intervention
PCL	-	Polycaprolactone
PDLLA	-	Poly(D,L-Lactide Acid)
PGA	-	Poly(Glycolic Acid)
PI3K	-	Phosphatidylinositol-3 Kinase
PLA	-	Poly(Lactic Acid)
PLGA	-	Poly(Lactic-*co*-Glycolic) Acid
POBA	-	Plain Old Balloon Angioplasty
POFS	-	Postoperative Fatigue Syndrome
PPD	-	Protopanaxadiol
PPT	-	Protopanaxatriol
PU	-	Polyurethane
PVA	-	Polyvinyl Alcohol
REKRG	-	Rg3 Enriched Korean Red Ginseng
RGE	-	Red Ginseng Water Extract
ROS	-	Reactive Oxygen Species
SEM	-	Scanning Electron Microscope
SS	-	Stainless Steel
TNF-α	-	Tumour Necrosis Factor-α
T-OA	-	3β-hydroxyolea-12-en-28-oic acid-3, 5, 6-trimethylpyrazin- 2-methyl
VEGF	-	Vascular Endothelial Growth Factor
VPSEM	-	Variable Pressure Scanning Electron Microscopy
VSMC	-	Vascular Smooth Muscle Cells

W/O	-	Water-in-Oil
WHO	-	World Health Organization
XPS	-	X-ray Photoelectron Spectroscopy

LIST OF SYMBOLS

%	-	Percentage
°	-	Degree
°C	-	Degree celcius
mA	-	Miliampere
dL/g	-	Decilitre per gram
mL	-	Mililitre
mm	-	Milimetre
mg	-	Miligram
×	-	Times
cm^2	-	Square centimetre
M	-	Molar
rpm	-	Rotation per minute
eV	-	Electron volt
nm	-	Nanometre
µm	-	Micrometre
=	-	Equal
rms	-	Root mean square
µL	-	Microlitre

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Cardiovascular disease (CVD) is a heart and blood vessel disease affecting entire arterial circulation related to pathologic process called atherosclerosis [1]. A build-up of plaques in the artery will contribute to atherosclerosis, further lead to blood vessel blockage. A statistic reported by Mozaffarian *et al.* [2] showed that CVD has caused more than 17.3 million deaths per year in 2013 and it depicted 31% of all global deaths in the same year. This statistic is expected to increase gradually by 2030, which up to more than 23.6 million [2]. In Malaysia, CVD has led to mortality and morbidity of 36% of total deaths in 2014 [3], accounting diabetes as the major causes of CVD [4]. Several other risk factors that contribute to CVD are obesity, hypertension and smoking habit [1]. Therefore, active prevention must be taken and more affordable treatment need to be offered to support the increasing number of CVD cases.

Coronary angioplasty or also known as percutaneous coronary intervention (PCI) is a common treatment used to treat blocked heart arteries [5]. It involves the insertion of a stent to remove thrombus and to support the shape of arterial wall from narrowing again in order to prevent heart attack from recurring [6]. There are several evolutions of stent including bare metal stent (BMS), degradable stent and drug-eluting stent (DES) [7]. Drug-eluting stent is a medicine/drug coated stent which acts to minimise the occurrence of restenosis and to reduce the formation of in-stent neointimal [8]. The main problems of DES are rapid release of drug and polymer hypersensitivity which subsequently lead to late endotheliasation and late-stent thrombosis [5]. Both problems occur due to retardation of vascular endothelial cells (EC) growth [9, 10]. Therefore, it is crucial to develop a functional drug coating which can induce the proliferation of vascular EC to prevent late endotheliasation.

Panax ginseng or also called as red ginseng in Korea is one of the herbal supplement products [11], widely used in Asia for thousands of years as medicine and food [12]. Originated from *Araliaceae* family, there are many types of ginsenosides such as Rb1, Rb2, Rf, Rg2, Rg3, Rg1 and Re [13] have been identified where each of them has different and interesting physiological effects [14]. One of the ability of these active compounds is to promote the growth of EC which gives a promising value in the development of functional drug coating on a stent [15-17].

In DES application, a drug/medicine is necessary to be incorporated within degradable polymer as a matrix to control the release of drug/medicine [18]. Poly(lactic-*co*-glycolic) acid (PLGA) is among the polymers that has been used as a coating material on DES other than poly(lactic acid) (PLA), poly(D,L-lactide acid) (PDLLA) and polycaprolactone (PCL) [19]. Since United States Food and Drug Administration (FDA) has approved PLGA to be used safely in the human body, this polymer is utilised in many biomedical devices and applications such as nanoparticles [20], drug-delivery carriers [21], implants and tissue engineering scaffolds due to its biodegradability, biocompatibility and proper mechanical properties [22].

