

**SYNTHESIS, FABRICATION, AND BIOCOMPATIBILITY OF HEXANOYL  
CHITOSAN**



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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,  
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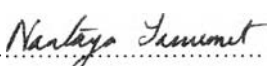
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**Thesis Advisors:** Assoc. Prof. Pitt Supaphol  
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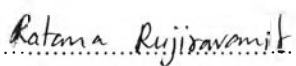
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## บทคัดย่อ

อาจพบ เนื้อขนาด : การสังเคราะห์ การขึ้นรูป และ ความเข้ากันได้ทางชีวภาพของเฮกซะโนอิลไคโตซาน (Synthesis, Fabrication, and Biocompatibility of Hexanoyl Chitosan) อ. ที่ปรึกษา : รองศาสตราจารย์ ดร.พิชญ์ สุขผล และ รองศาสตราจารย์ ดร.รัตนารุจิรวนิช 93 หน้า

งานวิจัยนี้ได้ศึกษาการสังเคราะห์ การขึ้นรูป และ ความเข้ากันได้ทางชีวภาพของเฮกซะโนอิลไคโตซานเพื่อประเมินความเป็นไปได้ในการนำไปใช้ในทางการแพทย์ ได้ทำการสังเคราะห์เฮกซะโนอิลไคโตซานโดยปฏิกิริยาเอซิชันจากผงไคโตซานกับคาโพรอิล คลอไรด์ (เฮกซะโนอิล คลอไรด์) และได้วิเคราะห์เฮกซะโนอิลไคโตซานที่ได้ด้วยเทคนิคฟูเรียร์ทรานฟอร์มอินฟราเรดสเปกโตรสโกปีและโปรตอนเอ็นเอ็มอาร์สเปกโตรสโกปีเพื่อยืนยันการเข้าแทนที่ของหมู่เฮกซะโนอิลในโมเลกุลไคโตซาน จากการวิเคราะห์ธาตุองค์ประกอบแสดงให้เห็นถึงจำนวนหมู่เฮกซะโนอิลที่เข้าแทนที่ต่อหนึ่งหน่วยกลูโคซามีน ได้ศึกษาเสถียรภาพทางความร้อนของเฮกซะโนอิลไคโตซาน โดยเครื่องศึกษาการเปลี่ยนแปลงน้ำหนักของสารโดยอาศัยคุณสมบัติทางความร้อน ส่วนการทดสอบความเข้ากันได้ทางชีวภาพนั้นได้ทำการศึกษา ความเป็นพิษของฟิล์มเฮกซะโนอิลไคโตซาน การเกาะ การเพิ่มจำนวน และการแผ่ของเซลล์ L929 ที่เพาะเลี้ยงบนฟิล์มเฮกซะโนอิลไคโตซาน นอกจากนั้นได้ทำการขึ้นรูปเส้นใยเฮกซะโนอิลไคโตซานด้วยเทคนิคกระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตและได้ศึกษาตัวแปรต่างๆที่มีผลต่อขนาดและลักษณะของเส้นใยที่ได้ จากลักษณะของแผ่นเส้นใยที่ได้เป็นโครงสร้างสามมิติที่ประกอบไปด้วยเส้นใยขนาดเล็กจำนวนมากและมีความเป็นรูพรุนสูงซึ่งมีลักษณะคล้ายกับโครงร่างของเนื้อเยื่อในร่างกายที่ประกอบไปด้วยเส้นใยคอลลาเจน เพื่อศึกษาความเป็นไปได้ในการนำแผ่นเส้นใยไปใช้เป็นวัสดุโครงร่างเนื้อเยื่อเทียมจึงได้ศึกษาความเข้ากันได้ทางชีวภาพโดยทดสอบความเป็นพิษ และการเกาะและการเพิ่มจำนวนของเซลล์ไฟโบรบลาสต์ (HFF) และเคราติโนไซต์ (HaCaT) บนแผ่นเส้นใย ภาพจากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดยังได้แสดงให้เห็นลักษณะและรูปร่างของเซลล์ที่เกาะอยู่บนวัสดุต่างๆในการศึกษานี้ด้วย

