FABRICATION AND CHARACTERIZATION ANALYSIS ON POLYURETHANE ELECTROSPUN NANOFIBERS INCORPORATED WITH CHITOSAN/ELASTIN FOR CARDIOVASCULAR GRAFTING

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DEDICATION

This study is wholehearted dedicated to my beloved parents for their inspiration during this journey and continually provide their emotional and financial support.

I am lucky to have you and I love you from the bottom of my heart

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ABSTRACT

Cardiovascular disease is the leading cause of death worldwide, where the damage of cardiac tissues is one of its major effects. Current synthetic graft scaffolds to repair and replace cardiac tissues have limitations to cover small blood vessels and have compliance mismatch which later contribute to scaffold failure. Therefore, the incorporation of natural polymers, such as elastin and chitosan, into a synthetic electrospun polymer of polyurethane (PU) is necessary to overcome these complications. Natural polymers such as chitosan and its derivatives are beneficial for wound healing while elastin has shown a successful approach in providing viscoelastic properties. Polyurethane is a synthetic polymer which is commonly used as a substitute of cardiovascular soft tissues due to its biocompatibility, elasticity and mechanical properties. In this study, PU was blended with chitosan nanoparticles and elastin at different elastin concentrations (1% and 1.5%) to be electrospun to form mat fiber scaffolds. The formation of chitosan nanoparticles was verified by using ultraviolet-visible (UV-Vis) spectrophotometer at an absorption peak of 290 nm. The physico-chemical properties of the electrospun PU incorporated with chitosan/elastin were investigated through scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and contact angle measurement. The SEM images were also subjected to fiber diameter, pore size and porosity analyses. The PU incorporated with chitosan/elastin nanofibers were homogenously electrospun with less beads at the flow rate of 0.5 mL/h and 12 kV voltage using 21-G stainless steel needle. The incorporation of chitosan and 1% elastin have increased the fiber diameter by 73.03%, the pore size by 21.05% and the porosity by 14.54% compared to the pure PU. The functional groups of chitosan and elastin (N–H and C–N) were presented in the FTIR spectral peaks. While the wettability of PU nanofibers incorporated with chitosan/elastin was increased by 22.53% compared to the PU nanofibers. The polymer scaffold that was made from natural and synthetic biomaterials in this study, is projected to be used in a range of cardiovascular applications, specifically to address the limitation on small blood vessels and to overcome the physiological properties mismatch.

ABSTRAK

Penyakit kardiovaskular adalah penyebab utama kematian di seluruh dunia, di mana kerosakan tisu jantung adalah salah satu kesan utamanya. Perancah sintetik terkini untuk memperbaiki dan mengganti tisu jantung mempunyai batasan untuk meliputi saluran darah kecil dan ketidaksesuaian pematuhan yang kemudiannya menyumbang kepada kegagalan perancah. Oleh itu, penggabungan polimer semula jadi, seperti elastin dan kitosan, ke dalam polimer elektrospun sintetik poliuretan (PU) diperlukan untuk mengatasi komplikasi ini. Polimer semula jadi seperti kitosan dan terbitannya bermanfaat untuk penyembuhan luka sementara elastin menunjukkan pendekatan yang berjaya dalam memberikan sifat viskoelastik. Poliuretan adalah polimer sintetik yang biasanya digunakan sebagai pengganti tisu lembut kardiovaskular kerana sifat keserasian bio, keanjalan dan sifat mekaniknya. Dalam kajian ini, PU diadunkan dengan partikel nano kitosan dan elastin pada kepekatan elastin yang berbeza (1% dan 1.5%) untuk dielektro putar bagi membentuk perancah serat tikar. Nanopartikel kitosan telah disahkan dengan menggunakan spektrofotometer pencerahan ultra ungu (UV-Vis) pada puncak resapan 290 nm. Sifat fisiko-kimia putaran elektro PU yang digabungkan dengan kitosan/elastin disiasat dengan imbasan mikroskop elektron (SEM), spektroskopi inframerah transformasi Fourier (FTIR) dan pengukuran sudut kontak. Imej SEM juga digunakan untuk menganalisis diameter serat, ukuran liang dan keliangan. Fiber nano PU yang digabungkan dengan kitosan/elastin telah dielektro putar dengan sekata dan kurang manik pada kadar aliran 0.5 mL/j dan voltan 12 kV menggunakan jarum keluli tahan karat 21-G. Penggabungan kitosan dan 1% elastin telah meningkatkan diameter serat sebanyak 73.03%, ukuran pori sebanyak 21.05% dan keliangan sebanyak 14.54% berbanding PU tulen. Kumpulan berfungsi kitosan dan elastin (N–H dan C–N) ditunjukkan dalam puncak spektrum FTIR. Manakala kebolehan serapan air fiber nano PU yang digabungkan dengan kitosan/elastin meningkat sebanyak 22.53% berbanding dengan fiber nano PU. Perancah polimer yang dibuat dari bahan bio semula jadi dan sintetik dalam kajian ini, dicadangkan untuk digunakan dalam pelbagai aplikasi kardiovaskular, khusus untuk mengatasi keterbatasan pada saluran darah kecil dan untuk mengatasi ketidak sesuaian sifat fisiologi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATR	-	Attenuated Total Reflection
bFGF	-	Basic Fibroblast Growth Factor
Ch	-	Chitosan
CB	-	Cardiovascular Biomaterial
CoCr	-	Cobalt Chromium
DCM	-	Dichloromethane
DMF	-	N, N - dimethylformamide
ECM	-	Extracellular Matrix
ECs	-	Endothelial cells
El	-	Elastin
FTIR	-	Fourier Transform Infrared Spectroscopy
LDL	-	Low-Density Lipoprotein
PCL	-	Polycaprolactone
PET	-	Polyethylene terephthalate
PPy	-	Polypyrrole
SEM	-	Scanning Electron Microscopy
SMCs	-	Smooth Muscle Cells
Ti	-	Titanium
TiO2	-	Titanium dioxide

