PROCESS OPTIMIZATION DESIGNS BASE ON SILK FIBROIN PROTEIN IN UNIQUE FIBROUS MORPHOLOGY FOR WOUND DRESSING APPLICATION

Jesada Chutipakdeevong

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy The Petroleum and Petrochemical College, Chulalongkorn University in Academic Partnership with The University of Michigan, The University of Oklahoma, and Case Western Reserve University

Thesis Title:	Process Optimization Designs Base on Silk Fibroin Protein in
	Unique Fibrous Morphology for Wound Dressing
	Application
By:	Jesada Chutipakdeevong
Program:	Polymer Science
Thesis Advisors:	Prof. Pitt Supaphol
	Dr. Uracha Ruktanonchai

Accepted by The Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

...... College Dean

(Asst. Prof. Pomthong Malakul)

Thesis Committee:

10

(Asst. Prof. Pomthong Malakul)

(Prof. Pitt Supaphol)

Uracha Ruktanonchai

(Dr. Uracha Ruktanonchai)

Hetha, have M.

(Asst.Prof.Hathaikarn Manuspiya)

molpun

(Dr. Pimolpun Niamlang)

ABSTRACT

5082002063: Polymer Science Program

Jesada Chutipakdeevong: Process Optimization Designs Base on Silk Fibroin Protein in Unique Fibrous Morphology for Wound Dressing Application

Thesis Advisors: Prof. Pitt Supaphol and Dr. Uracha Ruktanonchai,

118 pp.

Keywords: Silk/ Fibroin/ Electrospun/ Wound dressing

Nowadays, there are numerous researches regarding to the relative efficiency of the various kinds of material for effective wound healing. Biopolymers and fabrication techniques have been studied not only for covering in order to prevent infection but also have extraordinary properties which promote the healing process. In this work, Thailand domesticated, Bombyx mori, silk fibroin fibres, which is well known in the textile industry for centuries, was used to develop as active wound dressing. Silk fibroin from the silk cocoon is generally defined as an attractive biomaterial because of its unique characteristics such as high mechanical strength, excellent biocompatibility, controllable structure and morphology, and wide variety of constructive properties on tissue engineering. One of the most effective methods for this is electrospinning, a proven technique that precisely creates the fibrous structure that can mimic nanofibrillar structure and the biological functions of the natural extracellular matrix. Electrospun fibrous mats also combine extremely large surface area to volume ratios with high porosity, features that are needed for the application of these materials in wound healing. In this study, we aimed to optimize the preparation process of ultra-fine silk fibroin fibers for wound dressing application. These findings open an exciting opportunity to fabricate biocompatible scaffold structures that could be used as next generation effective wound healing materials.

บทคัดย่อ

เจษฎา ชุติภักดีวงศ์ : การออกแบบกระบวนการขึ้นรูปเส้นใยที่เหมาะสมสำหรับโปรตีนไหมไฟ โบรอินเพื่อการใช้งานเป็นวัสดุปิดแผล (Process Optimization Designs Base on Silk Fibroin Protein in Unique Fibrous Morphology for Wound Dressing Application) อ. ที่ ปรึกษา: ศาสตราจารย์ คร. พิชญ์ ศุภผล และ คร.อุรชา รักษ์ตานนท์ชัย 118 หน้า

