

2009

## Effective control of cell behavior on conducting polymers

Xiao Liu

*University of Wollongong*, xiaol@uow.edu.au

Follow this and additional works at: <https://ro.uow.edu.au/theses>

### University of Wollongong

#### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

### Recommended Citation

Liu, Xiao, Effective control of cell behavior on conducting polymers, Doctor of Philosophy thesis, Department of Chemistry - Faculty of Science, University of Wollongong, 2009. <https://ro.uow.edu.au/theses/3046>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)

## **NOTE**

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

## **UNIVERSITY OF WOLLONGONG**

### **COPYRIGHT WARNING**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

# **EFFECTIVE CONTROL OF CELL BEHAVIOR ON CONDUCTING POLYMERS**

A thesis submitted in fulfilment of the  
requirements for the award of the degree

**DOCTOR OF PHILOSOPHY**

from

**UNIVERSITY OF WOLLONGONG**

by

**XIAO LIU, B.Sc, M.Sc**

**DEPARTMENT OF CHEMISTRY**

**May, 2009**

To my husband, Hongwei Wang and my parents Ziqing Liu  
and Huifen Wu for their endless love.

## CERTIFICATION

I, Xiao Liu, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry at the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for any qualifications at any other academic institution.

Xiao Liu

May 2009

## ACKNOWLEDGEMENTS

I would like to thank my supervisors, Prof. Gordon G. Wallace, Dr. Kerry J. Gilmore and Dr. Simon E. Moulton for their guidance, insight and support throughout the course of this project. The assistance of Dr. Michael Higgins with AFM measurements, Dr. Yong Liu, Dr. Jun Chen and Dr. Dan Li with fiber electrospinning, Dr. Yong Liu and Peter Sherrell with SEM measurements and Dr. Simon E. Moulton with impedance measurement is highly appreciated.

I deeply appreciate helpful assistance from the staff and students at the Intelligent Polymer Research Institute. In particular, I would like to thank Brianna Thompson, Adrian Gestos, Dr. Scott McGovern and Dr. Elise Stewart and Phil Smugreski for helpful assistance, discussions and proof-reading.

The PhD scholarship provided by Intelligent Polymer Research Institute, ARC Centre of Excellence for Electromaterials Science and University of Wollongong is gratefully acknowledged.

Finally, the unending and loving support of my husband Hongwei, my parents, sister and my friends has meant so much to me. Thank you.

## PUBLICATIONS

Yong Liu, **Xiao Liu**, Jun Chen, Kerry J. Gilmore and Gordon G. Wallace 3D bio-nanofibrous PPy/SIBS mats as platforms for cell culturing. *Chemical Communication* 2008; 28(32):3729-3731.

**Xiao Liu**, Kerry J. Gilmore, Simon E. Moulton and Gordon G. Wallace Electrical stimulation promotes nerve cell differentiation on polypyrrole/poly (2-methoxy-5 aniline sulfonic acid) composites. *Journal of Neural engineering* (Accepted)

**Xiao Liu**, Jun Chen, Kerry J. Gilmore, Michael J. Higgins, Yong Liu, and Gordon G. Wallace Guidance of Neurite Outgrowth on Aligned Electrospun Polypyrrole/ Poly(styrene- $\beta$ -isobutylene- $\beta$ -styrene) (SIBS) Fiber Platforms. *Journal of Biomedical Material Research A* (Accepted)

## CONFERENCE PRESENTATIONS

**Xiao Liu**, Kerry J Gilmore, Simon E Moulton and Gordon G Wallace Electrical Stimulation Promotes PC12 Cell Differentiation On Polypyrrole Films (oral presentation), Australasian Society for Biomaterials and Tissue Engineering (ASBTE) 19th Annual Conference Sydney, Australia, Jan. 2009



## ABSTRACT

This study explored the potential biomedical applications of polypyrrole (PPy). Electrical and topographic cues have been delivered to cells via composites of these conducting polymers, resulting in the successful control of cell behaviour.