LIST OF SYMBOLS

%	-	Percent
ºC	-	Degree celsisus
µL	-	Microliter
µm	-	Micrometer
cm	-	Centimeter
kV	-	Kilovolt
min	-	Minute
ml	-	Mililiter
ml/h	-	Mililiter/hour
nm	-	Nanometer
w/v	-	Weight/volume
w/w	-	Weight/weight
Sec	-	Second
rpm	-	Revolutions per minute

CHAPTER 1

INTRODUCTION

1.1 Problem Background

Cardiovascular disease remains the leading cause of death in the world. Prevention and treatment of excessive bleeding following cardiac surgery are of major concern among cardiac surgeons (1). Many attempts have been made, to engineer various cardiac tissues (1). In order to maintain the blood flow and prevent cardiac bleeding, synthetic vascular grafts have been explored in cardiovascular applications and have been used in patients to recover from cardiovascular diseases (2).

In tissue engineering, the source of natural vascular grafts from native tissues can be classified into autogenic, allogenic and xenogeneic sources (3). Allografts and xenografts have some advantages such as long-term patency and reactivity, however, their utilization is limited by the high immunogenic response (3). In bypassing large diameter blood vessels (6 mm), synthetic grafts such as expanded polytetrafluoro or Dacron (polyethylene terephthalate fiber) have been used, in replacement to the natural vascular grafts (3). However, those synthetic grafts have demonstrated high failure rates on small diameter blood vessels (<6 mm) due to thrombosis, stenosis and occlusion (2).

In this study, polymer membrane scaffolds were fabricated using an electrospinning technique to address the research gap for small diameter blood vessels. Electrospinning is a broadly used technology which provides an electrostatic fiber formation, that uses electrical forces to form fibers, ranging from 2 nm to several μm diameter (4). In tissue engineering, electrospun fibers are known as one of the approaches to form polymer scaffolds (5). Such replacement tissues for the cardiovascular system may require biocompatible and suitable mechanical properties comparable to native vascular tissues (4, 5).

Polyurethane (PU) is a type of biomaterial, widely used in the medical field as wound dressings, artificial prosthetics and vascular grafts (6). It is biocompatible and does not cause inflammatory reactions on tissues. Besides, it is biodegradable under certain conditions (4). Polyurethane has a wide range of physico-chemical, thermal an mechanical properties due to its segmented structure, which is composed of hard and soft segments (4). Moreover, it is easy to modify the properties of PU by changing its solvent reagents or by blending PU with other materials, such as natural materials (4). Therefore, PU was selected to be the main matrix for the incorporation of chitosan and elastin. The incorporation of chitosan and elastin is necessary to enhance the biological properties of PU for nanofibers vascular graft production.