ในปัจจุบันได้มีงานวิจัยจำนวนมากศึกษาเกี่ยวกับประสิทธิภาพของวัสดุหลากหลายชนิด ้เพื่อช่วยในการรักษาแผล โพลิเมอร์ชีวภาพและเทคนิคในการขึ้นรูปวัสดุจึงได้มีการศึกษาเพิ่มเติม ไม่เพียง แค่สำหรับป้องกันแผลจากสิ่งแวคล้อมภายนอก หรือเพื่อป้องกันการติคเชื้อ แต่ยังมี คุณสมบัติ พิเศษที่ช่วยส่งเสริมกระบวนหายของแผล ในงานวิจัยนี้ ได้ทำการปรับปรุงพัฒนา เส้น ในไหมสายพันธุ์ท้องถิ่นในประเทศไทย ซึ่งเป็นที่รู้จักกันดีในอุตสาหกรรมสิ่งทอมานานหลาย ศตวรรษ เพื่อใช้ในการพัฒนาวัสดุปิดแผลที่มีประสิทธิภาพ เส้นใยไหมที่สาวออกจากรังไหมแล้ว ้จะถูกนำมาสกัดอยู่ในรูปของโปรตีนไหมไฟโบรอิน ซึ่งโดยทั่วไปว่าเป็นวัสดุที่มีความน่าสนใจ สนใจมาก เนื่องจากคุณสมบัติที่เป็นเอกลักษณ์ เช่น ความแข็งแรง, มีความเข้ากันได้ทางชีวภาพที่ดี, มีโครงสร้างและสัณฐานวิทยาที่สามารถควบคุมได้, และมีความหลากหลายของคุณสมบัติที่ สร้างสรรค์ทางค้านวิศวกรรมชีวภาพ หนึ่งในวิธีการที่มีประสิทธิภาพมากที่สุดสำหรับการขึ้นรูป สำหรับแผ่นปิดแผล คือ เทคนิคอิเล็ค โทรสปินนิ่ง ซึ่งมีการการพิสูงน์แล้วว่า มีความแม่นยำในการ สร้างโครงสร้างเส้นใย ที่สามารถเลียนแบบโครงสร้างเส้นใยนาโน และการทำงานทางชีวภาพ ของเอ็กตราเซลลูล่า เมทริค ในร่างกาย เส้นใยอิเล็คโทรสปั่น่ยังมีความสามารถรวมถึงในเรื่องของ พื้นที่ผิวที่มีขนาคใหญ่ เทียบกับอัตราส่วนโคยปริมาตร และมีความเป็นรูพรุนสูง ซึ่งเป็นคุณสมบัติ ์ที่มีความจำเป็นสำหรับการใช้งานวัสคุเหล่านี้ในรูปแบบของวัสคุที่ใช้ในการรักษาแผล ในงานวิจัย นี้ เรามีวัตถุประสงค์เพื่อ คิคค้นวิธีเพิ่มประสิทธิภาพของกระบวนการเตรียมเส้นใยไหมไฟโบรอิน สำหรับทำแผ่นปิดแผล ซึ่งการค้นพบนี้ จะเปิดโอกาสในการคิดค้นเพิ่มเติมสำหรับการสร้างวัสดุ โครงสร้างที่มีความเข้ากันได้ทางชีวภาพ และสามารถนำมาใช้เป็นวัสดุปิดแผลที่มีประสิทธิภาพ ต่อไป

ACKNOWLEDGEMENTS

Appreciation is expressed to those who have made contributions to this dissertation. First the author gratefully acknowledges his advisors, Prof. Pitt Supaphol from The Petroleum and Petrochemical College, Chulalongkorn University, and Dr. Uracha Ruktanonchai from National Nanotechnology Center, National science and Technology Development Agency for giving their invaluable knowledge, meaningful guidance and encouragement all along the way.

He gratefully acknowledges all faculty members and staffs at The Petroleum and Petrochemical College. Chulalongkorn University for their knowledge and assistance. Moreover he would like to give his special thanks to all members in his research group and all of his friends for their kind assistance, continual encouragement and wonderful friendship.

Asst. Prof. Pomthong Malakul, Prof. Pitt Supaphol, Dr. Uracha-Ruktanonchai, Asst. Prof. Hathaikarn Manuspiya, and Dr. Pimolpun Niamlang are further acknowledged for being his dissertation committees, making valuable comments and suggestions.

He wishes to express his deep gratitude to his family for their unconditioned love, understanding and very supportive during all these years spent for his Ph.D. study.

