University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Bauquier, SH;McLean, KJ;Jiang, JL;Boston, RC;Lai, A;Yue, Z;Moulton, SE;Halliday, AJ;Wallace, G;Cook, MJ

Title:
Evaluation of the Biocompatibility of Polypyrrole Implanted Subdurally in GAERS

Date:
2017-05-01

Citation:
Bauquier, S. H., McLean, K. J., Jiang, J. L., Boston, R. C., Lai, A., Yue, Z., Moulton, S. E., Halliday, A. J., Wallace, G. & Cook, M. J. (2017). Evaluation of the Biocompatibility of Polypyrrole Implanted Subdurally in GAERS. Macromolecular Bioscience, 17 (5), https://doi.org/10.1002/mabi.201600334.

Persistent Link:
https://hdl.handle.net/11343/191155

Full Paper

Evaluation of the biocompatibility of polypyrrole implanted sub-durally in GAERS

Sebastien H. Bauquier [a*], Karen J. McLean [b] , Jonathan L. Jiang [b], Ray C. Boston [b], Alan Lai [b], Zhilian Yue [c], Simon E. Moulton[‡c], Amy J. Halliday [b], Gordon Wallace [c], Mark J. Cook [b]

[a] Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 250 Princes Hwy, Werribee, Victoria 3030, Australia

[b] Centre for Clinical Neurosciences and Neurological Research, St. Vincent's Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia

[c] Intelligent Polymer Research Institute and ARC Centre of Excellence for Electromaterials Science, AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, New South Wales 2522, Australia

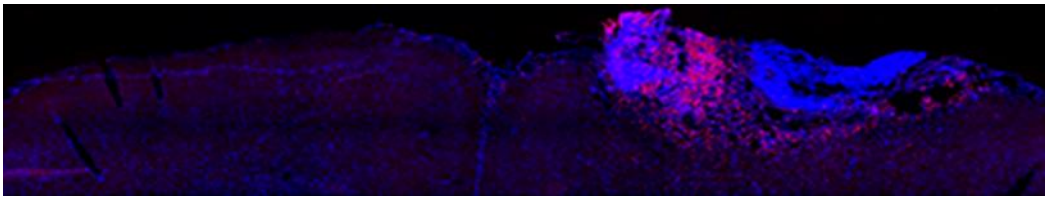
*Corresponding author. Tel.: +61 3 9731 2311; fax: +61 3 9731 2366. E-mail address: bauquier@unimelb.edu.au (S.H. Bauquier).

‡ Current Address: Biomedical Engineering, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, VIC, 3122, Australia.

Abstract

This blinded controlled prospective randomized study investigated the biocompatibility of polypyrrole (PPy) polymer that will be used for intracranial triggered release of anti-epileptic drugs (AEDs). Three by three millimeters PPy were implanted subdurally in six adult female GAERS (Genetic Absence Epilepsy Rat from Strasbourg). Each rat had a polymer implanted on one side of the cortex and a sham craniotomy performed on the other side. After a period of 7 weeks, rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Four series of fifteen sections were histological (H&E) and immunohistochemically (NeuN, GFAP and ED1) stained and evaluated by 3 investigators. The results showed that implanted PPy mats did not induce obvious inflammation, trauma, gliosis and neuronal toxicity. Therefore we conclude the PPy used offer good histocompatibility with central nervous system cells and that PPy sheets could be used as intracranial, AED delivery implant.

A brain slice from a rat with a confirmed cortical abscess



1. Introduction

The incidence of epilepsy ranges from 50 and 100 cases per 100,000 population per year in most developed countries though it is estimated that up to 5% of a population will experience non-febrile seizures at some point in life.[1-3] Because treatment with conventional anti-epileptic drugs (AEDs) provides adequate seizure control in only two third of patients, more novel therapy is urgently needed.[1-3]

Alternative therapies aiming at improving the availability of AEDs such as the intracranial implantation of polymer-based drug delivery systems are being investigated.[4, 5] This targeted drug delivery approach has shown some successes in the treatment of animal models of several neurological disorders such as Parkinson's disease, Huntington's disease and Alzheimer's disease.[6] This approach has been published once for the treatment of epilepsy.[4] The results of this study indicated that drug-eluting poly(D,L-lactide-co-glycolide) (PLGA) polymer implants represents a promising evolving treatment option for intractable epilepsy. The PLGA polymers used is a biodegradable copolymer that is very well tolerated by the brain.[7] However this polymer can only be used as a passive release vehicle. Work by Cook et al, 2013 has shown that prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy is becoming reality.[8] Synchronizing the AED release from a polymer with the seizure advisory system could be technically feasible when using a conductive polymer such as polypyrrole (PPy) that can change physical properties when being applied electrical stimulation. The physical changes associated with the mechanism for release is proposed to involve electrostatic interactions as the application of electrical stimulation protocol increases the amount of drug release. However, both the ionic and hydrophobic properties of polypyrrole have been shown