It was found that a clinically-relevant electrical stimulation protocol (250 Hz biphasic pulsed-current) delivered directly via PPy/poly(2-methoxy-5-aniline sulfonic acid) (PMAS) films can significantly promote PC12 nerve cell differentiation in the presence of nerve growth factor (NGF), and can initiate reversible neurite sprouting from PC12 cell in the absence of NGF. The ability to promote neural outgrowth on PPy/PMAS has important implications for improving the neural/electrode interface, and this may be used to effect in nerve regeneration.

The same biphasic 250 Hz electrical stimulations were applied to a monolayer of endothelial cells on PPy/heparin films, and significantly enhanced endothelial cell migration was observed as a result. Combined with the ease of fabrication on metallic stents and the antithrombotic function of heparin, these materials may be utilized for modification of stents to improve the re-endothelialization process after implantation.

Finally, aligned PPy/poly(styrene- $\beta$ -isobutylene- $\beta$ -styrene) (SIBS) nanofibrous scaffolds were fabricated by vapor phase depositing PPy onto electrospun SIBS fibrous mats. It was shown that this novel material provided a conductive and biocompatible platform for PC12 cell adhesion and differentiation. Neurite

outgrowth was significantly influenced by the aligned fibers. High resolution AFM provided a closer inspection of the neurite outgrowths and revealed interesting physical interactions between the neurites and the aligned fibers. Aligned electroactive PPy/SIBS fibers have potential applications for improving the electrode-cellular interface of neural electrodes by encouraging guided neurite outgrowth toward the electrode through the use of electrical stimulation.

The knowledge gained during the course of this study could form the basis for improving the cellular interface of neural electrodes and stents using conducting polymers.

## ABBREVIATIONS

RGD	Arginine-Glycine-Asparagine
AFM	atomic force microscopy
BDNF	brain-derived neurotrophic factor
i	current
CV	cyclic voltammetry
DEHS	di(2-ethylhexyl) sulfosuccinate
DBSA	dodecylbenzene sulfonic acid
DBS	dodecylbenzene-sulfonate
DRG	dorsal root ganglia
DMEM	Dulbecco's modified Eagle's medium
EIS	electrochemical impedance spectroscopy
ECM	extracellular matrix
FBS	fetal bovine serum
HUVEC	human umbilical vein endothelial cell
HA	hyaluronic acid
LDH	lactate dehydrogenase
NGF	nerve growth factor
NT3	neurotrophin-3
pTS	<i>para</i> -toluene sulphonic acid
PNA	peptide nucleic acid
PMAS	poly (2-methoxy-5 aniline sulfonic acid)
PEDOT	poly (3,4-ethylenedioxy-thiophene)
PCL	poly (epsilon-caprolactone)
PLCL	poly(L-lactid- <i>co</i> -ε-caprolactone)
PLGA	poly(lactide- <i>co</i> -glycolide acid)
PEO	poly(ethylene oxide)
PLA	poly(L-lactic acid)
PMMA	poly(methyl methacrylate)

PVP	poly (vinylpyrrolidone)
PANi	polyaniline
PET	polyethylene terephthalate
PLCL	poly(l-lactid-co-ε-caprolactone)
PPy	polypyrrole
PSS	polystyrenesulfonate
PTn	polythiophene
SEM	scanning electron microscopy
SD	standard deviation
SEM	standard error of the mean
SIBS	poly(styrene-β-isobutylene-β-styrene)
THF	tetrahydrofuran
t	time
TC plastic	tissue culture plastic
UV-vis	ultraviolet visible
VPP	vapor phase polymerization