Chitosan and elastin have been applied as topical dressings in wound management, due to their haemostatic wound healing promotion (7). Elastin is an insoluble extracellular matrix protein that provides elasticity and resilience to the arteries, lungs and skin (7). It has sufficient mechanical strength for the elasticity function of certain biological organs (8). Chitosan, whilst is an aminopolysaccharide, found in the exoskeleton of crustaceans and the cell wall of fungi (8). Chitosan is considered as the second most abundant biopolymer, which has been used in gels and nanoparticles, to meet the preferable conditions in wound dressing applications (9).

In this study, PU electrospun nanofibers were incorporated with chitosan and elastin for the potential use as vascular grafts. Specifically, the physico-chemical properties of the fabricated electrospun membranes were characterized by scanning electron microscopy (SEM). The SEM images were subjected to fiber diameter, pore size and porosity analyses. Other two characterization analyses were Fourier transform infrared spectroscopy (FTIR) and contact angle measurement.

1.2 Problem Statement

In recent years, demand for tissue engineering is increasing in medical applications and facing challenges towards treating and repair vascular damages as well as cardiac tissues. In the current treatment, patients with carotid artery stenosis are treated with synthetic vascular patches known as Dacron (10, 11). Late wound complications and inflammatory reactions have occurred due to the limited properties of Dacron (10). In the same context, another research demonstrated that the treatments using synthetic materials such as Dacron, following aorta surgical repair have caused serious risk where 20 - 51% of aneurysm formation and rupture were reported (11). Furthermore, a high rate of vessel blockages had been referred following the implantation of Dacron due to discrepancy in mechanical characteristics between synthetic vascular grafts and arteries (6).

Available synthetic materials have also been identified with cellular interaction limitations, which cause disappointment in clinical routines, mainly due to low hemo- or biocompatibility, lack of compliance and normal physiologic responsiveness that lead to inflammatory reaction and blockage (2). Several decades of efforts in bioengineering and tissue engineering, along with a growing understanding of cardiovascular pathologies, have driven researchers to develop regenerative approaches, focusing on new materials that imitate native tissues, including degradable and natural polymers for temporary scaffolding. (12).

1.3 Research Objectives

The objectives of the research are:

(a) To electrospun PU incorporated with chitosan/elastin nanofibers by varying the concentration of elastin.

(b) To characterize the electrospun PU incorporated with chitosan/elastin nanofibers through several physico-chemical analyses of SEM, FTIR and contact angle.

1.4 Scope of the study

In this study, electrospun PU blended with chitosan/elastin scaffolds were fabricated at different concentrations of elastin. These scaffolds are intended to be used in soft tissue reconstruction, to be specific for cardiac and vascular tissues. The electrospinning parameters such as voltage, flow rate, working distance and syringe needle were kept constant during the electrospinning process. The PU incorporated with chitosan/elastin electrospun nanofibers were subjected to four physico-chemical characterization analyses.

There are three analysis methods were conducted including SEM, FTIR, contact angle measurement. The SEM was conducted by observing the morphology structure and fiber diameter, while the FTIR was performed to determine the chemical composition and functional groups of the electrospun membranes. In order to assess the surface wettability of the electrospun membranes, the contact angle data were recorded. With regards to the porosity analysis, it was utilized to forecast the flow transport of nutrients, oxygen and metabolic waste products.

1.5 Significance of the Study

Loss of cardiac function caused by damaged heart tissues and vessels is considered as threating remark to patients' health (1). Cardiologists and researchers have engineered tissues for possible solutions and methods to replace or repair different areas of the heart (1). The significant advantage of this study is to fabricate PU electrospun scaffold to be used as cardiovascular replacement tissues, to enhance the biological function of the scaffold by using elastin and chitosan. Furthermore, the blending of PU with natural polymers such as elastin will provide necessary viscoelastic properties which requires further exploration (4). Additionally, the incorporation of chitosan into the PU matrix will provide hydrophilicity and better cell-tissue interaction (9).

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