to vary upon application of electrical stimulus.[9] The expansion and contraction (actuation) of polypyrrole is also a well-documented property of polypyrrole upon oxidation and reduction and these processes may be involved in the release of incorporated drug.[10, 11] As these properties change simultaneously it is not possible to separate them to determine which process is the driving force for release. These mechanisms of drug release are the same for all polypyrrole despite the chemical composition of the polymer and has been reported for a wide range of incorporated drugs such as dexamethasone, fos-phenytoin] and the anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH).[12-14]

PPy sheets have been shown to be well tolerated by the brain tissue *in vitro* and they seem to be as compatible as Teflon when implanted into the cerebral cortex.[15] The aim of the present study is to test the in-vivo biocompatibility of PPys implanted sub-durally in GAERS (Genetic Absent Epilepsy Rat from Strasbourg) on top of their cerebral cortex.

2. Experimental Section

The study was designed as blinded controlled prospective randomized experiments. To evaluate the in-vivo biocompatibility of the PPy sheets, the rats underwent surgery for bilateral craniotomy; a PPy sheet was implanted subdurally on one side of the cortex and the other side was used as a sham control. After a period of 7 weeks, the rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Hematoxylin and Eosin (H&E) staining was used to evaluate inflammation, and general histological and architectural changes.[16] Immunohistochemical (IHC) staining for glial fibrillary acidic protein (GFAP) was used to evaluate possible materials glial reaction (gliosis), whilst the ED1 (CD68) antibody and the neural marker NeuN antibody were used to evaluate rat

macrophages and neuronal toxicity respectively.[16-18]

2.1. Evaluated materials

Pyrrole (Py) was obtained from Merck (Frenchs Forest, NSW, Australia) and distilled prior to use. The electrolytes used for electropolymerisation were 2 mg/mL dextran sulphate (DS, M_W 9000-20,000, Sigma–Aldrich, Castle Hill NSW, Australia). The PPy was synthesized from a monomer solution containing 0.2 M pyrrole and the aforementioned electrolyte. The synthesis method employed was galvanic polymerisation using a current density of 0.2 mA/cm^2 applied for 180 min using a 3 electrode cell containing an auxiliary electrode (stainless steel mesh – 5cm × 5cm), reference electrode (RE: Ag/AgCl - 3.0M NaCl) and a working electrode (WE: polished stainless steel plate 5cm × 5cm). After polymerisation, the WE was rinsed with Milli-Q water overnight to remove any unreacted monomer and dopant. The PPy film standing film was obtained by carefully cutting around the edges of the deposited PPy film and gently removing it from the WE. The free standing film was then laid down on a KimWipeTM tissue sheet that was pre-wet with Milli-Q water. The films were left on the tissue paper and placed into a petri dish, covered and left at 2^oC until required for implantation.

The morphology of the as-prepared PPy film was examined using a Field Emission Scanning Electron Microscope (FESEM, JEOL JSM-7500FA). The thickness of the sample was determined by analyzing the cross sectional micrographs of the sample (n = 20). The mean static contact angle of the polypyrrole free standing film was measured using a Dataphysics optical contact angle goniometer. A total of 20 measurements were made on the sample using deionized water droplets of 1 µl for the reported value.

2.2. Animals

Six 2-month-old female GAERS were obtained from the University of Melbourne (Parkville, Victoria, Australia) and were housed individually in 12 hours light/dark cycles with *ad libitum* access to food and water. All experiments were approved by St Vincent's Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.3. Animal surgery

Immediately prior to surgery, rats were weighed and anaesthetized with an intra-peritoneal injection of ketamine (ketamine hydrocloride 100 mg/mL, Ceva Animal Health, Glenorie NSW, Australia) (75 mg/kg) and xylazine (Ilium xylazine hydrochloride 20 mg/mL, Troy Laboratories Pty Ltd, Glendenning NSW, Australia) (10 mg/kg). Following anesthesia induction, rats were placed in a stereotaxic apparatus, and administered isoflurane (Isoflo™, Abbott Australasia Pty Ltd, Botany NSW, Australia) (0.5 to 1% in oxygen, 1 L/min) via a nose-cone, and were administered subcutaneous (SQ) carprofen (Tergive carprofen 50 mg/mL, Parnell Australia Pty Ltd, Alexandria NSW, Australia) (5 mg/kg) for pain relief and 0.9% sodium chloride (Baxter, Old Toongabble NSW, Australia) (2 mL) for cardiovascular support. Over the scalp of the rats, the hair was clipped and the skin aseptically prepared. A single incision was made down the midline, the skull cleared of tissue and the exposed bone dried with 3% hydrogen peroxide (Sanofi, Virginia QLD, Australia). Implants measuring 3 mm by 3 mm were cut from the polymer sheets described above. Placements of the implants were performed after 5 mm by 4 mm craniotomies were created bilaterally at the level of the coronal suture (over the motor cortices) and after excising the dura to expose the brain

