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The controlled release of neurotrophic proteins from the conducting polymer polypyrrole to improve the nerve/cochlear implant interface

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**THE CONTROLLED RELEASE OF NEUROTROPHIC PROTEINS
FROM THE CONDUCTING POLYMER POLYPYRROLE TO
IMPROVE THE NERVE/COCHLEAR IMPLANT INTERFACE**

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

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

BRIANNA THOMPSON, B. Biotech (Adv.) (Hons)

SCHOOL OF CHEMISTRY

(2009)

CERTIFICATION

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

Brianna C. Thompson

20th January, 2009

ABSTRACT

The cochlear implant has restored hearing to hundreds of thousands of profoundly deaf individuals since the early 1980s. The implant works by providing electrical stimulation directly to the auditory nerves, bypassing the damaged sensory processes. As the electrodes of the cochlear implant are required to directly stimulate the auditory nerves, the interface between these electrodes and the nervous system is a vital part of the function of the bionic device. However, in cases of long-term hearing loss the auditory nerve degenerates and auditory nerve processes, or neurites, retract away from the cochlea and the intra-cochlear electrodes. This leads to a loss of effectiveness of the cochlear implant. Currently the only remedy for this problem is to amplify the electrical signals provided to the implant, but this causes shorter battery life due to the higher currents and voltages and can potentially be damaging to the tissues in and surrounding the cochlea. This work describes attempts to develop a material which can be coated onto cochlear electrodes to preserve auditory nerves, potentially improving the interface between the cochlear implant and the auditory nerve.

The material used throughout this work, polypyrrole, is a conducting polymer. Due to its unique properties, polypyrrole can be used to incorporate and release charged molecules with different electrical stimuli. The work presented within this thesis describes the development of polypyrrole to release nerve growth factors, which are also known as neurotrophins, in order to improve auditory nerve survival and growth. With delivery of nerve growth factor in response to the electrical stimuli provided by the cochlear implant, it is hoped that better nerve survival will lead to an improved nerve/cochlear electrode interface, and better function of the implant. Extensive tests on the release of radiolabelled neurotrophins from polypyrrole substrates were performed under a variety of conditions in order to optimise both the material and the electrical stimulation used for controlled release. The focus of most work was neurotrophin-3, the member of the neurotrophin family of proteins that has the most widespread action on cell receptors.

Testing of the response of auditory nerve tissue dissected from rats showed that neurotrophins released from polypyrrole promoted the survival and growth of nerves *in*

vitro. From these experiments, it was determined that electrical stimulation was effective in controlling the release of neurotrophins to improve neurite outgrowth to a significant degree. Based on these results *in vivo* experiments were undertaken, in which guinea pigs were implanted with cochlear electrodes coated with polypyrrole. From these experiments, it was determined that electrical stimulation to promote neurotrophin release helped not only to prevent implantation trauma, but also to prevent deafening-related degeneration of auditory nerves observed in untreated cochleae.

Additionally, some further optimisation of the materials was completed. Another neurotrophin, brain derived neurotrophic factor, has been shown to work synergistically with neurotrophin-3, and the release of both neurotrophins from a single polypyrrole film was investigated. While the release of the proteins was not as well controlled, the effect of release on auditory nerve explants cultured *in vitro* was significantly greater than either neurotrophin alone. Studies exploring release of neurotrophin-3 from PPy coated on several novel substrates were performed, suggesting that biodegradable and nanostructured materials can be used to further tailor the release of therapeutic proteins from polypyrrole. An implantable power source - a “biobattery” - was also used to electrically control the release of neurotrophin-3, highlighting the potential for a totally implantable system for the controlled release of therapeutic molecules using the polypyrrole materials developed.

The insights gained in the course of this work may have implications for the controlled release of other therapeutic molecules from polypyrrole. Additionally, the information contained within this thesis could also have relevance beyond the scope of the cochlear implant/neural interface, with potential for use in other bionic devices or in nerve repair applications. The work described here demonstrates the potential to enhance nerve/electrode interfaces using polypyrrole to electrically control the release of neurotrophins.

TABLE OF CONTENTS

Certification.....	ii
Abstract	iii
Table of Contents	v
List of Figures	xi
List of Tables.....	xix
Abbreviation and Notation.....	xx
Publications	xxii
Conference Presentations.....	xxii
Acknowledgements.....	xxiv
1. INTRODUCTION.....	1
1.1 Conducting Polymers.....	2
1.1.1 Conductivity and electroactivity of conducting polymers	3
1.2 Polypyrrole.....	4
1.2.1 Synthesis	5
1.2.2 Polymer properties	6
1.2.3 Electrochemistry	7
1.3 Biomaterials	9
1.3.1 Polypyrrole as a Biomaterial.....	12
1.4 Controlled Release	13
1.4.1 Controlled release from polypyrrole	15
1.5 The Bionic Ear and Cochlear Implants	19
1.6 Neurotrophins.....	23
1.6.1 Neurotrophins for inner ear therapies	26
1.7 Aims of the project.....	28
1.8 References	29
2. GENERAL EXPERIMENTAL	38
2.1 Introduction	39
2.2 Reagents and Materials	39
2.2.1 Polymer materials.....	39
2.2.1.1 Monomer.....	39
2.2.1.2 Dopants	39
2.2.1.3 Materials for vapour phase polymerisation.....	40
2.2.2 Neurotrophins.....	41
2.2.2.1 Radio-labelling.....	41
2.2.3 Solutions.....	42
2.2.3.1 Polymerisation solutions	42
2.2.3.2 Release media.....	43
2.3 Polypyrrole synthesis	43
2.3.1 Electrochemical growth	43
2.3.1.1 Galvanostatic synthesis	43
2.3.1.2 Potentiodynamic growth	45
2.3.2 Chemical synthesis.....	46
2.3.2.1 Vapour phase growth	46

2.4	Polypyrrole Characterisation.....	47
2.4.1	Electrochemical characterisation	47
2.4.2	Scanning Electron Microscopy	47
2.4.3	Contact Angle Measurements	47
2.4.4	Profilometry	47
2.4.5	Atomic Force Microscopy.....	48
2.5	Neurotrophin Release	48
2.5.1	Electrochemical Cell Setup	48
2.5.2	Instrumentation	49
2.6	Culture of primary auditory neurons on polypyrrole	50
2.6.1	Sterile synthesis of PPy for SGN culture	50
2.6.2	Preparation of surfaces for SGN culture	51
2.6.3	Animals used for SGN dissection	51
2.6.4	Dissection and culture of SGN explants	52
2.6.5	Electrical stimulation of PPy and explants under culture conditions.....	53
2.6.6	Fixation and immunohistochemistry of explants	53
2.6.7	Quantifying explant response to NT-3	53
2.7	References	54