## TABLE OF CONTENTS

CERTIFICATION.....	III
ACKNOWLEDGEMENTS.....	IV
PUBLICATIONS.....	V
ABSTRACT.....	VII
ABBREVIATIONS.....	IX
TABLE OF CONTENTS.....	XI
LIST OF FIGURES.....	XVII
LIST OF TABLES.....	XXIII
<b>CHAPTER 1</b>	
<b>GENERAL INTRODUCTION.....</b>	<b>1</b>
<b>1.1 Motivation and contribution of this project.....</b>	<b>2</b>
<b>1.2 Background of this project.....</b>	<b>2</b>
<i>1.2.1 What are conducting polymers?.....</i>	<i>2</i>
<i>1.2.2 Biomedical applications of conducting polymers.....</i>	<i>5</i>
1.2.2.1 Applications of conducting polymers for tissue engineering.....	5
1.2.2.1.1 Modification of conducting polymers for tissue engineering applications.....	6
1.2.2.1.2 Application of three-dimension conducting polymer composites for tissue engineering application.....	8
1.2.2.2. Application of conducting polymers for Neural Electrodes.....	11
1.2.2.3. Biosensors.....	14
1.2.2.3.1 Modification of conducting polymers for biosensor applications.....	15

1.2.2.3.2 Applications of conducting polymers for nano-structured biosensors.....	16
1.2.2.4. Other biomedical applications of conducting polymers.....	16
1.2.2.4.1 Controlled release from conducting polymers.....	17
1.2.2.4.2 Artificial muscles.....	17
1.2.3 <i>Electrospinning and biomedical applications of electrospun fibers</i> .....	19
1.2.3.1 Electrospinning.....	19
1.2.3.2 Biomedical applications of electrospun fibers.....	21
1.2.4 <i>Cellular responses to extracellular stimuli</i> .....	24
1.2.4.1 Cellular responses to electrical stimuli.....	25
1.2.4.2 Cellular responses to topographic cues.....	27
<b>1.3 Overview of thesis</b> .....	29
<b>1.4 References</b> .....	30
 <b>CHAPTER 2</b>	
<b>EXPERIMENTAL TECHNIQUES</b> .....	48
<b>2.1 Introduction</b> .....	49
<b>2.2 Electrochemical polymerization conditions</b> .....	49
2.2.1 <i>Polymerization cell design and polymer solution processing</i> .....	49
2.2.2 <i>Electrochemical polymerization</i> .....	50
<b>2.3 Vapor phase polymerization of conductive polymers</b> .....	51
<b>2.4 Electrospinning</b> .....	52
<b>2.5 Techniques used for characterization of electroactive materials</b> .....	53
2.5.1 <i>Cyclic voltammetry</i> .....	53
2.5.2 <i>Electrical conductivity</i> .....	54

2.5.3 Electrochemical impedance spectroscopy (EIS).....	55
2.5.4 Surface wettability.....	56
2.5.5 Ultraviolet visible spectrophotometry.....	57
2.5.6 Scanning electron microscopy (SEM).....	57
2.5.7 Raman Spectroscopy.....	58
2.5.8 Atomic force microscopy (AFM).....	59
<b>2.6 Routine cell culture.....</b>	<b>60</b>
<b>2.7 Cell number assay.....</b>	<b>62</b>
<b>2.8 Fluorescence labeling and microscopy.....</b>	<b>63</b>
<b>2.9 Electrical stimulation.....</b>	<b>63</b>
<b>2.10 References.....</b>	<b>66</b>
<b>CHAPTER 3</b>	
<b>SYNTHESIS AND CHARACTERIZATION OF POLYPYRROLE FILMS.....</b>	<b>69</b>
<b>3.1 Introduction.....</b>	<b>70</b>
<b>3.2 Materials and methods.....</b>	<b>70</b>
3.2.1 Synthesis and characterization of PPy films.....	70
3.2.2 Sterilization of PPy films.....	72
3.2.3 Cell Studies.....	72
<b>3.3 Results .....</b>	<b>73</b>
3.3.1 Characterization of PPy films.....	73
3.3.1.1 Surface morphology.....	73
3.3.1.2 UV-visible spectra.....	74
3.3.1.3 Electrochemical properties of PPy films.....	75
3.3.1.4. Surface wettability of PPy films.....	79