There are several techniques have been used to coat stents such as electrospinning, dip-coating, rolling coating, electro-treated coating, plasma treated coating and spray coating [23, 24]. In this study, an electrodeposition technique was used to deposit a coating material on metal substrate as this technique is able to provide a stable coating in controlling and prolonging drug delivery [25]. Besides, electrodeposition is a simple and low expense technique to be performed in a room temperature [26]. Electrodeposition parameters such as time, voltage, distance and current are tolerable for the adjustment of coating's chemical composition, coating thickness and coating homogeneity [26].

In depositing drug/polymer based materials on a metal substrate using an electrodeposition technique, a conducting element is required to be incorporated into the coating material to drive the attachment of polymer coating onto the substrate. Several types of conducting polymers which have potential to initiate the

electrodeposition process are identified including polyaniline (PANI), PCL and polypyrrole [27]. Polyaniline is a unique conducting polymer, mostly used in the area of electrical, electrochemical and anti-corrosion [28-30]. It has also been studied as an electroactive conductive polymer in cell and tissue culture applications due to its biocompatibility, greater cell attachment and proliferation [29].

Therefore, the aim of this study was to deposit ginseng encapsulated PLGA/PANI microcapsules on electropolished stainless steel 316L by using an electrodeposition technique. The characterisations of the coating were conducted through attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscope (SEM), atomic force microscope (AFM) and contact angle analyses. The stability tests were performed to forecast the performance of the coating following an implantation. Finally, the *in-vitro* cell test was carried out to evaluate the biological reaction on the coating, specifically in enhancing EC proliferation.

1.2 Problem Statement

Percutaneous coronary intervention has evolved rapidly and has been nominated as one of the most widely performed medical procedures, worldwide, with over 3 million annually since it was first conducted in 1977 [31]. As PCI is a major contributor to the health care cost area [32, 33], it is important to explore a low cost and affordable treatment to support the increasing number of PCI. Stent is a medical device commonly combined with coronary angioplasty or PCI to treat cardiovascular diseases. Drug-eluting stent is introduced following the development of BMS to overcome the malfunction of BMS. In Malaysia, DES is currently a preferred stent for PCI treatment with 58% procedures compared to BMS with 39% procedures as reported in the National Cardiovascular Disease Database [34]. Although DES has suppressed the restenosis to large extent and outperformed BMS by incorporating the antiproliferative drugs, other serious drawbacks are reported following the implantation of DES [35]. The drawbacks include late endotheliasation and polymer

hypersensitivity that later produce impaired EC, local hypersensitivity and late-stent thrombosis [35].

In previous research, several improvements have been made on DES, in the area of coating design, technique and drug function that specifically address EC growth [36, 37]. However, those previous research are concentrating on synthetic coating and thin layer coating [38, 39] where the exploration on herbal-based polymer microcapsules coating is essential for natural therapeutic delivery and better physiological performances. An electrodeposition technique is viewed as a technique to form the polymer microcapsules coating. However, previous studies are mostly highlighting its utilisation in depositing ceramic/metal coating [39-41]. Therefore, the ability of electrodeposition technique to form a herbal-based polymer microcapsules coating is remained unknown and requires specific investigation on related parameters. Among the electrodeposition parameters, current and deposition time are two main determinants in achieving optimum electrodeposition procedure [42]. For example, insufficient current and deposition time could affect coating formation while excessive current and deposition time may give impact on coating morphology and homogeneity [40, 43]. Conductive materials are also one of the determinants to drive coating formation onto substrate effectively. However, the safety concerns of conductive materials need to be clarified. For example, PANI had shown toxicity at higher concentration and may influence the biocompatibility of cell performance [44].

Another concern in DES implantation is restricted to the limited coating on the surface of stent struts which often lead to roughening of the stent surfaces [45]. The rough surfaces will increase the strut thickness and cause lack of uniformity, thus interrupting the coating stability and elevate the risk of restenosis [46]. Besides, bridging formation is another issue as it can provide sites to activate platelet adhesion and it can complicate endotheliasation due to the prevention of cell migration [47]. Another factor of delayed endotheliasation is burst and rapid release of drug that led to non-uniform local drug distribution [5, 46], causing insufficient amount of drugs to be released at the implanted lesion. The burst release scenario will arrest EC growth and prolong the healing process of implanted heart's vessel wall. The burst

release of drug will also project plaque reformation inside the implanted stent which known as in-stent restenosis [48]. Therefore, coating strategies with good surface chemistry and adequate stability that can enhance EC growth and resolve late-stent thrombosis should be explored and developed.