3. OPTIMISATION OF INCORPORATION AND RELEASE OF NEUROTROPHIN-3 FROM POLYPYRROLE FILMS 55

3.1	Introduction	56
3.1.1	Previous work in release of therapeutic proteins from polypyrrole	56
3.1.2	Choice of therapeutic protein	56
3.1.3	Polypyrrole	57
3.2	Aims	58
3.3	Methods	59
3.3.1	Growth of PPy/pTS/NT-3	59
3.3.2	Characterisation of PPy/pTS/NT-3	59
3.3.3	Release of NT-3 from PPy/pTS	60
3.3.3.1	Charge calculations	61
3.4	Results and Discussion	62
3.4.1	Optimising incorporation of NT-3 in polypyrrole films	62
3.4.1.1	Polypyrrole synthesis method	62
3.4.1.2	Optimising galvanostatic growth	64
3.4.1.2.1	Current density	64
3.4.1.2.2	Polymerisation solution.....	65
3.4.1.2.3	Neurotrophin concentration	66
3.4.1.2.4	Two-layer deposition	67
3.4.1.2.5	Polymerisation Time	67
3.4.2	Optimising release of NT-3 from polypyrrole films	72
3.4.2.1	Polypyrrole synthesis method	72
3.4.2.2	Growth time	73
3.4.2.3	The stimulation protocol	75
3.4.2.3.1	Short term	75
3.4.2.3.2	Long term	78
3.4.2.4	Dual electrode release	82
3.4.2.5	Interdigitated micro-electrodes	83
3.5	Conclusions	85

3.6	References	86
4.	EFFECTS OF NEUROTROPHIN-3 RELEASE FROM PPy/PTS ON PRIMARY AUDITORY NERVE CELLS	88
4.1	Introduction	89
4.1.1	NT-3 effects on auditory nerves.....	89
4.1.2	Spiral ganglion neuron cells.....	90
4.2	Aims.....	91
4.3	Methods.....	92
4.3.1	Sterile growth of PPy on ECIS plates	92
4.3.2	Preparation of surfaces for SGN culture	92
4.3.3	Animals	92
4.3.4	Dissection and culture of SGN explants	92
4.3.5	Electrical stimulation of PPy and explants under culture conditions.....	93
4.3.6	Fixation and immunohistochemistry of explants.....	94
4.3.7	Quantification of explant response to NT-3	94
4.3.8	SEM of SGN explants on PPy	95
4.3.9	Determining NT-3 release under cell culture conditions.....	96
4.4	Results and Discussion.....	96
4.4.1	PPy/pTS/NT-3 growth on ECIS plates under sterile conditions.....	96
4.4.2	Validation of neurite outgrowth method.....	96
4.4.3	Toxicity testing of PPy/pTS components.....	99
4.4.4	Neurite outgrowth from SGN explants on PPy.....	100
4.4.5	Neural and cellular interactions on PPy.....	102
4.4.6	Electrical Stimulation of SGN explants on PPy/pTS/NT-3 films.....	104
4.4.6.1	Impedance	104
4.4.6.2	NT-3 release data	105
4.4.6.3	Effect of short term electrically stimulated NT-3 release from PPy films on SGN explants	107
4.5	Conclusions	110
4.6	References	111
5.	CHANGING DOPANT DURING POLYPYRROLE SYNTHESIS: MODULATION OF NT-3 INCORPORATION, RELEASE AND EFFECT ON PRIMARY AUDITORY NERVE CELL RESPONSES	114
5.1	Introduction	115
5.1.1	Growth of PPy with different dopants	115
5.2	Aims.....	117
5.3	Methods.....	117
5.3.1	Growth of polypyrrole with various dopants	117
5.3.2	Characterisation of PPy grown with various dopants	118
5.3.3	Incorporation and release of NT-3	118
5.3.4	Gel electrophoresis of PPy/dopant mixtures.....	118
5.3.4.1	Polyacrylamide gel electrophoresis.....	119
5.3.4.2	Agarose gel electrophoresis	120
5.3.5	SGN explant culture on PPy grown with various dopants.....	120
5.4	Results and Discussion.....	121

5.4.1	Growth of polypyrrole with various dopants	121
5.4.2	Characterisation of PPy films.....	123
5.4.2.1	Scanning electron microscopy	123
5.4.2.2	Cyclic voltammetry	124
5.4.2.3	Water contact angle measurements.....	127
5.4.3	Incorporation and release of NT-3 from polypyrrole with various dopants 128	
5.4.3.1	Comparing NT-3 incorporation in variously-doped PPy films.....	128
5.4.3.2	Comparing NT-3 release from variously-doped PPy films	130
5.4.3.3	Investigation of interaction between NT-3 and dopant molecules....	136
5.4.4	Response of SGN explants to variously-doped polypyrrole surfaces...	139
5.4.4.1	Effects of NT-3 release from variously-doped PPy on SGN explants 143	
5.5	Conclusions	145
5.6	References	146

6. INTRACOCHELEAR RELEASE OF NEUROTROPHIN-3 FROM POLYPYRROLE IN GUINEA PIGS 148

6.1	Introduction	149
6.2	Aims	150
6.3	Methods.....	150
6.3.1	Growth of PPy on GP cochlear electrodes.....	150
6.3.2	Release experiment details.....	151
6.3.3	Guinea pig procedures.....	151
6.3.3.1	Guinea pig deafening	152
6.3.3.2	Implantation of PPy-coated GP cochlear electrode	152
6.3.3.3	Electrical stimulation for NT-3 release <i>in vivo</i>	153
6.3.3.4	Perfusion and cochlea processing	154
6.3.3.5	Assessing fibrous tissue response to PPy.....	154
6.3.3.6	SGN counts	154
6.3.3.7	Statistical analysis	155
6.4	Results and Discussion.....	155
6.4.1	Growth of PPy on GP cochlear electrodes.....	155
6.4.2	Incorporation and release of NT-3 from PPy on GP cochlear electrodes	158
6.4.3	Tissue response to PPy-coated GP cochlear electrodes.....	160
6.4.4	Electrically-stimulated release of NT-3 from PPy-coated GP cochlear electrode	161
6.5	Conclusions	162
6.6	References	163

7. INCORPORATION AND RELEASE OF ANOTHER NEUROTROPHIN, BRAIN DERIVED NEUROTROPHIC FACTOR, FROM POLYPYRROLE..... 165

7.1	Introduction	166
7.1.1	Brain-derived neurotrophic factor.....	166
7.1.2	Neurotrophin effects on auditory nerves.....	168
7.2	Aims	169

7.3	Methods	170
7.3.1	Incorporation and release of BDNF	170
7.3.2	Incorporation and release of both NT-3 and BDNF.....	170
7.3.3	SGN explant culture.....	171
7.3.3.1	Assessing SGN explant response to BDNF	171
7.3.4	Culture of SGN explants on PPy/pTS/BDNF/NT-3 films	171
7.4	Results and Discussion	172
7.4.1	Incorporation and release of BDNF from PPy.....	172
7.4.1.1	Incorporation of BDNF into PPy/pTS	172
7.4.1.2	Release of BDNF from PPy/pTS	172
7.4.2	Effects of BDNF release on SGN explants.....	174
7.4.2.1	Assessing response of SGN explants to BDNF in culture media	174
7.4.2.2	Effect of BDNF release from PPy.....	175
7.4.3	Dual Neurotrophin incorporation and release from PPy/pTS/BDNF/NT-3	178
7.4.3.1	Simultaneous incorporation of NT-3 and BDNF into PPy	178
7.4.3.2	Simultaneous release of BDNF and NT-3 from PPy	179
7.4.4	Effects of dual neurotrophin release from PPy on SGN explants.....	183
7.5	Conclusions	186
7.6	References	187