3.3.2 Effect of sterilization methods on PPy films .....	80
3.3.3 Compatibility of PPy films with L929 cells.....	88
<b>3.4 Conclusion.....</b>	<b>94</b>
<b>3.5 References.....</b>	<b>95</b>
 <b>CHAPTER 4</b>	
<b>ELECTRICAL STIMULATION PROMOTES PC12 CELL DIFFERENTIATION ON POLYPYRROLE FILMS.....</b>	<b>99</b>
<b>4.1 Introduction.....</b>	<b>100</b>
<b>4.2 Materials and methods.....</b>	<b>103</b>
4.2.1 Materials.....	103
4.2.2 Synthesis and characterization of polypyrrole films .....	103
4.2.3 PC12 cell culture .....	103
4.2.4 Fluorescence labeling and microscopy .....	104
4.2.5 Electrical stimulation .....	104
4.2.6 Neurite length measurement.....	105
<b>4.3 Results .....</b>	<b>106</b>
4.3.1 Nerve cell differentiation on PPy/PMAS films.....	106
4.3.2 Selection of the electrical stimulation protocol .....	107
4.3.3 The effect of electrical stimulation frequency on PC12 cell differentiation.....	109
4.3.4 The effect of electrical stimulation duration on PC12 cell differentiation.....	110
4.3.5 Influence of electrical stimulation on PC12 cell differentiation in the absence of NGF.....	112
<b>4.4 Discussion.....</b>	<b>113</b>
<b>4.5 Conclusion.....</b>	<b>118</b>



4.6 References.....	119
---------------------	-----

## CHAPTER 5

<b>ELECTRICAL STIMULATION PROMOTES ENDOTHELIAL CELL MIGRATION ON POLYPYRROLE/HEPARIN FILMS.....</b>	<b>123</b>
---	------------

5.1 Introduction.....	124
-----------------------	-----

5.2 Materials and methods.....	126
--------------------------------	-----

5.2.1 Materials.....	126
----------------------	-----

5.2.2 Synthesis of polypyrrole films .....	126
--	-----

5.2.3 HUVEC cell culture and cell proliferation assay.....	126
--	-----

5.2.4 HUVEC Cell migration assay.....	127
---------------------------------------	-----

5.2.5 Fluorescence labeling and microscopy .....	128
--	-----

5.2.6 Electrical stimulation setup.....	128
---	-----

5.2.7 Statistical analysis.....	129
---------------------------------	-----

5.3 Results .....	129
-------------------	-----

5.3.1 HUVEC cell proliferation on PPy/heparin films.....	129
--	-----

5.3.2 Electrical stimulation promotes endothelial cell migration on PPy/heparin films.....	134
--	-----

5.4 Discussion.....	136
---------------------	-----

5.5 Conclusion.....	139
---------------------	-----

5.6 References.....	140
---------------------	-----

## CHAPTER 6

<b>FABRICATION OF 3D PPY/SIBS NANOFIBROUS SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS.....</b>	<b>145</b>
--	------------

6.1 Introduction.....	146
-----------------------	-----

<b>6.2 Materials and methods</b> .....	148
6.2.1 <i>Materials</i> .....	148
6.2.2 <i>Fabrication of random PPy/SIBS nanofibrous mats</i> .....	148
6.2.3 <i>Fabrication of aligned PPy/SIBS nanofibers</i> .....	150
6.2.4 <i>Characterization of PPy/SIBS nanofibers</i> .....	151
6.2.5 <i>PC12 cell culture</i> .....	151
6.2.6 <i>Fluorescence labeling and microscopy</i> .....	151
6.2.7 <i>Atomic Force Microscopy (AFM)</i> .....	152
6.2.8 <i>Quantitation of fiber alignment</i> .....	152
<b>6.3 Results</b> .....	152
6.3.1 <i>Characterization of random PPy/SIBS nanofibers</i> .....	152
6.3.2 <i>PC12 cell adhesion and differentiation on random PPy/SIBS nanofibrous mats</i> .....	154
6.3.3 <i>Characterization of aligned PPy/SIBS nanofibers</i> .....	156
6.3.4 <i>Contact guidance of neurites on aligned PPy/SIBS fibers</i> .....	158
<b>6.4 Discussion</b> .....	162
<b>6.5 Conclusion</b> .....	166
<b>6.6 References</b> .....	167
<b>CHAPTER 7</b>	
<b>CONCLUSIONS AND RECOMMENDATIONS</b> .....	171
<b>7.1 General conclusion</b> .....	172
<b>7.2 Future recommendations</b> .....	174
<b>7.3 References</b> .....	176