## ABSTRACT

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Hexanoyl chitosan (H-chitosan) was synthesized via a heterogeneous acylation reaction between chitosan powder and caproyl chloride. Successful substitution of hexanoyl side chains onto chitosan was confirmed by means of Fourier-transformed infrared spectrometry (FT-IR) and proton-nuclear magnetic resonance spectrometry ( $^1\text{H-NMR}$ ). An elemental analysis result indicated the degree of substitution of hexanoyl groups per glucosamine unit of chitosan. Further biocompatibility evaluations of H-chitosan film comprised the cytotoxicity testing, and the attachment, proliferation, and spreading of L929 cells cultured on the surface of H-chitosan film were determined *in vitro*. Moreover, a H-chitosan non-woven mat composed of submicrofibers was successfully fabricated by electrospinning technique. The size and morphology of the as-spun fibers was dependent on several variables including solution concentration, applied electric potential, and salt addition as revealed by scanning electron microscope (SEM) images. The possible use of the electrospun mat as a tissue scaffold or a wound dressing material was further assessed *in vitro*. Non-toxicity of the electrospun mat was revealed by indirect cytotoxicity test with L929 cells. The electrospun mat was evaluated in terms of attachment and proliferation of human keratinocytes (HaCaT) and human foreskin fibroblasts (HFF) that were seeded and cultured on the scaffolds at different time points. In addition, the interactions of the cells cultured on the fibrous scaffolds with each other and with the surrounding fibers were investigated through SEM images.

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## TABLE OF CONTENTS

	<b>PAGE</b>
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	viii
List of Figures	ix
Abbreviations	xii
List of Symbols	xiii
 <b>CHAPTER</b>	
<b>I INTRODUCTION</b>	<b>1</b>
 <b>II LITERATURE REVIEW</b>	 <b>4</b>
 <b>III <i>IN VITRO</i> BIOCOMPATIBILITY OF HEXANOYL CHITOSAN FILMS</b>	  <b>20</b>
3.1 Abstract	20
3.2 Introduction	20
3.3 Experimental	22
3.4 Results and Discussion	25
3.5 Conclusion	29
3.6 Acknowledgements	29
3.7 References	30

<b>CHAPTER</b>	<b>PAGE</b>
<b>IV ELECTROSPINNING OF HEXANOYL CHITOSAN</b>	<b>40</b>
4.1 Abstract	40
4.2 Introduction	40
4.3 Experimental	42
4.4 Results and Discussion	45
4.5 Conclusion	48
4.6 Acknowledgements	48
4.7 References	49
<b>V IN VITRO BIOCOMPATIBILITY OF ELECTROSPUN HEXANOYL CHITOSAN FIBROUS SCAFFOLDS TOWARDS HUMAN KERATINOCYTES AND FIBROBLASTS</b>	<b>62</b>
5.1 Abstract	62
5.2 Introduction	62
5.3 Experimental	64
5.4 Results and Discussion	68
5.5 Conclusion	72
5.6 Acknowledgements	73
5.7 References	73
<b>VI CONCLUSION AND RECOMMENDATIONS</b>	<b>81</b>
<b>REFERENCES</b>	<b>84</b>
<b>CURRICULUM VITAE</b>	<b>92</b>

## LIST OF TABLES

<b>TABLE</b>		<b>PAGE</b>
<b>CHAPTER IV</b>		
4.1	Viscosity, conductivity, surface tension of hexanoyl chitosan solutions in chloroform and observed average diameter and bead density of as-spun hexanoyl chitosan fibers. The applied electrostatic field strength was 12 kV/12 cm	52
4.2	Average diameter of as-spun hexanoyl chitosan fiber from 8% w/v hexanoyl chitosan solution in chloroform as a function of varying applied electric potential ranging between 8 and 18 kV over a fixed collection distance of 12 cm	53
4.3	Conductivity of 8% w/v hexanoyl chitosan solution in chloroform with addition of pyridinium formate in varying amount ranging between 0 and 10.0% w/v and observed average diameter, bead density, and observed average diameter to bead density ratio of as-spun hexanoyl chitosan fibers. The applied electrostatic field strength was 12 kV/12 cm	54
<b>CHAPTER V</b>		
5.1	Selected SEM images of HaCaT that were either seeded (during 2-24 h) or cultured (during 24 h-7 d) on H-chitosan fibrous and film scaffolds at different times of cell seeding or cell culturing	75
5.2	Selected SEM images of HFF that were either seeded (during 2-24 h) or cultured (during 24 h-7 d) on H-chitosan fibrous and film scaffolds at different times of cell seeding or cell culturing	76