1.3 Research Objectives

The aim of this study was to deposit ginseng encapsulated PLGA/PANI microcapsules on electropolished stainless steel 316L using an electrodeposition technique for drug-eluting stent application. Thus, following objectives were designed:

1. To assess the effect of current in electrodepositing ginseng encapsulated PLGA/PANI microcapsules on stainless steel 316L in terms of its chemical composition, morphology, wettability and electrolyte colour changes.

2. To assess the effect of deposition times in electrodepositing ginseng encapsulated PLGA/PANI microcapsules on stainless steel 316L in terms of its chemical composition, morphology, wettability and electrolyte colour changes.

3. To analyse the effect of different PANI compositions in electrodepositing ginseng encapsulated PLGA/PANI microcapsules on stainless steel 316L in terms of its chemical composition, morphology, wettability and surface roughness.

4. To evaluate the stability of the ginseng encapsulated PLGA/PANI microcapsules coating through one month immersion procedure with regard to its chemical composition, morphology and wettability properties.

5. To justify the biocompatibility of the ginseng encapsulated PLGA/PANI microcapsules coating with human umbilical vein endothelial cells (HUVEC) through cytotoxicity, cell proliferation and cell attachment analyses.

1.4 Scope of the Study

The scope of the study is divided into five phases where each phase is addressing each objective of the study. The first phase of the study includes the fabrication of microcapsules by encapsulating 30 mg ginseng within 400 mg PLGA and 1 mg PANI microcapsules using a double emulsion (water/oil/water) technique. The microcapsules were characterised through ATR-FTIR, SEM and particle size analyses. The metal substrates of medical grade stainless steel 316L were then cleaned and electropolished, prior to the deposition of microcapsules coating onto the metal surfaces. The microcapsules were deposited using an electrodeposition technique at different current amplitudes of 1 mA, 2 mA and 3 mA.

The coating surfaces were subjected to ATR-FTIR, SEM and contact angle analyses. The colour changes of electrolyte solution were also observed and analysed using an ImageJ software, before and after the electrodeposition. The second phase of the study involves the deposition of microcapsules coating at different deposition times (20 seconds, 40 seconds and 60 seconds). The coating surfaces were subjected to similar characterisation analyses and colour changes evaluation.

The selected current of 2 mA and deposition time of 40 seconds from the first and the second phases of the study were then applied and standardised in the third phase of the study where only PANI compositions were varied at 0.5, 1.0, 1.5 and 2.0 mg. The surface characterisations were conducted through ATR-FTIR, XPS, SEM, AFM and contact angle analyses. The coating stability test is covering the fourth phase of the study where the microcapsules coating were immersed in phosphate buffer saline (PBS) solution for one month to forecast the performance and ability of the microcapsules coating to retain its properties after stent implantation. The ATR-FTIR, SEM and contact angle instruments were used to evaluate the performances of the aged coated metals.

The last phase of the study involves the conduction of *in-vitro* cell test at 1, 3 and 7 days of incubation to justify the biocompatibility of the microcapsules coating with HUVEC through both qualitative and quantitative assessments. For the

quantitative assessment, cell toxicity and cell proliferation were evaluated using MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium) assay while for the qualitative assessment, the morphology of cell attachment was viewed under variable pressure SEM (VPSEM).

1.5 Significance of the Study

The stainless steel 316L coated with ginseng encapsulated PLGA/PANI microcapsules is projected to have great potential and significance values in DES application. The novelty of this study is based on the utilisation of electrodeposition technique to form ginseng encapsulated PLGA/PANI microcapsules coating on metal substrate. The microcapsules coating is offering great advantage in controlling ginseng drug release as previously, there was no attempt to coat polymer microcapsules with fusion of drug on implants by using this technique.

Furthermore, the implementation of electrodeposition technique with selectable parameters in depositing the ginseng encapsulated PLGA/PANI microcapsules coating, is associated to tolerable coating stability. The improvement of coating stability will prevent coating degradation and delamination, thus lessen the possibility of coating failure. Long-term and stable release of ginseng on the site of stent restoration will further prevent late endotheliasation through the promotion of vascular EC growth. In a greater view, the microcapsules coating will accelerate vessel healing and help the vessel to remain open [49].

Besides, the Global Vascular Stent Market Forecast has shown that the global market for coronary artery stents was $5371 million, with DES accounting for $4658 million in 2016 [50]. This DES global market is expected to grow at compound annual growth rate (CAGR) of 3.2% over the next five years [50]. This report presented the needs of coronary artery stents due to the vast increment and demand for stents to treat diseases related to the blood vessels and the heart. The World Health Organization (WHO) also estimates the increasing of cardiac mortality rates to 22.2 million in 2030 from 17.5 million in 2012 [50]. Therefore, in an economic

view, the microcapsules coating is recognised as an attempt for the development of less cost DES due to the implementation of natural herb compared to the utilisation of synthetic commercialised drugs.

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LIST OF PUBLICATION

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