## LIST OF FIGURES

- Figure 1.1** Chemical structure of (A) polypyrrole, (B) polythiophene, and (C) polyaniline: leucoemeraldine ( $\gamma = 1$ ), emeraldine ( $\gamma = 0.5$ ), and pernigraniline ( $\gamma = 0$ );  $m$  determines the molecular weight..... 3
- Figure 1.2** Scheme of polypyrrole polymerization,  $A$  represent the dopant,  $n$  is determined by the degree of doping and  $m$  is determined by molecular weight..... 4
- Figure 1.3** Patterning of PPy to create microchannels for contact guidance of neurons. Phase-contrast (A, C) and fluorescence (B, D) photomicrographs of hippocampal neurons on PPy. (A, B) Cells cultured on 2  $\mu\text{m}$  wide and 200 nm deep PPy microchannels. (C, D) Cells cultured on unmodified PPy. The green labeling (Alexa 488) corresponds to Tau-1 (axonal marker) immunostaining. Scale bars represent 20  $\mu\text{m}$ . Reproduced from Gomez et al. [8]..... 9
- Figure 1.4** A) procedure used to fabricate microscale patterns of PPy chemically modified with plys or Imn. B) Scanning electron micrograph of microscale patterns of PPy (dark area). C) Phase contrast and D) fluorescent images of DRG adhered to a surface of PPy. Neurites stained positive for GAP-43 (green fluorescence). Reproduced from Song et al. [53]..... 10
- Figure 1.5** a) a stained slice at 6 weeks post-surgery with neurons (blue) and glia (green), scale bar: 100  $\mu\text{m}$ , No severe gliosis is seen surrounding the implant, scale bar: 200  $\mu\text{m}$ . Arrows indicate region where cells are extending into the PPy lumen and black is the space occupied by PPy/NGF/BDNF implant. ( b) PPy/NaDBS film, scale bars: 200  $\mu\text{m}$ , (c) fluorescently labeled explanted cortical neurons growing and forming networks on a PPy/NaDBS surface after 21 days, scale bar: 50  $\mu\text{m}$ . green: neurons, red: glia, blue: nuclei. Reproduced from George et al..... 13
- Figure 1.6** A) PEDOT (rough, nodular texture) polymerized around living nerve cells on Au/Pd electrode surface, reveals that PEDOT uses the cell membrane as a scaffold for polymerization. (B) Diagram representing the process of polymerizing PEDOT directly into brain tissue from a neural electrode device. Reproduced from Richardson-Burns et al..... 14