surface. Only one of the two craniotomy sites was used to implant the polymer. The site of the polymer implantation was randomly allocated using the random function in Microsoft Excel 2007. Following implant placements, although neither the dural flap nor the bone pieces were replaced, an approximately 20 mm by 10 mm silicone sheet was placed on top of the skull to cover both craniotomy sites and the scalp was sutured closed (3/0 Monosyn® B Braun, Rubi, Spain). Silicone sheets were prepared using implant grade liquid silicone rubber (Cochlear Ltd), LSR 30, which comprises a polymer precursor base and a crosslinker. The polymer precursor base and crosslinker were dissolved in hexane at a ratio of 10:1, cast in a Teflon mould at room temperature, and cured by crosslinking at 110 ^{o}C for 30 min and then at 200 ^{o}C for 4 h. The silicone sheets were finally extracted with hexane for 72 h to remove any residual oligomers, and dried in vacuo at 60 ^{o}C overnight.

The animals were placed on heat pads for recovery. Post-operative treatments included SQ buprenorphine (Temgesic® 0.324 mg/mL buprenorphine hydrochloride, Reckitt Benckiser Healthcare Ltd, Hull, UK) every twelve hours (0.03mg/kg, twice a day), saline (2mL, once a day) and carprofen (5mg/kg, once a day) for up to 3 days.

2.4. Animal post-operative health monitoring

For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss, mobility and grooming twice a day (Table 1). At each assessment, a debilitation score was calculated by cumulating weight loss score, mobility scores and grooming score. A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, mildly affected, moderately affected, severely affected by the surgery/polymer implantation respectively.

2.5. Sacrifice and sample collection

Seven weeks after surgery, rats were weighed, anesthetized with isoflurane in oxygen and euthanized with pentobarbital (Lethabarb, pentobarbitone sodium 325 mg/mL, Virbac Pty Ltd, Milperra NSW, Australia) (100 mg/kg, intraperitoneal). The brain was immediately carefully dissected and frozen in liquid nitrogen-cooled isopentane and stored at - 80°C. At a later stage, brains were sectioned using a Leica CM 1850 cryostat. Parallel series of coronal sections were cut at 10 µm throughout the implant site, mounted onto chrome-gel-alum subbed microscope slides, air-dried then stored at -80°C until further processing.

2.6. Histological and immunohistochemical analysis

For each of the histological or IHC staining, the sections from all brains were processed simultaneously to ensure similarity in the experimental conditions.

2.6.1. H&E staining

H&E staining was performed similarly to previously published protocol.[16] In brief, slices were removed from the -80°C freezer and leave to air dry for 10 to 30 min. They were then placed into room temperature (RT) 10% Neutral Buffered Formalin (NBF, Sigma–Aldrich, Castle Hill NSW, Australia) for 15 minutes, rinsed with tap water (3*1 min), dipped in Hematoxylin (Gills 3, Sigma–Aldrich, Castle Hill NSW, Australia) for 4 minutes, rinsed with tap water for 5 minutes, differentiated by dipping in ethanol 70% (containing 0.5% HCl) for 20 seconds, rinsed in tap water (1min) and allowed bluing for 15s (0.1% ammonium hydroxide solution in distilled water). After another rinse in tap water (3 min), the slides were dipped into ethanol (50 and 70 %, 1 min each), stained in Eosin 0.6% (Alcoholic, Fisher Scientific, Pittsburgh, PA) for 30 seconds and dipped in

ethanol (95% and 100 % for 3 minutes and 2 *1minute respectively). Finally the slices were cleared in Xylene *(*Ajax Finechem, Taren Point NSW, Australia) for 2* 5 minutes, mounted in DPX (Sigma–Aldrich, Castle Hill NSW, Australia), and coverslipped.