8. TECHNOLOGIES FOR NEXT-GENERATION CONTROLLED RELEASE DEVICES 189

8.1	Introduction	190
8.1.1	Applications of controlled release of neurotrophins	190
8.1.1.1	Future cochlear implant development.....	190
8.1.1.2	Spinal and other applications	191
8.1.2	Carbon nanotubes.....	192
8.1.2.1	3D aligned MWNT forest structures.....	193
8.1.2.2	2D aligned MWNT structures.....	193
8.1.3	PLGA	194
8.1.4	Vapour phase polymerisation of polypyrrole.....	194
8.1.5	Biodegradable batteries.....	195
8.2	Aims	196
8.3	Methods	196
8.3.1	Vapour phase polymerisation of polypyrrole.....	196
8.3.2	Preparation of 3D PPy-coated MWNT material	197
8.3.2.1	Incorporation of NT-3 into VPP layer of 3D MWNT substrates.....	199
8.3.2.2	CV coating of 3D MWNT substrates.....	199
8.3.3	Preparation of 2D PPy-coated MWNT material	199
8.3.3.1	VPP coating of 2D MWNT material.....	199
8.3.4	Preparation of PPy-coated electrospun PLGA material.....	200
8.3.5	Characterisation of materials.....	202
8.3.5.1	Scanning electron microscopy	202
8.3.5.2	NT-3 release	202
8.3.6	Biodegradable battery	202
8.4	Results and Discussion	205
8.4.1	Novel substrates	205
8.4.1.1	Substrates coated with PPy using CV method	205

8.4.1.1.1	Flat films	205
8.4.1.1.2	3D aligned MWNT forest substrates.....	209
8.4.1.1.3	Biodegradable PLGA electrospun substrates.....	213
8.4.1.1.4	Comparison of incorporation and release of NT-3 from CV-grown PPy using various substrates	217
8.4.1.2	Substrates coated by PPy using vapour phase polymerisation method... ..	220
8.4.1.2.1	Flat films	220
8.4.1.2.2	3D aligned MWNT forest substrates.....	222
8.4.1.2.3	2D MWNT aligned substrates.....	225
8.4.1.2.4	Comparison of incorporation and release of NT-3 from VPP-grown PPy using various substrates.....	228
8.4.1.3	Summary of NT-3 release from novel substrates.....	230
8.4.2	Biobattery-controlled release of NT-3	231
8.5	Conclusions	234
8.6	References	235

9. CONCLUSIONS..... 237

LIST OF FIGURES

- Figure 1.1** – Structures of several undoped conducting polymers. (a) polyacetylene, (b) polyaniline, (c) polythiophene, (d) polypyrrole and (e) poly(3,4-ethylenedioxythiophene). 3
- Figure 1.2** – Schematic of oxidative polymerisation of polypyrrole incorporating a dopant anion A^- . Reproduced from Wallace *et al.* [5]. 5
- Figure 1.3** – De-doping and re-doping of PPy films with reduction and re-oxidation. A^- represents the anionic dopant incorporated into the PPy film during synthesis of the oxidised polymer. Note that at each stage, the net charge on the polymer or polymer-dopant complex is neutral. 8
- Figure 1.4** – Cation exchange with oxidation and reduction of PPy films. A^- represents a large, immobile anionic dopant incorporated into the PPy film during synthesis of the polymer. X^+ represents a mobile cation present in the electrolyte. Note that after reduction of the PPy, the net charge on the polymer-anion complex is negative, driving the incorporation of the X^+ cation to charge-balance the PPy. 9
- Figure 1.5** - Cross section of a whole cochlea, illustrating direction of fluid flow in the chambers of the spiral-shaped organ. Labels are as follows: 1 - scala media, filled with endolymph; 2 - scala vestibule, filled with perilymph flowing towards the apex of the cochlea; 3 - scala tympani, filled with perilymph flowing toward to base of the cochlea and the round window; 4 - spiral ganglion, containing auditory nerve bodies; and 5 - auditory nerve fibre. The organ of Corti and the hair cells are shown in pink. Image reproduced with permission from from "Promenade around the cochlea" EDU website <http://www.cochlea.org> by Rémy Pujol *et al.* INSERM and University Montpellier (date accessed: 1st December 2008). 20
- Figure 1.6** – The cochlear implant inserted into a patient, showing the external and internal components. The microphone detects noise, sending the signal to the speech processor which encodes the correct nerve stimulation. This signal is delivered to the auditory nerves by the electrode array which is implanted within the cochlea. Source: ABC news website <http://www.abc.net.au/rn/boyerlectures/images/2007/image.htm> (date accessed: 1st December 2008). 21
- Figure 1.7** – Cross section of one turn of the cochlea, showing the positioning of the cochlear electrode array within the scala tympani relative to SGNs and the Organ of Corti, with a deafened Organ of Corti pictured inset. Reproduced from [97]. 23
- Figure 1.8** – Schematic of the production, release, and signalling of neurotrophins to effect changes in target neurons. In this example, neurotrophin is produced by a neuron and a non-neural cell, an astrocyte. After release from these cells, the neurotrophin binds to a Trk receptor on the target neuron and is internalised, causing a signalling pathway to be activated. Source: Society for Neuroscience Brain Briefing “Neurotrophic Factors” published online at http://www.sfn.org/index.cfm?pagename=brainBriefings_neurotrophicFactors (date accessed: 12th November 2008). 25
- Figure 1.9** – Neurotrophin interactions with cell surface receptors and activation of intracellular signalling cascades. Mature NT-3 and BDNF interact primarily with TrkC and TrkB, respectively, increasing the signals for survival, differentiation and synaptic plasticity. Adapted from [113]. 26
- Figure 1.10** – Preservation of SGNs within the cochlea of guinea pigs with neurotrophin treatment. (a) shows a normal, undeaftened cochlea, (b) shows a deafened, neurotrophin-treated cochlea and (c) shows a deafened, untreated cochlea. The SGN cells (indicated by the arrows) were preserved when BDNF and NT-3 were delivered by a mini-osmotic pump for 28 days. Scale bars shows 20 μm . Adapted from [124]. 27
- Figure 2.1** – Pyrrole monomer unit. 39
- Figure 2.2** – Structures of dopant molecules used in PPy synthesis in this project (PMAS structure from [1] and HA and CS structures from [2]). 40
- Figure 2.3** – Two electrode electrochemical cell for galvanostatic polypyrrole synthesis on a Au-coated mylar electrode. 44