- Figure 1.7** Movements of a polypyrrole/nonconducting polymer bilayer (c) produced by the local stress gradients and bending originating at the interface between the two layers owing to reversible conformational changes in polypyrrole during oxidation (a) and reduction (b) processes. Reproduced from Otero et al..... 18
- Figure 1.8** A) Schematic illustration of the setup used to electrospun nanofibers as uniaxially aligned arrays. The collector was composed of two conductive substrates separated by a void gap. B) Dark-field optical micrograph of PVP nanofibers collected across a void gap formed between two silicon strips. C) SEM image of a 2x2 array of crossbar junctions constructed by sequentially transferring two layers of PVP nanofibers onto the same substrate. Reproduced from Li et al..... 20
- Figure 1.9** A) Electrospun nylon nanofibers are collected on a rotating copper wire drum during electrospinning. B) SEM images of axially-aligned polymer nanofibers on a conductive copper wire drum. Reproduced from Katta et al ..... 20
- Figure 1.10** Effects of poly(L-lactide) (PLLA) fiber alignment on neurite extension. DRG grown for 3 days and then stained for neurofilaments are shown on randomly aligned fibers (A), intermediate fibers (B), and highly aligned fibers (C). Scale bar represents 100  $\mu\text{m}$ . Reproduced from Joseph et al..... 23
- Figure 1.11** A) Randomly oriented and B) aligned substrates after 7 days showing the global alignment and length of myotubes. Arrows indicate direction of PLLA nanofibers. Scale bars are 100  $\mu\text{m}$ . Reproduced from Huang et al..... 23
- Figure 1.12** Xenopus neurons that landed fortuitously at the boundary between grooved regions (right) and flat regions (left) of the slides during plating. grooves are 1  $\mu\text{m}$  wide and 320 nm deep (a) or 520 nm deep (b). Note that for each cell neurites on grooves are straighter and longer than those on the flat region. bar, 50  $\mu\text{m}$ . Reproduced from Rajnicek et al..... 28
- Figure 2.1** Schematic diagram of the electrochemical polymerization cell setup..50
- Figure 2.2** Schematic diagram showing the vapor phase polymerization technique..... 52
- Figure 2.3** Schematic diagram showing the electrospinning technique..... 53

- Figure 2.4** a) Cyclic potential sweep, (b) resulting cyclic voltammogram..... 54
- Figure 2.5** Schematic diagram showing 4-point probe configuration..... 55
- Figure 2.6** Contact angle ( $\Theta$ ) of a 2  $\mu$ L water droplet on a polymer surface..... 57
- Figure 2.7** SEM image of an AFM cantilever (A) and schematic diagrams showing DC mode (B), AC mode (C) AFM and Force-vs.-distance curve(D). Reproduced from <http://www.nanoscience.com/education/AFM.html>..... 60
- Figure 2.8** Electrical stimulation setup: (A) top view of the custom made 4-well culture slide, (B) front view of 4-well culture slide and current waveforms of 10Hz (C1), 100Hz (C2) and 250 Hz (C3) electrical stimulation signal..... 65
- Figure 2.9** An example of neurite length measurement. The yellow lines in the right image identify measured neurites..... 65
- Figure 3.1** SEM micrographs of PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C) and PPy/NO<sub>3</sub> (D) films. The insert images were taken at high magnification; the scale bars represent 1  $\mu$ m..... 74
- Figure 3.2** UV-vis spectra PPy/PMAS (A), PPy/NO<sub>3</sub> (B), PPy/heparin (C) and PPy/pTS (D) films..... 75
- Figure 3.3** Cyclic voltammetry of PPy/PMAS (A), PPy/heparin (B), PPy/pTS (C), and PPy/NO<sub>3</sub> (D) films in 0.1 M NaNO<sub>3</sub> at a scan rate of 100 mV/s. The arrows indicate the direction of potential scan..... 78
- Figure 3.4** Impedance spectra for PPy/NO<sub>3</sub> (A), PPy/heparin (B), PPy/pTS (C) and PPy/PMAS (D) films recorded in PBS (pH = 7.2) at +50.0 mV (vs. Ag|AgCl)..... 77
- Figure 3.5** SEM images of PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C) and PPy/NO<sub>3</sub> (D) films before (1) and after (2) autoclaving. The scale bars represent 5  $\mu$ m..... 82
- Figure 3.6** Contact angle of H<sub>2</sub>O on PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C) and PPy/NO<sub>3</sub> (D) films before treatment (1) and following sterilization using Ethanol-UV treatment (2) and autoclaving (3)..... 84
- Figure 3.7** Contact angle of H<sub>2</sub>O on different PPy films following sterilization using ethanol-UV treatment (labelled as EtOH-UV) and autoclaving.... 84