Finally, he is grateful for the partial support received from The Petroleum and Petrochemical College, The National Nanotechnology Center; and The Center of Excellence for Petrochemicals and Materials Technology, Thailand and a doctoral scholarship received from the Thailand Graduate Institute of Science and Technology (TGIST) (TG-55-09-51-035D). This work would not be carried out successfully without all financial supports.

TABLE OF CONTENTS

				PAGE
Title	Page			i
Abst	tract (in English)			iii
Abst	ract (in Thai)			iv
Ack	nowledgements			v
Tabl	e of Contents			vi
List	of Tables			viii
List	of Figures			х
Abb	reviations			x v
CHAPTE	R			
I	INTRODUCTION	n 		1
II	THEORITICAL BACKGROU	ND AND LI	FERATURE	
	REVIEW			4
	2.1 Silkworm (B. Mori) Silk			4
	2.2 Wound Healing			9
	2.3 Update on Wound Dressing		1.12	14
	2.4 Biomimetic Morphology Des	ign :		21
	2.5 Biocompatible Polymers and	Surface		
	Functionalization for Enhanc	e Biological	Activity -	22
	2.6 Wound Dressing Material Ba	sed on		
	Electrospinning of Nanofiber	Ś		24
	2.7 The Future of Wound Dressin	ngs	-	30
III	PREPARATION AND CHARA	CTERIZAT	ION OF	
	ELECTROSPUN SILK FIBRO	IN FOR TIS	SUE	
	REGENERATION			
	3.1 Abstract		0	32
	3.2 Introduction			33

3.3	Experimental	34
3.4	Results and Discussion	37
3.5	Conclusions	41
3.6	Acknowledgements	41
3.7	References	42

IVPROCESS OPTIMIZATION OF ELECTROSPUN SILKFIBROIN FIBER MAT FOR ACCELERATEWOUND HEALING4.1 Abstract4.2 Introduction

4.3	Experimental	56
4.4	Results and Discussion	60
4.5	Conclusions	66
4.6	Acknowledgements	66
4.7	References	66

V	HYBRID BIOMIMETIC ELECTROSPUN FIBROUS MATS		
	DERIVED FROM POLY (E-CAPROLACTONE) AND	D SILK	
	FIBROIN PROTEIN FOR WOUND DRESSING		
	APPLICATION		
	5.1 Abstract		80
	5.2 Introduction		81
	5.3 Experimental		83
	5.4 Results and Discussion		87
	5.5 Conclusions		92
	5.6 Acknowledgements		93

5.7 References

PAGE

53

54

VI	CONCLUSIONS AND RECOMMENDATIONS	109
	6.1 Conclusions	110
	6.2 Recommendations	111
	REFERENCES	110
	CURRICULUM VITAE	116

-

LIST OF TABLES

PAGE TABLE

CHAPTER II

2.1	Classification of Wounds Based on the Appearance	12
2.2	Topical wound dressing type, important characteristics and	
	commercially available products	15
2.3	Typical biological materials with function integration	23

CHAPTER III

	CHAPTER III		
3.1	Electrospun SF fibers diameters.	46	1.00
3.2	SEM micrographs of HFF cells attached on the surface of SF		1.
	film and electrospun SF fiber mat comparison with control	51	
3.3	SEM micrographs of HFF cells proliferated on the surface of		
	SF film and electrospun SF fiber mat comparison with control	52	

CHAPTER IV

	CHAPTER IV	
4.1	XPS surface chemical compositions and elemental ratio of	
	eSF fibers before and after modification with fibronectin	74
4.2	SEM images of human dermal fibroblasts (NHDF)	
	attachment on glass slide (i.e., control), electrospun silk	
	fibroin fibers (eSF) and surface-modified eSF fibers for 2	
	h, 4 h and 8 h. (magnification = $500x$)	77
4.3	SEM images of human dermal fibroblasts (NHDF)	
	proliferation cultured on glass slide (i.e., control),	
	electrospun silk fibroin fibers (eSF) and surface-modified	
	eSF fibers for 1 d, 2 d and 3 d. (magnification = $1000x$)	78