## LIST OF FIGURES

FIGURE	PAGE
<b>CHAPTER II</b>	
2.1	Structure of cellulose, chitin, and chitosan <span style="float: right;">5</span>
2.2	Schematic route for deacetylation of chitin <span style="float: right;">6</span>
2.3	Schematic drawing of the electrospinning process (Dan, 2004) <span style="float: right;">14</span>
<b>CHAPTER III</b>	
3.1	A synthesis route for perfect H- chitosan <span style="float: right;">32</span>
3.2	TGA curves for chitosan and H-chitosan films. The heating rate was 10°Cmin <sup>-1</sup> <span style="float: right;">33</span>
3.3	SEM micrographs of films made of (a) H-chitosan. Mag = 500x; (b) chitosan. Mag = 500x <span style="float: right;">34</span>
3.4	Number of living cells after cultured with extraction medium for a period of 24 hours for H-chitosan film <span style="float: right;">35</span>
3.5	Attachment of L 929 cells on TCPS, chitosan and H-chitosan films after cell culture for 1, 3, 5, and 24 hours <span style="float: right;">36</span>
3.6	L929 cells proliferation on TCPS, chitosan film, and H-chitosan film <span style="float: right;">37</span>
3.7	SEM micrographs of L929 cells proliferated on H-chitosan film (a) at day 1, Mag = 500x; (b) at day 2, Mag = 500x; (c) at day 3, Mag = 500x; (d) at day 3, Mag = 3,500x; and on chitosan film (e) at day 1, Mag = 500x; (f) at day 2, Mag = 500x; (g) at day 3, Mag = 500x <span style="float: right;">38</span>
3.8	Cell spreading of L929 cells on H-chitosan and chitosan films <span style="float: right;">39</span>

<b>FIGURE</b>	<b>PAGE</b>
<b>CHAPTER IV</b>	
4.1 A synthetic route for perfect hexanoyl chitosan	55
4.2 Schematic diagram of electrospinning set-up device	56
4.3 IR spectra of (a) chitosan and (b) hexanoyl chitosan.	57
4.4 <sup>1</sup> H-NMR spectrum of hexanoyl chitosan in deuterated chloroform	58
4.5 SEM images of as-spun products from (a) 4, (b) 6, (c) 8, (d) 10, and (e) 14% (w/v) hexanoyl chitosan solutions in chloroform (magnification = 400x and scale bar = 10 μm). The applied electric field strength was 12 kV/12 cm	59
4.6 SEM images of as-spun fibers from 8% w/v hexanoyl chitosan solution in chloroform (a) without addition of pyridinium formate salt and (b) with 7.5% w/v pyridinium formate salt addition (magnification = 400x and scale bar = 10 μm). The applied electric field strength was 12 kV/12 cm	60
4.7 Surface morphology of the as-spun fibers 8% w/v hexanoyl chitosan solution in chloroform (a) without addition of pyridinium formate salt and (b) with 7.5% w/v pyridinium formate salt addition (magnification = 30000x and scale bar = 1 μm). The applied electric field strength was 12 kV/12 cm	61
<b>CHAPTER V</b>	
5.1 Chemical structure of fully-substituted hexanoyl chitosan (H-chitosan)	77
5.2 Selected SEM images of electrospun H-chitosan fiber mat from 8% w/v H-chitosan solution in chloroform with 7.5% w/v pyridinium formate	78

**FIGURE****PAGE**

- 5.3 Indirect cytotoxicity evaluation of H-chitosan fiber mats and films based on the viability of mouse fibroblasts (L929) that were cultured in the extraction media ( $10 \text{ mg}\cdot\text{mL}^{-1}$ ) from these materials. The viability of the cells cultured in fresh media was used as control. (\*)  $p < 0.05$  compared with control
- 5.4 Attachment and proliferation of (a) HaCaT or (b) HFF that were either seeded or cultured on tissue-culture polystyrene plate (TCPS; positive control) and H-chitosan fibrous and film scaffolds at various time points after cell seeding or cell culturing. 2-24 h represents the attachment period and 24-72 h represents the proliferation period. (\*)  $p < 0.05$  compared with TCPS and (#)  $p < 0.05$  compared between the fibrous and the film scaffolds at a given time point

79

80

**ABBREVIATIONS**

<sup>1</sup> H-NMR	<sup>1</sup> H-Nuclear magnetic resonance
d	Day
DD	Degree of deacetylation
DMEM	Dulbecco's modified Eagle's medium
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DS	Degree of substitution
EA	Elemental analysis
ECM	Extracellular matrix
FBS	Fetal bovine serum
FT-IR	Fourier-transformed infrared spectrometry
h	Hour
H-chitosan	Hexanoyl chitosan
HFF	Human foreskin fibroblast
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide
PBS	Phosphate buffer saline
PF	Pyridinium formate
SEM	Scanning electron microscopy
SFM	Serum-free medium
TCPS	Tissue-culture polystyrene plate
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran

**LIST OF SYMBOLS**

$\gamma$	Surface tension
$\rho$	Density
$V^*$	Critical Potential
$V_c$	Critical Voltage
$T_m$	Melting temperature
$T_d$	Degradation temperature
$[\eta]$	Intrinsic viscosity
$\bar{M}_v$	Viscosity-average molecular weight