- Figure 3.8** Cyclic voltammetry scans of PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C) and PPy/NO<sub>3</sub> (D) films before (solid line) and after ethanol-UV treatment (dashed line) in 0.1 M NaNO<sub>3</sub>, at a scan rate of 100mV/s... 85
- Figure 3.9** Cyclic voltammetry scans of PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C) and PPy/NO<sub>3</sub> (D) films before (solid line) and after autoclaving (dashed line) in 0.1 M NaNO<sub>3</sub>, at a scan rate of 100mV/s. ....86
- Figure 3.10** L-929 cell number assay on different PPy films and polypropylene cell culture plates over a period of 72 h. Error bars represent the standard error of triplicate cell cultures..... 87
- Figure 3.11** Time course for growth of L929 cells on different PPy films and TC plastic over a period of 72 h. Error bars represent the standard error of triplicate cell cultures..... 89
- Figure 3.12** Optical images of L929 cells on PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C), PPy/NO<sub>3</sub> (D) films and tissue culture plastic (E). Images were taken at 72 h. The scale bars represent 20 μm.....91
- Figure 3.13** Fluorescence images of Calcein-stained L929 cells on PPy/heparin (A), PPy/PMAS (B), PPy/pTS (C), PPy/NO<sub>3</sub> (D) films and tissue culture plastic (E). Images were taken 72 h after seeding. The scale bars represent 50 μm.....92
- Figure 3.14** SEM images of L929 cells on PPy/heparin (A) and PPy/PMAS (B) films after 72 h of seeding.....93
- Figure 4.1** The chemical structure of PMAS, m determines the molecular weight..... 102
- Figure 4.2** Waveforms of 200 Hz (A), 250 Hz (B), 100 Hz (C) and 10 Hz (D) electrical stimulation signal..... 105
- Figure 4.3** Fluorescence (A and C) and SEM (B and D) images of PC12 cells after 144 h culture in proliferation medium (A and B) or differentiation medium (C and D) ..... 106
- Figure 4.4** Potential response of PPy chamber slider to 200 Hz biphasic ±1 mA pulsed current after 1 min (A) and 59 min (B) ..... 108
- Figure 4.5** Potential response of PPy chamber slider of 250 Hz biphasic ±1 mA pulsed current..... 109

- Figure 4.6** Neurite length distribution of PC12 cells on PPy/PMAS film without (A) and with 3 days of 8 h per day stimulation at 10 Hz (B), 100 Hz (C) and 250 Hz (D) on PPy/PMAS films, and phase-contrast images of PC12 cells on PPy/PMAS films without (E) and with (F) 250 Hz electrical stimulation. The scale bars represent 20  $\mu\text{m}$ .....111
- Figure 4.7** Percentage of neurite longer than 60  $\mu\text{m}$  after different durations of electrical stimulation..... 112
- Figure 4.8** Phase-contrast images of PC 12 cells on PPy/PMAS films without (A) and with (B) electrical stimulation in the absence of NGF. The scale bars represent 20  $\mu\text{m}$ ..... 113
- Figure 5.1** Structure of heparin..... 124
- Figure 5.2** Waveform of 250 Hz electrical stimulation signal..... 129
- Figure 5.3** The proliferation of HUVEC cells on PPy films and tissue culture plastic (TCP) over a period of 192 h. Data points are the mean  $\pm$  SEM for triplicate determinations in three independent cell cultures. Asterisk shown for 144 h data represents significant difference from controls ( $p < 0.05$ , Student's T test)..... 131
- Figure 5.4** Phase contrast images of HUVEC cells on (A) TCP, (B) PPy/heparin, (C) PPy/pTS and (D) PPy/ $\text{NO}_3$  films 144 h after seeding. The scale bars represent 20  $\mu\text{m}$ ..... 132
- Figure 5.5** Fluorescence image of Alexa Fluor 488 phalloidin-stained HUVEC cells on PPy/heparin film 144 h after seeding. The scale bar represents 20  $\mu\text{m}$ ..... 132
- Figure 5.6** SEM micrographs of a HUVEC cell on a PPy/heparin film (A). At high magnification, the strong adhesion point (arrows) of the cells to the substrate could be seen (B)..... 133
- Figure 5.7** Images of typical wounds of HUVEC cells monolayers on PPy/heparin films without (A) and with (B) electrical stimulation at 0 h (1) and 6 h (2). The scale bars represent 100  $\mu\text{m}$ ..... 135
- Figure 5.8** The effect of electrical stimulation on HUVEC cell wound closure on PPy/heparin films. Data points represent the mean  $\pm$  SEM of eight determinations. This experiment has been repeated three times and the same trends were observed..... 135