CHAPTER V

5.1	Electrospun Fiber diameters	97
5.2	Representative SEM images of human dermal fibroblast	
	attachment on different types of electrospun fibers after 2h, 4	
	h and 8 h (magnification = $1000x$)	107
5.3	Representative SEM images of human dermal fibroblast	
	proliferation on different types of electrospun fibers after 1	
	d, 2 d and 3 d (magnification = $400x$, $1000x$)	108

PAGE

LIST OF FIGURES

FIGURE

CHAPTER II

2.1	A) Photograph of a Bombyx mori silkworm. B) Electron	
	micrograph of partially degummed B. mori silkworm cocoon	
	fibers, in which the two brins of fibroin and the coating of	
	sericins C) Schematic cross-section of the composite	
	structure of a cocoon fiber, in which the two brins of fibroin	
-	and the coating of sericins. Adapted with the permission of	
	both the authors and publisher, Copyright 2008, Elsevier	
	Science Ltd., Oxford, UK.	4
2.2	(A) Silk fibroin is purified from sericins by boiling in an	+
	alkaline solution. Non-woven silk mats by partial	. ÷
	solubilization; or dissolved in lithium bromide, dialyzed and	* 5
	formed into aqueous silk fibroin solution for preparation of	+
	other material morphologies. (B) Processing of silk	. u.
4	morphologies from aqueous silk fibroin solution into non-	
	woven silk fibers; aqueous- and solvent-based porous	
	sponges; hydrogels and films.	.7.
2.3	(a) Schematic of electrospinning process (b) Shape evolution	:
	of poly(ethylene oxide) solution in the electrospinning	14
	process which time zero is when the first eruption appears.	28
	CHAPTER III	

3.1 SEM images of electrospun SF fibers at different concentrations: a) 10%, b) 20%, c) 30%, and d) 40% (w/v). The EFS was applied at 20 kV/10 cm. (magnification = 5000x, scale bar = 5 μm).

FIGURE

3.2	SEM images of electrospun SF fibers from 40% (w/v) SF solution after methanol treatment at different applied EFS: a)	
	15 kV, b) 20 kV, and c) 25 kV/10 cm. (magnification =	
	$10000x$, scale bar = 1 μ m).	45
3.3	ATR-FTIR spectra of electrospun SF fiber mat: (a)	
	electrospun SF fiber mat (b) electrospun SF fiber mat after	
	methanol treatment.	47
3.4	The viability of L929 cells that were cultured on electrospun	
	SF fiber mats for indirect cytotoxicity evaluation with	
	various extract media concentration for 24 hr.	48
3.5	Attachment of HFF cell on control, SF film and electrospun	
	SF fiber mat as a function of time in culture.	49
3.6	Proliferation of HFF cell on control, SF film and electrospun	
	SF fiber mat as a function of time in culture.	50

CHAPTER IV

4.1	SEM micrographs of electrospun SF/PEO fibers at different	
	humidity: (a) RH \sim 60%, (b) RH \sim 45% and (c) RH $<$ 30%.	
	Weight ratio of SF/PEO is 70:30 in 10 %(w/v) of aqueous.	69
4.2	The morphology and fiber diameter at various states of	
	electrospinning of silk fibroin nanofibers of: (a) electrospun	
	SF/PEO fibers, (b) MeOH treated SF/PEO fibers, (c) eSF	
	fibers after PEO extraction, and (d) surface-modified eSF	
	fibers. Applied electric field strength was 14 kV/15 cm.	70
4.3	FTIR spectrums of different states silk fibroin proteins: (a)	
	silk fibroin film, (b) electrospun SF/PEO fiber mat, (c) eSF	-
	fiber mat after PEO extraction, and (d) surface-modified eSF	
	fiber mat.	71