Figure 2.4 – Three electrode electrochemical cell for cyclic voltammetric deposition of polypyrrole on a Au-coated mylar electrode.....	45
Figure 2.5 – Two electrode electrochemical cell for stimulating release of neurotrophins from polypyrrole films.	48
Figure 2.6 - Waveform applied by experimental cochlear implant stimulator. Current levels of 0.2, 1 or 2 mA (peak), and pulse widths of 50, 100 or 20 μ sec were used in the present study. Pulse rates used included 2.5, 25 or 250 Hz (achieved by varying the short circuit period between current pulses). The open circuit potential (OCP) period was not varied.	50
Figure 2.7 – ECIS plate used for sterile PPy deposition and subsequent culture of cells. The grey represents the common reference electrode, while the black circles indicate the working electrodes, onto which PPy was deposited. Each well was individually addressable, and the plates could be used to perform electrical stimulation of polymers and cells under tissue culture conditions.....	51
Figure 2.8 – Procedure for dissection of SGN explants from Day 4-6 rat pups.	52
Figure 3.1 – Electrochemical basis of drug delivery from PPy, showing release of the dopant A- during redox cycling of PPy.....	58
Figure 3.2 – Electrochemical cell for “dual electrode” stimulation of NT-3 release from PPy/pTS/NT-3.	61
Figure 3.3 – Cyclic voltammetric growth of PPy/pTS without (a) and with (b) NT-3 added to the polymerisation solution. The first 10 scans are shown. Scan rate = 50 mV/sec. Arrows show the direction of the applied voltage.	63
Figure 3.4 – Cyclic voltammogram obtained using PPy/pTS as the working electrode in 0.2 M PBS (pH 7.4). The third scan is shown. Scan rate = 100 mV/sec. Arrows show the direction of the applied voltage sweep.	65
Figure 3.5 – Proposed scheme for incorporation of NT-3 into polypyrrole using a co-dopant. A ⁻ represents a dopant anion.....	67
Figure 3.6 – Film thickness of polymers grown under galvanostatic conditions at 2 mA/cm ² for various times from a monomer solution containing 0.2 M pyrrole/0.05 M pTS and 2 μ g/mL NT-3.....	68
Figure 3.7 – The effect of polymerisation time on the mass of NT-3 incorporated into PPy/pTS films. Polymerisation was performed using a current density of 2 mA/cm ² with 0.2 M pyrrole and 0.05 M pTS and NT-3 at 2 μ g/mL. Each point represents the average of two samples, and the error bars show the range.	69
Figure 3.8 – Comparison of NT-3 adsorption onto bare gold-mylar and PPy/pTS-coated electrodes. The PPy/pTS layer was grown at 2 mA/cm ² for 90 seconds.	70
Figure 3.9 – AFM images of (a) bare gold mylar electrode and (b) 90 second-grown PPy/pTS coated electrode. The image analysis statistics for surface area of each area analysed are shown.	70
Figure 3.10 – Amount of NT-3 incorporated into PPy/pTS films grown using a 2 layer approach from monomer solution containing 0.2 M pyrrole, 0.05 M pTS and 2 μ g/mL NT-3. Each point represents the average of 24 samples and the error bars represent one standard deviation of the mean.....	71
Figure 3.11 - SEM images of PPy/pTS films grown for 10 and 60 minutes \pm NT-3 in the polymerisation solution. The scale bar represents 20 μ m.....	71
Figure 3.12 – Comparison of long term release of NT-3 from PPy/pTS films synthesised by cyclic voltammetric and galvanostatic methods. Each point represents the average of two replicates and the error bars show the range.	73
Figure 3.13 – (a) Mass and (b) percentage of NT-3 released from PPy/pTS/NT-3 grown for different times after one hour of clinical stimulator-induced release.	74
Figure 3.14 - Mass of NT-3 released by (a) 10 min grown and (b) 60 min grown PPy/pTS/NT-3 polymers in 0.9% NaCl by various electrochemical release methods. Each point represents the average of 3 measurements, and the 60 min value presented is the sum of the 12 min average and the next 48 min average.....	76

- Figure 3.15** - Variation in electrically stimulated (a) mass and (b) percent NT-3 release from PPy/pTS/NT-3 films by various protocols with increasing charge (in 0.9% NaCl). The total positive charge was taken as the total charge passed in the positive direction in one hour of stimulation of the 1 cm². In all cases, the total negative charge was almost identical, and thus the total charge was approximately twice the values presented here. 78
- Figure 3.16** – Comparison of NT-3 release from PPy/pTS/NT-3 films over one week from unstimulated films, films stimulated using a low-frequency \pm 0.5 mA pulsed current stimulation or a cochlear implant mimicking stimulation protocol (“clinical stimulator”). Each point represents the mean of 3 replicates and the error bars show one standard error of the mean. 79
- Figure 3.17** – Linear regression of NT-3 release from days 4-7 using three different stimulation protocols – unstimulated release, a low-frequency \pm 0.5 mA pulsed current stimulation and a clinically-relevant stimulation protocol. 80
- Figure 3.18** - Optimisation of stimulation parameters using clinical stimulator. (a) The pulse current, (b) pulse rate, and (c) pulse width were varied and release of NT-3 from polypyrrole films over 1 week was monitored..... 81
- Figure 3.19** – Comparison of release of NT-3 from a dual PPy electrode cell to release from an asymmetric PPy electrode/stainless steel mesh electrode electrochemical cell. Release was stimulated using a clinical stimulator in 0.9% NaCl over 1 hour, and error bars show one standard error of the mean of three replicates. 82
- Figure 3.20** - Micro-interdigitated electrodes used for PPy deposition. (a) shows the whole chip, and (b) shows a close view of the interdigitated electrodes. 83
- Figure 3.21** – Comparison of release of NT-3 from PPy/pTS on microstructured, interdigitated electrodes and a flat film electrode. Each data point shows the average of two measurements of release stimulated by a clinical stimulator over 1 hour in 0.9% NaCl, and the error bars show the range. ... 84
- Figure 4.1** – Anatomy of the cochlear, showing spiral ganglion neurons in cross section. Source: University of Western Ontario Department of Anatomy and Cell Biology website - <http://instruct.uwo.ca/anatomy/530/earcoch.gif>. (date accessed: 12th December 2008). 90
- Figure 4.2** – An example of the resulting voltage waveform when biphasic current pulses were applied to gold electrodes. Impedances were calculated from the voltage waveform using peak voltage output (V_i) as well as components of V_i (V_a and V_p). 94
- Figure 4.3** – NT-3-induced neurite outgrowth from SGN explants. Representative images of SGN explants grown on CAM-coated tissue culture plastic with various concentrations of NT-3 added to the culture media. (a) In the absence of NT-3, very few neurites were observed from explants, while explants grown in 20 ng/mL NT-3 (b), 40 ng/mL NT-3 (c) and 60 ng/mL NT-3 (d) demonstrated increased numbers of sprouting neurites. Scale bars are 100 μ m. 97
- Figure 4.4** - Validation of explant assay. SGN explants were plated onto plastic tissue culture slides coated with CAMs and cultured in media containing 0–80 ng/mL NT-3. The number of neurites growing out from SGN explants was counted proximal to the explant edge for each condition. Error bars represent standard error of the mean of over 15 replicates per data point (* p<0.05 compared to the 0 ng/mL NT-3 control). 98
- Figure 4.5** – Neurite outgrowth from SGN explants on PPy. (a-c) show explant growth on PPy/pTS, while (d-f) show growth on PPy/pTS/NT-3. (a) and (d) are uncoated, untreated polymers, while (b), (c), (e) and (f) have been coated with CAMs. Additionally, extra NT-3 was included in culture media at 40 ng/mL in (c) and (f). Scale bars are 100 μ m. 101
- Figure 4.6** – Comparison of neurite out growth on tissue culture plastic (Plas), PPy/pTS (PPy), and PPy/pTS/NT-3 (PPy/NT-3), with and without CAMs and NT-3 included in culture media at 40 ng/mL. Growth of explants on PPy/pTS/NT-3 enhanced neurite outgrowth compared to explants grown on tissue culture plastic or PPy/pTS (p<0.02). In addition, the presence of NT-3 in the culture media or the addition of CAMs to plastic, PPy/pTS or PPy/pTS/NT-3 also resulted in greater neurite outgrowth compared to the respective controls (p<0.01). Error bars represent standard error of the mean. 101
- Figure 4.7** – SEM images of explants grown on (a, b, d) PPy/pTS or (c, e) PPy/pTS/NT-3. (a) Under low power, explants appear as cells and neurites radiating from a central mass of tissue. (b) A fibroblast growing on PPy/pTS surface appears as a flat cell adhering to raised globules of PPy/pTS. (c) A

neurite extending from an explant (out of view on the left side of the picture) grows alongside another neurite in a fascicle (arrowhead) and then becomes a single neuron. A nerve branch is visible (arrow). (d) Closer to the explant, nerve fibres can grow very densely, interacting with both the PPy and other cells. (e) Growth cones are often visible at the growing tip of neurites; in this case the growth cone terminates on a raised PPy/pTS/NT-3 nodule and is extending a filopodium (arrow). 103