- Figure 6.1** Electrospinning setup demonstrating the synthesis of random PPy/SIBS nanofiber mats.....149
- Figure 6.2** Electrospinning setup demonstrating the synthesis of aligned PPy/SIBS nanofibers..... 150
- Figure 6.3** SEM micrograph of an electrospun PPy/SIBS nanofibrous mat..... 153
- Figure 6.4** A) High magnification SEM image of an electrospun PPy/SIBS fiber. (B) AFM image of fibers, indicating the positions of the corresponding height cross-sections shown in (C).....153
- Figure 6.5** Raman spectra of (A) PPy/SIBS electrospun fibers, (B) the PPy film prepared by VPP and (C) SIBS fibers. Excitation wavelength was 632.8 nm.....154
- Figure 6.6** SEM (A) and fluorescence (B) micrograph of differentiated PC12 on PPy/SIBS nanofibrous mat after 144 h of culture. Cells were stained using Alexa Fluor 488 phalloidin..... 155
- Figure 6.7** Optical images of electrospun PPy/SIBS nanofibers obtained on a rotating drum with different rotation speeds and collection times, a) 1000 rpm, 1 min; b) 1500 rpm, 1 min; c) 2000 rpm, 1 min and d) 2000 rpm, 5 min. The scale bars represent 20  $\mu\text{m}$ ..... 157
- Figure 6.8** A) SEM image of the aligned electrospun PPy/SIBS fibres. (B) Histogram of the measured angles between the aligned fibres and rotation direction of the collection drum.....158
- Figure 6.9** A and B) Fluorescence images of PC12 cells on the aligned electrospun PPy/SIBS fibres. The PC12 cell and neurite outgrowths (arrows in A) are indicated by their Alexa Fluor 488 phalloidin (green) staining. (C) Histogram of the neurite orientation (angle) on the aligned PPy/SIBS fibres. The fitted Gaussian curve gives a mean of  $2.4 \pm 4.2^\circ$  (mean  $\pm$  SEM). (D) Fluorescence images of PC12 cells on PPy films without PPy/SIBS fibres. (E) Histogram of the neurite orientation (angle) on the PPy films. Scale bars represent 10  $\mu\text{m}$ .....160
- Figure 6.10** A) Optical and (B) corresponding AFM images of the boxed region in (A) showing a neurite (arrows) extending along a PPy/SIBS fibre (black asterisks). Scale bar in (A) = 20  $\mu\text{m}$ . Scale bar in (B) = 5  $\mu\text{m}$ . (C) High resolution AFM image showing small finger-like lateral outgrowths (arrows) making contact with a PPy/SIBS fibre (black asterisks). Scale in (C) =1  $\mu\text{m}$ ..... 161



**Figure 7.1** Schematic of a combination of electrical, biochemical and topographical cues for neural tissue engineering scaffolds..... 175

## LIST OF TABLES

**Table 3.1** Summary of the dopant concentrations.....71

**Table 3.2** Conductivity of PPy films containing different dopants..... 75

**Table 3.3** Contact angle of water on different PPy films..... 80