2009

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

Brianna C. Thompson
University of Wollongong, brianna@uow.edu.au

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

LIST OF FIGURES

LIST OF TABLES

ABBREVIATION AND NOTATION

2D	two dimensional
3D	three dimensional
μg	microgram, a measure of mass. 1 μg = 1 X 10^{-6} g
μL	microlitre, a measure of volume. 1μL = 1 X 10^{-6} 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	40'6-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 mCi = 37 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-ethylenedioxythiphene)
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	*para*-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

PUBLICATIONS

Thompson, Brianna C., Moulton, Simon E., Ding, Jie, Richardson, Rachael T., Cameron, Adrian, O'Leary, Stephen, Wallace, Gordon G., Clark, Graeme M. **Optimising the incorporation and release of a neurotrophic factor using conducting polypyrrole.** *Journal of Controlled Release* (2006) vol. 116 (3) pp. 285-94

Richardson, Rachael T., Thompson, Brianna, Moulton, Simon, Newbold, Carrie, Lum, May Ghee, Cameron, Adrian, Wallace, Gordon, Kapsa, Robert, Clark, Graeme, O'Leary, Stephen. **The effect of polypyrrole with incorporated neurotrophin-3 on the promotion of neurite outgrowth from auditory neurons.** *Biomaterials* (2007) vol. 28 (3) pp. 513-23

Evans, Alison J., Thompson, Brianna C., Wallace, Gordon G., Millard, Rodney, O'Leary, Stephen J., Clark, Graeme M., Shepherd, Robert K., Richardson, Rachael T. **Promoting Neurite Outgrowth from Neural Explants Grown on Electrically Stimulated Ppy/BDNF Polymers.** *Journal of Biomedical Materials Part A* (2009) vol. 91A (1) 241-250

Thompson, Brianna C., Moulton, Simon E., Gilmore, Kerry J., Higgins, Michael J., Wallace, Gordon G. **Carbon Nanotube Biogels**. *Carbon* (2009) vol. 47 (5) 1282-1291

Richardson, Rachael T., Wise, Andrew K., Thompson, Brianna C., Flynn, Brianna O., Atkinson, Patrick J., Fretwell, Nicole, Fallon, James B., Wallace, Gordon G., Shepherd, Robert K., Clark, Graeme M., O'Leary, Stephen J. **Polypyrrole-Coated Electrodes for the Delivery of Charge and Neurotrophins to Cochlear Neurons**. *Biomaterials* (2009) vol. 30 (13) pp. 2614-2624

CONFERENCE PRESENTATIONS

Thompson, Brianna C., Moulton, Simon E., Wallace, Gordon G. **Using Conducting Polymers for Controlled Release Devices and Biofuel Cells.** University of Wollongong Chemistry Departmental Conference, 23-26 October 2005, Kioloa, New South Wales, Australia. (oral presentation)

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)

Thompson, Brianna C., Moulton, Simon E., Richardson, Rachael T., Cameron, Adrian, O'Leary, Stephen, Wallace, Gordon G., Clark, Graeme M.**Controlled Release of NT-3 from a Conducting Polymer.** ARC Centre of Excellence for Electromaterials Science Full Centre Meeting, 10 May 2006, Melbourne, Australia. (oral presentation)

Thompson, Brianna C., Moutlon, 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., Moutlon, 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)

Thompson, B.C., Moulton, S.E., Richardson, R.T., Wallace, G. G and Clark, G.M. **Release of Neurotrophins from Conducting Polymer Substrates to Promote Nerve Growth**. Australian Society for Biomaterials, 17th Annual Conference, 11-13 April 2007, Mt Eliza, Victoria, Australia. (oral presentation)

Thompson, Brianna C., Richardson, Rachael T., Moulton, Simon E., Wallace, Gordon G., Clark, Graeme M. **Neurotrophin release from polypyrrole: Enhancing the nerve-electrode interface of the cochlear implant.** MacDiarmid Symposium on Organic Conductors, May 16 2007, Wollongong, Australia. (oral presentation)

Thompson, Brianna C., Richardson, Rachael T., Moulton, Simon E., Wallace, Gordon G., Clark. **Effect of dopant on release of NT-3 from polypyrrole films and growth of auditory nerve explants.** ARC Centre of Excellence for Electromaterials Science Full Centre Meeting, 10 October 2007, Melbourne, Australia.(poster presentation)

Thompson Brianna C., Moulton Simon E., Richardson Rachael T., Ding Jie, O'Leary Stephen, Clarke Graeme M., Wallace Gordon G. **Release of a Neurotrophic Growth Factor from a Conducting Polymer Enhances Nerve Growth.** First HOPE Meeting – Advanced Course on Nanoscience and Nanotechnology. 24-29 February 2008, Tsukuba, Japan. (poster presentation)

Thompson, Brianna C., Chen, Jun, Moulton, Simon E., Wallace, Gordon G., Clark, Graeme M. **Enhancing release of the protein NT-3 from polypyrrole by using a MWNT aligned substrate.** Asia-Pacific Symposium on Nanobionics, 22-25 June 2008, Wollongong, Australia. (poster presentation)

ACKNOWLEDGEMENTS

The completion of this work would not have been possible without the hard work and input of several individuals. The input, assistance and patience of my supervisors is gratefully acknowledged. Without the day-to-day supervision of Dr. Simon Moulton there would have been a lot of time lost trying to figure out the ins-and-outs of a materials and electrochemistry lab. Thankyou immensely for helping me at pretty much every step of the way. Another enormous thankyou goes to my primary supervisor, Professor Gordon Wallace. His ability to see the larger picture and keep me on track has allowed this project to come to completion. He is also a truly inspirational role model.

Another person who has contributed greatly to this work is Dr. Rachael Richardson of the Bionic Ear Institute. Rachael has been a fantastic person to collaborate with, and her professionalism and extraordinary flair for scientific writing are something that I aspire to. She has been a great person to work with, and a great friend. Dr. Kerry Gilmore has also provided help, support and an open ear at many stages throughout this project, and I am grateful for our late evening chats.

The Intelligent Polymer Research Institute is a fantastic place to work, and the assistance of many individuals has been invaluable throughout my PhD. In particular, the technical input of George Tsekouras, Violeta Misoska, Phil Whitten, Carol Lynam and Peter Sherrell for their assistance in SEM, TEM and AFM is gratefully acknowledged. Additionally, I want to thank the people who kept me sane with chats, beers, hugs, proofreading and helpful advice – Jenny, Adrian, Shannon, Scotty, Xiao, Luke and Jakub (and Brianna, Alison and Patrick from the Bionic Ear Institute). Assistance from Mark, Amy, Elise, Alan, Joy and Laura from the School of Biological Sciences at University of Wollongong was so important to me – thankyou very much for helping when you didn't have to.

Finally, to the people who had to put up with my at home when experiments didn't work and equipment broke – my lovely family and friends. Thankyou to Mum and Dad, who have supported me through my whole education, have always let me make my own mistakes, and have been there to pick me up and dust me off when I have fallen. And for sitting through every presentation I have practised over and over again without falling asleep. To my brother Fletcher, thanks for patiently explaining electrical things to me, and for letting me bounce weird ideas off you (and calmly explaining why they wouldn't work). To Sam, my partner-in-crime – thanks for your patience and calming words when they were needed. And to my grandmother and grandfather – thankyou for encouraging me to achieve whatever could be achieved my whole life. I love you all.