Figure 4.8 – Impedance of gold and PPy-coated electrodes. Impedances were calculated from the applied 1.5 mA amplitude waveform, voltage waveform (inset) and Ohm’s law. There was no difference in Z_t , R_a or Z_p between plain gold electrodes, PPy/pTS-coated gold electrodes and PPy/pTS/NT-3-coated gold electrodes when measured in PBS at room temperature. Error bars denote the standard error of the mean. Values shown are representative of 4 independent experiments. 105

Figure 4.9 – Comparison of cumulative release of I-125-labelled NT-3 from stimulated (grey) and unstimulated (black) 1 cm² PPy/pTS/NT-3 polymers at various timepoints. Stimulated polymers received only 1 hour of stimulation at 24-25 hours after being placed in release media. Error bars represent standard error of the mean of three replicates. 106

Figure 4.10 – Effect of short-term electrically-stimulated release of NT-3 from PPy on explant neurite outgrowth. Explants were grown for 4 days on CAM-coated PPy/pTS or PPy/pTS/NT-3 and subjected to a biphasic current pulse stimulus for 1 hour after 24 hours. (a) shows explants grown on stimulated PPy/pTS/NT-3 and (b) shows stimulated PPy/pTS. Scale bars are 100 μm. (c) compares the number of neurites per explant on both PPy/pTS and PPy/pTS/NT-3 with and without electrical stimulation. While no difference was observed with stimulation on PPy/pTS ($p = 1.0$), electrical stimulation significantly increased neurite outgrowth on PPy/pTS/NT-3 ($* = p < 0.01$). Error bars represent standard error of the mean. PPy refers to PPy/pTS, PPy/NT-3 refers to PPy/pTS/NT-3 and ‘stim’ refers to 1 hour stimulation. 108

Figure 5.1 – Chronopotentiograms of galvanostatic PPy growth with various dopants, as shown in the key. PPy/pTS, PPy/DBS and PPy/PSS were grown at 2 mA/cm² and PPy/PMAS, PPy/HA and PPy/CS were grown at 0.25 mA/cm². 122

Figure 5.2 – Photographs of 2 cm X 1 cm strips of PPy synthesised with various dopants. (a) PPy/pTS, (b) PPy/DBS and (c) PPy/PSS were grown for 10 minutes at 2 mA/cm² and (d) PPy/PMAS, (e) PPy/HA and (f) PPy/CS were grown for 80 minutes at 0.25 mA/cm². 123

Figure 5.3 – Scanning electron microscope images of PPy synthesised with various dopants. (a) PPy/pTS, (b) PPy/DBS and (c) PPy/PSS were grown at 2 mA/cm² and (d) PPy/PMAS, (e) PPy/HA and (f) PPy/CS were grown at 0.25 mA/cm². All polymers were grown for 60 minutes. The scale bar represents 20 μm. 124

Figure 5.4 – Cyclic voltammogram using PPy films synthesised with various dopants as the working electrode. Films were synthesised for 10 min at 2 mA/cm² (pTS, DBS and PSS dopants) or 80 min at 0.25 mA/cm² (PMAS, HA and CS) to standardise the amount of charge passed during deposition. The potential was scanned between +0.6 V and -0.8 V vs. Ag/AgCl using a platinum counter electrode in 1 M NaNO₃ at a scan rate of 10 mV/sec for 5 cycles. The third scan is shown. 125

Figure 5.5 – Peak voltage separation in cyclic voltammograms recorded at 10 mV/sec in 1 M NaNO₃ of PPy films grown with various dopants. 127

Figure 5.6 – Contact angle measurements of water on PPy films grown with various dopants. Each value represents the mean of three measurements taken on separate films, and the error bars show one standard error of the mean. # indicates that no accurate measure of contact angle of PPy/HA could be taken, and that the value is <10°. 128

Figure 5.7 – Amount of NT-3 incorporated into polypyrrole films grown with various dopants. Polymers were grown for 1 hour at 2 mA/cm² (pTS, DBS, PSS dopants) or 8 hours at 0.25 mA/cm² (PMAS, HA, CS) so that the same amount of charge was passed per cm² for constant current growth. Each point shows the average of four replicates, with the error bars showing one standard error of the mean. 129

Figure 5.8 – (a) Electrically stimulated and (b) unstimulated cumulative mass release of NT-3 from PPy films grown with various dopants. Note that the y-axis of (b) is at a different scale to (a) to allow the unstimulated release from PPy films to be easily compared. 131