PAGE

FIGURE

÷

	4.4	XPS photoelectron peaks of C 1s of: (a) eSF and (b) surface-			
		modified eSF fiber mat. The different chemical			
		compositions obtained from peak fitting are shown.	72		
	4.5	Water retention capacity (\bullet) and dissolution (\circ) of: (a) eSF			
		and (b) surface-modified eSF fiber mat at various time			
		points in PBS buffer at 37 °C.	73		
	4.6	Indirect cytotoxicity evaluation of the neat and modified eSF			
		fiber mat based on the viability of normal human dermal			
		fibroblasts (NHDF) cultured with various extraction media			
		concentration for 24 hours.	75		
	4.7	7 Cell viability of NHDF (a) attachment and (b) proliferation.			
		The NHDF were either seeded or cultured on control, eSF			
		fiber mat, and surface-modified eSF fiber mat as a function			
		of time.	76		
	4.8	Cross-section images of: (a,b) neat eSF fibers mat and (c,b)			
		surface-modified eSF fibers mat before and after cell culture			
	<u> </u>	for 3 days respectively (magnification = $3000x$).			
	1				
-		CHAPTER V			
	5.1	SEM micrographs of (a) neat PCL electrospun fibers, (b)			
		· .			

2	hybrid electrospun fibers and (c) surface-modified hybrid	
	electrospun fibers at 5000x, 2500x and 22000x of	
	magnification. The applied EFS were 21 kV/20 cm.	96
5.2 :	The % SF content on the surface of hybrid electrospun fibers	
	as a function of SF solution concentration.	98
5.3	FTIR spectra for different electrospun fiber mats made from:	
	(a) neat PCL electrospun fibers, (b) hybrid electrospun	
	fibers, (c) MeOH treated hybrid electrospun fibers and (d)	
	surface-modified hybrid electrospun fibers.	99

PAGE

FIGURE

- 5.4 XPS spectra of (a) neat PCL electrospun fibers, (b) hybrid electrospun fibers and (c) surface-modified hybrid electrospun fibers.
- 5.5 XPS photoelectron peak fitting of C 1s of (a) neat PCL electrospun fibers, (b) hybrid electrospun fibers and (c) surface-modified hybrid electrospun fibers. The different chemical compositions obtained from the peak fitting are shown.
- 5.6 Water retention capacity behavior of PCL electrospun fibers
 (°), hybrid electrospun fibers (•) and surface-modified
 hybrid electrospun fibers (Δ) in PBS buffer at 37°C.
- 5.7 Degradation of PCL electrospun fibers (○), hybrid electrospun fibers (●) and surface-modified hybrid electrospun fibers (△) as a function of incubation time in PBS buffer at 37°C.
- 5.8 Indirect cytotoxicity evaluations for different types of electrospun fibers based on the viability of normal human dermal fibroblasts (NHDF).
- 5.8 Attachment (a) and proliferation (b) of NHDF seeded or cultured on control, PCL electrospun fibers, hybrid electrospun fibers and surface-modified hybrid electrospun fibers as a function of culture time. * Indicates significant difference at p-values of < 0.05.

xiv

100

102

103

104

105

ABBREVIATIONS

AAS	Atomic absorption spectroscope
DMEM	Dulbecco's modified Eagle's medium
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
EDC	One-ethly-3-(dimethylaminoproply)
÷	carbodiimide hydrochloride
EFS	Electric field strength
e-spinning	Electrospinning
e-spin	Electrospun
eSF	Electrospun silk fibroin
FT-IR	Fourier-transform infrared spectroscopy
HFF	Human foreskin fibroblast
HPLC	High pressure liquid chromatography
MTT	Methyl 4-hydroxybenzoate
NHS	N-hydroxysuccinimide
NHDF	Normal human dermal fibroblast
PBS	Phosphate buffer saline
PCL	Poly (ε-caprolactone)
PEO ·	Poly(ethylene oxide)
RH :	Relative humidity
SEM	Scanning electron microscopy
SF	Silk fibroin
SFM	Serum-free medium
TCPS	Tissue-culture polystyrene plete
UV	Ultra-violet
XPS	X-ray photoelectron spectroscopy