- Figure 5.9** – (a) Electrically stimulated and (b) unstimulated cumulative % release of NT-3 from PPy films grown with various dopants. Note that the y-axis of (b) is at a different scale to (a) to allow the unstimulated release from PPy films to be easily compared. 134
- Figure 5.10** – Native polyacrylamide gel electrophoresis of NT-3 with various components of PPy films. Well 1 shows unmodified NT-3, well 2 shows NT-3 mixed with pyrrole, wells 3-8 show NT-3 mixed with dopant molecules (3: pTS, 4: DBS, 5: PSS, 6: PMAS, 7: HA, 8: CS). 136
- Figure 5.11** – Native agarose gel electrophoresis of NT-3 with various components of PPy films. Well 1 shows unmodified NT-3; well 2 shows NT-3 mixed with pyrrole; wells 3-8 show NT-3 mixed with dopant molecules (3: pTS, 4: DBS, 5: PSS, 6: PMAS, 7: HA, 8: CS). The directions of the positive and negative electrode within the gel electrophoresis setup are also indicated. 138
- Figure 5.12** – Number of neurites extending from explants grown on various substrates including tissue culture plastic and PPy films grown with various dopants. For all experiments NT-3 was added exogenously into the media at 40 ng/mL. The error bars show one standard error of the mean for over 20 replicates per point. 139
- Figure 5.13** – Microscope images of SGN explants grown on various substrates, including (a) tissue culture plastic, (b) PPy/pTS, (c) PPy/DBS, (d) PPy/PSS, (e) PPy/PMAS, (f) PPy/HA and (g) PPy/CS. NT-3 was added exogenously into the media at 40 ng/mL. 141
- Figure 5.14** – Increased neurite outgrowth from SGN explants with electrical stimulation of PPy grown with various dopants to release NT-3. Each point represents the average of 6-43 replicates, and the error bars represent the standard error of the mean. 143
- Figure 5.15** – Images of SGN explants grown on electrically-stimulated PPy synthesised with various dopants - (a) PPy/pTS, (b) PPy/DBS, (c) PPy/PSS, (d) PPy/PMAS, (e) PPy/HA and (f) PPy/CS. 145
- Figure 6.1** - Four-ring platinum electrode array for implantation in GPs. The electrode array consisted of four active tapering electrodes individually wired for stimulation as electrode pairs and a dummy electrode as a marker for insertion depth. Diagram not to scale. 151
- Figure 6.2** – A guinea pig electrode array coated with PPy/pTS implanted into a GP cochlea. The fourth electrode can be seen protruding from the cochleostomy in this example. The fifth uncoated platinum extracochlear electrode is also visible. 153
- Figure 6.3** – Images of PPy/pTS growth on GP cochlear electrodes at several points during synthesis of the PPy. 40 minutes was determined to be the maximum deposition period for PPy/pTS growth at 2 mA/cm², as growth for longer than this led to bridging of the individual Pt electrodes. 155
- Figure 6.4** – Chronopotentiograms of galvanostatic PPy/pTS growth on GP cochlear electrodes. PPy was synthesised in a 2-electrode electrochemical cell at 2 mA/cm² on the four platinum electrodes (with a total area of 1.658 mm²) simultaneously. 156
- Figure 6.5** – Post-growth cyclic voltammogram of PPy/pTS synthesised on GP cochlear electrodes. CV was performed at 10 mV/sec in 1 M NaNO₃ in a 3 electrode cell with a Pt mesh counter electrode and a Ag|AgCl reference electrode. 157
- Figure 6.6** – Scanning electron microscope images of PPy/pTS grown on a platinum GP cochlear electrode. Scale bars are 100 μm, 10 μm and 2 μm as marked. 158
- Figure 6.7** – NT-3 release from PPy/pTS/NT-3-coated GP cochlear electrodes. Release was electrically stimulated for the first week (as marked), and then left unstimulated for a further week. Each point represents a single measurement. 159
- Figure 6.8** – Cross sectional images of the lower basal turn of guinea pig cochleae, showing examples of tissue response to PPy-coated GP cochlear electrode implantation. (a) shows little to no fibrous tissue response (category 1 in Table 6.1), while (b) shows a large amount of fibrous tissue almost filling the scala tympani in which the electrodes were inserted (category 4 in Table 6.1). Scale bars are 200 μm as indicated. 160
- Figure 6.9** – Comparison of change in average SGN densities between implanted and unimplanted ears of guinea pigs in different treatment groups. All groups except for PPy/NT-3 + stim showed a decrease in SGN density with implantation of the cochlear electrodes due to insertion trauma. In the PPy group, there was a lower density of SGNs in the implanted cochlea compared to the unimplanted cochlea (first bar). This loss of SGNs was lessened in the PPyNT3 group, but not to significant levels (second bar). In PPyNT3-stim GPs, there was significant survival of SGNs compared to the

unimplanted cochlea and compared to unstimulated groups, while there was a high degree of variability in the PPy-stim group. Error bars indicate the standard error of the mean. * $p < 0.05$ compared to the PPy group by ANOVA. 161

Figure 6.10 – Spiral Ganglion Neurons of cochleae implanted with PPy/pTS or PPy/pTS/NT-3 coated GP cochlear electrodes without (top) or with (bottom) electrical stimulation. The density of SGNs was improved with stimulation and with NT-3 inclusion in PPy coating of GP electrodes. 162

Figure 7.1 - Sequence and structural summary for NT-3 (top) and BDNF (bottom), showing 3 disulphide bonds typical of cysteine knot growth factors (dotted green lines), strong beta-sheet content (blue arrows – 53% of sequence for NT-3 and 55% for BDNF) and alpha-helix structure in BDNF (red curved lines – 5% of sequence for BDNF). Images from Protein Data Bank analysis of data provided by [3]. 167

Figure 7.2 - Images of (a, c) NT-3 and (b, d) BDNF rendered from X-ray crystallography of the proteins by [3]. a) and b) show space fill renderings and c) and d) show ribbon cartoons illustrating structural features. The colouring of the images is explained in the colour key. 168

Figure 7.3 – Comparison of mass of BDNF from PPy/pTS/BDNF with and without electrical stimulation. Each data point shows the mean of three replicates, and the error bars show one standard error of the mean. 173

Figure 7.4 – Response of SGN explants grown on tissue culture plastic with various concentrations of BDNF added to the culture media. (a-d) Representative images of SGNs. In the absence of BDNF (a) and with the addition of 1 ng/mL (b) and 10 ng/mL BDNF (c), very few neurites were observed from explants. (d) Explants grown in 100 ng/mL BDNF demonstrated greater numbers of resprouting neurites. Scale bar is 100 μm and applies to all images. (e) Graph of mean neurites per SGN explant after 3 days culture in 0, 1, 10 or 100 ng/mL BDNF. Error bars represent the standard error of the mean. * $p < 0.05$ compared to the 0 ng/mL BDNF control. 175

Figure 7.5 - Response of SGN explants to BDNF released from PPy/pTS/BDNF with and without electrical stimulation. * $p < 0.01$ compared to the PPy/pTS controls. Although there was an increase with electrical stimulation, there was no significant difference between the unstimulated and stimulated BDNF data. 176

Figure 7.6 – Comparison of incorporation of BDNF and NT-3 into PPy films. No significant difference was seen between PPy films containing only one neurotrophin and PPy films containing both neurotrophins at 2 $\mu\text{g/mL}$ (PPy/pTS/BDNF/NT-3). There was a slight decrease in the incorporation with 1 $\mu\text{g/mL}$ of each neurotrophin in the polymerisation solution (1 μg PPy/pTS/BDNF/NT-3) rather than 2 $\mu\text{g/mL}$, which was significant for BDNF incorporation (* $p < 0.05$). Each point is the average of 6 replicates, and the error bars show one standard error of the mean. 179

Figure 7.7 – Release of BDNF from PPy/pTS films with and without electrical stimulation. The release of BDNF alone is compared to the release of BDNF from dual neurotrophin films. Films were grown with both neurotrophins at 2 $\mu\text{g/mL}$ (“BDNF/NT3”), or with both neurotrophins at 1 $\mu\text{g/mL}$ (“1 μg BDNF/NT3”). Each point represents the average of three measurements, and error bars are not shown for clarity. 180

Figure 7.8 - Release of NT-3 from PPy/pTS films with and without electrical stimulation. The release of NT-3 alone is compared to the release of NT-3 from dual neurotrophin films. Films were grown with both neurotrophins at 2 $\mu\text{g/mL}$ (“NT-3/BDNF”), or with both neurotrophins at 1 $\mu\text{g/mL}$ (“1 μg NT-3/BDNF”). Each point represents the average of three measurements, and error bars are not shown for clarity. 181

Figure 7.9 – SGN response to neurotrophins released from PPy/pTS. Neurite extension was significantly increased with electrical stimulation for all polymers except PPy/pTS and PPy/pTS/BDNF ($p < 0.03$). Both PPy samples containing both BDNF and NT-3 significantly increased the neurite outgrowth over either of the neurotrophins alone with electrical stimulation ($p < 0.01$). Each point shows the average of 25-60 measurements, with the error bars showing one standard error of the mean. 183

Figure 7.10 – Representative images of SGN explants grown on PPy films containing various neurotrophins, with (right) and without (left) electrical stimulation. 185

Figure 8.1 – Cross-section of one turn of the cochlea, illustrating the position of the basilar membrane relative to the scala tympani, where the cochlear implant is inserted, and the cochlear nerve fibres

are situated. Source: Wikipedia article on the cochlea http://en.wikipedia.org/wiki/Cochlea (date accessed: 1 st December 2008).....	191
Figure 8.2 – Schematic diagram showing the steps involved in the process for deposition of PPy on 3D aligned MWNT forests.	198
Figure 8.3 – Schematic diagram showing the steps involved in the process used for deposition of VPP PPy layers on 2D aligned MWNT substrates.	200
Figure 8.4 – Schematic of electrospinning apparatus.....	201
Figure 8.5 – Schematic diagram showing the steps involved in the process used for deposition of CV PPy layer on electrospun PLGA substrates.....	201
Figure 8.6 - General schematic image of Mg/conducting polymer battery.....	203
Figure 8.7 - Details of the Mg/PEDOT battery used for release experiments.....	203
Figure 8.8 – Fabrication of PEDOT electrode on one side of a Goretex membrane.....	204
Figure 8.9 - Cyclic voltammetric growth of PPy/pTS/NT-3 on a bare gold mylar electrode. 15 scans at 50 mV/sec between -0.6 and +1.0 V were performed to deposit PPy on the 1 cm x 1 cm substrate. Grey arrows show direction of scans.....	206
Figure 8.10 – Scanning electron microscope images of CV-grown PPy/pTS/NT-3 on Au-mylar substrates. Two magnifications of films before and after one week of electrical stimulation are shown, with the bars representing 100 μm for the 400 X magnification images, and 10 μm for the 5000 X magnification images.....	207
Figure 8.11 – NT-3 release from flat CV-grown PPy/pTS films. (a) shows cumulative mass release from the films, and (b) shows cumulative % release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.....	209
Figure 8.12 – Cyclic voltammetric growth of PPy/pTS/NT-3 on 3D aligned MWNT forest substrate. 15 scans at 50 mV/sec between -0.6 and +1.0V were performed to deposit PPy on the 1 cm X 1 cm substrate. Grey arrows show direction of scans.....	210
Figure 8.13 - Scanning electron microscope images of 3D aligned MWNT forest substrates, either uncoated or coated with CV-grown PPy/pTS/NT-3. Three magnifications of substrates are shown, with the bars representing 100 μm for the 400 x magnification images, 10 μm for the 5000 x magnification images, and 2 μm for the 20000 x magnification images.....	211
Figure 8.14 - NT-3 release from CV-grown PPy/pTS films on 3D aligned MWNT forests. (a) shows cumulative mass release from the films, and (b) shows cumulative % release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.....	212
Figure 8.15 - Cyclic voltammetric growth of PPy/pTS/NT-3 on PLGA electrospun fibre-coated gold electrodes. 15 scans at 50 mV/sec between -0.6 and +1.0 V were performed to deposit PPy on the 1 cm X 1 cm substrate. Grey arrows show direction of scans.....	214
Figure 8.16 – Scanning electron microscope images of electrospun PLGA biodegradable substrate. Substrates shown are uncoated, coated with CV-grown PPy/pTS/NT-3 after growth, and PPy-coated substrates after 1 week of NT-3 release. Three magnifications are shown, with the bars representing 100 μm for the 400 X magnification images, 10 μm for the 5000 X magnification images, and 2 μm for the 20000 X magnification images.....	215
Figure 8.17 – NT-3 release from CV-grown PPy/pTS films on electrospun PLGA biodegradable substrates. (a) shows cumulative % release from the films, and (b) shows cumulative mass release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.....	216
Figure 8.18 – Comparison of incorporation of NT-3 into CV-grown PPy/pTS/NT-3 deposited on various substrates using identical conditions.....	218
Figure 8.19 – Release of NT-3 from CV-grown PPy layers deposited on three different substrates. (a) shows the average cumulative mass release from the films, and (b) shows % release over 1 week. Each data point represents the mean of 3 data points. Error bars are not shown for clarity.....	219

Figure 8.20 - Scanning electron microscope images of VPP-grown PPy/pTS/NT-3 on Au-mylar substrates. Two magnifications of films before and after one week of electrical stimulation are shown, with the bars representing 100 μm for the 400 X magnification images, and 10 μm for the 5000 x magnification images.	221
Figure 8.21 - NT-3 release from flat VPP-grown PPy/pTS films. (a) shows cumulative mass release from the films, and (b) shows cumulative % release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.	222
Figure 8.22 - Scanning electron microscope images of 3D aligned MWNT forest substrate, without (top) and with (middle + bottom) NT-3 incorporated into the VPP PPy layer. Substrates that have had NT-3 stuffed into the VPP PPy layer are shown immediately after incorporation (middle) and after 1 week of NT-3 release (bottom). Three magnifications are shown, with the bars representing 100 μm for the 400 X magnification images, 10 μm for the 5000 X magnification images, and 2 μm for the 20000 X magnification images.	223
Figure 8.23 - NT-3 release from VPP-grown PPy/pTS films in 3D aligned MWNT forest substrates. (a) shows cumulative mass release from the films, and (b) shows cumulative % release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.	225
Figure 8.24 – Scanning electron microscope images of 2D aligned MWNT substrates. Substrates shown are uncoated, coated with VPP-grown PPy/pTS/NT-3 after growth, and PPy-coated substrates after 1 week of NT-3 release. Three magnifications are shown, with the bars representing 100 μm for the 400 x magnification images, 10 μm for the 5000 x magnification images, and 2 μm for the 20000 x magnification images.	226
Figure 8.25 - NT-3 release from VPP-grown PPy/pTS films on 2D aligned MWNT substrates. (a) shows cumulative % release from the films, and (b) shows cumulative mass release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.	227
Figure 8.26 – Comparison of incorporation of NT-3 into VPP-grown PPy/pTS/NT-3 deposited on various substrates using identical conditions.	228
Figure 8.27 - Release of NT-3 from VPP-grown PPy layers deposited on three different substrates. (a) shows cumulative mass release from the films, and (b) shows cumulative % release over 1 week. Each point represents the mean of 3 data points, and the error bars show one standard error of the mean.	229
Figure 8.28 – Comparison of mass NT-3 release from all novel substrates. (a) show release with electrical stimulation, whole (b) shows passive, unstimulated release from the substrates. Each point represents the mean of 3 experiments.	231
Figure 8.29 – Preliminary study on release of NT-3 from PPy using biobattery with paper spacer and LiCl electrolyte. Each point represents the mean of 2 data points, and the error bars shown the range.	232
Figure 8.30 – Mass of NT-3 released from PPy stimulated by biobatteries with various electrolytes. Each data point represents the average of two data points, with the error bars showing one standard error of the mean.	233
Figure 8.31 – Current and voltage values measured during electrically-stimulated release of NT-3 from PPy using various electrolytes in a biobattery. Each point represents the mean of two data points, and large standard error bars have been omitted for clarity.	234

LIST OF TABLES

Table 1.1 – Property changes typically observed upon electrical stimulation to switch conducting polymers between oxidised and reduced states. Reproduced from [5].	4
Table 1.2 – Comparison of advantages and disadvantages of different materials used in implantable devices. Reproduced from [24].	11
Table 1.3 – Summary of literature involving incorporation and release of biomolecules or therapeutic agents from PPy.	18
Table 2.1 – Basic properties of the dopants used in PPy synthesis in this project. Theoretical logP calculated using molinspiration logP calculator (http://www.molinspiration.com/services/logp.html), using the monomer unit of polymeric anions for calculations. Note that these values do not give a good measure of hydrophobicity, and are intended for comparison only.	40
Table 2.2 – Composition of polymerisation solutions for synthesis of polypyrrole with different dopants.	42
Table 3.1 – Parameters of different electrochemical stimulation protocols used to release NT-3 from PPy/pTS/NT-3.	60
Table 3.2 – Comparison of mass of NT-3 incorporated into 1 cm ² PPy/pTS films using two different electrochemical synthesis methods. All films were grown from identical polymerisation solutions consisting of 0.2 M pyrrole, 0.05 M pTS and 1 µg/mL NT-3.	64
Table 3.3 – Mass NT-3 incorporated into 1 cm ² PPy/pTS polymers grown from different polymerisation solutions for various periods of time. All polymerisation solution contained 1 µg/mL NT-3.	66
Table 4.1 - Numbers of explants cultured on various surfaces with different treatments, including presence of cell adhesion molecules and concentration of NT-3 added to cell culture media.	95
Table 5.1 – Concentrations of NT-3 and dopant/pyrrole in solutions used for gel electrophoresis, along with the mass of protein and dopant loaded onto the gels in a 50 µL aliquot.	119
Table 5.2 - Number of explants analysed on PPy surfaces in various treatment groups.	121
Table 5.3 – Summary of rates of release of NT-3 from PPy films grown with various dopants, with R ² values and the ratio of stimulated:unstimulated release for mass release of NT-3 over 1 week.	132
Table 5.4 - Summary of rates of release of NT-3 from PPy films grown with various dopants, with R ² values and the ratio of stimulated:unstimulated release for % release of NT-3 over 1 week.	135
Table 6.1 – Guinea pig numbers in different treatment groups.	152
Table 6.2 - Fibrous tissue reaction and insertion damage in implanted GPs.	160
Table 7.1 – Numbers of explants cultured on PPy surfaces in various treatment groups described in dual neurotrophin release work.	172
Table 7.2 – Summary of rates of release of BDNF from PPy/pTS/BDNF, with R ² values and the ratio of stimulated:unstimulated release for release of BDNF from Day 1-7.	174
Table 7.3 – Summary of release rates of neurotrophins from PPy films grown with both BDNF and NT-3 at various concentrations (either 2 µg/mL “PPy/pTS/BDNF/NT-3”, or 1 µg/mL “1 µg PPy/pTS/BDNF/NT-3”), with R ² values and the ratio of stimulated:unstimulated release over 1 week.	182

ABBREVIATION AND NOTATION

2D	two dimensional
3D	three dimensional
μg	microgram, a measure of mass. $1 \mu\text{g} = 1 \times 10^{-6} \text{g}$
μL	microlitre, a measure of volume. $1 \mu\text{L} = 1 \times 10^{-6} \text{L}$
Ag AgCl	silver silver chloride reference electrode
Au	gold
BDNF	Brain-derived Neurotrophic Factor
CAM	Cell Adhesion Molecule
CNT	carbon nanotube
CS	chondroitin sulfate
CV	cyclic voltammetry or cyclic voltammogram
CVD	chemical vapour deposition
DAPI	4',6-diamidino-2-phenylindole
DBS	dodecylbenzene sulfonate
DMEM	Dulbecco's Modified Eagles Medium
ECIS	Electric Cell-substrate Impedance Sensing
EDTA	ethylene diamine tetra acetic acid
EtOH	ethanol
Fe	iron
Fe(III).pTS	iron (III) para-toluene sulfonate
FeCl_3	iron chloride
GP	guinea pig
H_2O_2	hydrogen peroxide
HA	hyaluronic acid
HEM	HEPES-buffered Eagle's media
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
I-125	iodine-125, a radioisotope of iodine
LiCl	lithium chloride
mCi	milli Curie, measure of radiation strength ($1 \text{ mCi} = 37 \text{ MBq}$)
Mg	magnesium
MgCl	magnesium chloride
min	minute
MPa	megapascals
MWNT	multi-walled carbon nanotube
NaOH	sodium hydroxide
NT-3	Neurotrophin-3
PBS	phosphate-buffered saline
PEDOT	poly(3,4-ethylenedioxythiophene)
PEG	poly(ethylene glycol)
pI	isoelectric point
PLGA	poly(lactic-co-glycolic acid)
PMAS	poly(2-methoxyaniline-5-sulfonic acid)
PPG	poly(propylene glycol)
PPG-PEG-PPG	poly(propylene glycol)-poly(ethylene glycol)-poly(propylene glycol)
PPy	polypyrrole
PSS	poly(4-styrenesulfonate)
Pt	platinum

pTS	<i>para</i> -toluene sulfonate
PVC	polyvinylchloride
PVDF	polyvinylidene fluoride
QCM	Quartz crystal microbalance
QSPR log D	Quantitative Structure-Property Relationship algorithm estimation of the distribution coefficient (log D)
R ²	R-squared value, the square of the correlation coefficient of linear regression
rpm	revolutions per minute
SEM	scanning electron microscopy/microscope
SGN	spiral ganglion neuron
TrkB	receptor tyrosine kinase B
TrkC	receptor tyrosine kinase C
UV	ultraviolet
VPP	vapour phase polymerisation

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Thompson, Brianna C., Moulton, Simon E., Ding, Jie, Richardson, Rachael T., Cameron, Adrian, O'Leary, Stephen, Wallace, Gordon G., Clark, Graeme M. **Controlled Release of Neurotrophins from Inherently Conducting Polymers.** Combined 28th Australasian Polymer Symposium / Australasian Society for Biomaterials 16th Annual Conference, 2006, 5-9 February 2006, Rotorua, New Zealand. (oral presentation)

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Thompson, Brianna C., Moulton, Simon E., Richardson, Rachael T., Wallace, Gordon G. **Using conducting polymers to enhance nerve growth.** University of Wollongong

Chemistry Departmental Conference, 23-24 October 2006, Jervis Bay, New South Wales, Australia. (oral presentation)

Thompson, Brianna C., Moulton, Simon E., Richardson, Rachael T., Wallace, Gordon G., Clark, Graeme M. **Release of neurotrophins from polypyrrole films: optimisation of electrical stimulation and effect on auditory nerve growth.** ARC Centre of Excellence for Electromaterials Science, ARC Centre Meeting, 6-7 February 2007, Wollongong, Australia. (oral presentation)

Thompson, Brianna C., Moulton, Simon E., Richardson, Rachael T., Cameron, Adrian, O'Leary, Stephen, Wallace, Gordon G., Clark, Graeme M. **Release of a neurotrophic factor from a conducting polymer enhances nerve growth.** ARC Centre of Excellence for Electromaterials Science Electromaterials Science Symposium, 8-9 February 2007, Wollongong, Australia (poster presentation)

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