2.6.2. NeuN/GFAP-IHC staining

NeuN/GFAP staining was performed similarly to previously published protocol.[7] In brief, immediately after taking the slices out of the freezer, they were placed into room temperature (RT) 10% NBF for 15 minutes. Sections were then washed in 0.1M phosphate-buffered saline (PBS, pH=7.4) (3 x 5 min) on a rocking platform and incubated in 10% normal goat serum (NGS) for 1 h RT in a humidified chamber. The slices were washed again in PBS (3 x 5 min) on a rocking platform, before overnight incubations in primary antibodies for neuron-specific nuclear protein (NeuN) and glial fibrillary acidic protein (GFAP) NeuN/GFAP (1/500 and 1:1000 respectively; NeuN - mouse monoclonal MAB377 and GFAP – rabbit polyclonal AB5804; both from Millipore Merck Millipore, Bayswater VIC, Australia) at 4^{o}C in a humid chamber. The following day slides were washed in PBS (3 x 5 min) on a rocking platform, incubated in two secondary antibodies (each at 1/1500; goat anti-mouse IgG (H+L) secondary Antibody, Alexa Fluor® 594 conjugate and goat anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor® 488 conjugate; both from Life technologies, Scoresby VIC, Australia) for 1 h RT in a dark humid chamber, and washed again in PBS (3 x 5 min) on a rocking platform. The slices were then incubated in 4',6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich, Castle Hill NSW, Australia ; 1/5000) for 5 min in the dark on a rocking platform and washed again in PBS (3 x 5min) on a rocking platform. Finally the slices were coverslipped with an aqueous mounting medium (Fluoromont™, Sigma–Aldrich, Castle Hill NSW, Australia) and stored in the dark.

2.6.3. ED1-IHC staining

The ED1 staining was performed following the protocol than NeuN/GFAP staining except for the following points: The overnight incubation was performed in primary antibody for anti-CD68 (ED1) (1/1000; mouse monoclonal from Serotec, Bio-Rad Laboratories Pty, Gladesville NSW, Australia) at 4^{o}C in a humid chamber and the secondary antibody used was GαM Alexa Fluor 594 (1/1500). A brain slice from a rat with a confirmed cortical abscess was also ED1 stained and used as a positive control.

2.6.4. Histological and IHC evaluations

Images were obtained using a Dot Slide Olympus system using an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) and captured using Virtual Slide System Software (VS-ASW-S1 FL 2.5, Olympus Soft Imaging Solution GmbH, Hamburg, Germany).

Both sides (polymer implantation side and sham surgery side) of 15 cortexes' parallel coronal sections cut throughout the surgery site were evaluated independently by three investigators for each histological and immunohistochemical staining. At the time of the evaluations, the investigators were unaware of the side of the polymer implantation. H&E stained sections were evaluated to assess macroscopic consequences of polymer implantation. The degree of damage was rated following a published scale: minor = incomplete disruption of the meninges and disruption of molecular cortical layer; moderate = complete disruption of the meninges and molecular cortical layer; severe = damage extending beyond the meninges and

molecular cortical layer.[7] Immunohistochemically stained sections were subjectively rated from 0 to 3 (0 = no change in immunoreactivity, 1 = subtle changes in immunoreactivity, 2 = clear changes in immunoreactivity but only affecting a very limited part of the cortex, 3 = clear changes in immunoreactivity affecting an extended area of the cortex).

2.7. Statistical methods

Differences in immunoreactivity between the two sides of the cortexes were evaluated using a multilevel mixed-effects ordered logistic regression using commercially available statistical software (Stata 11.0; StataCorp, College Station, TX). Graphics were made using commercially available software (Prism 6 for Windows, GraphPad Inc., San Diego CA, USA). *P*-Value of 0.05 was used to distinguish statistically significant differences. Unless specified, the results are reported as median and range.

3. Results and discussion

The thickness of the PPy mats was determined to be 3.1 ± 0.3 µm (**Figure 1**) with a contact angle of 67.8 ± 3.9 deg (n = 20) measured for the free standing film.

The median of the post-operative health monitoring scores was 0.5 (0 to 4) with only one rat receiving a score above 2 (i.e. score of 4 at the first evaluation of day 2 post-operatively). The median pre-surgical and pre-euthanasia weight of the 6 rats were 152 g (136 to 156) and 186.5 g (168 to 207) respectively representing a weight gain over the 7 weeks implantation period of 24 % (21 to 33). Samples of parallel series of coronal sections representative of the changes seen after H&E, NeuN, GFAP and ED1-IHC staining are presented in **Figure 2**.

3.1. Histological changes in H&E staining:

The three evaluators were in agreement for the following results: there was no appreciable differences between the sham and implanted sides of the cortex, and besides the fact that part of the stratum 1 was missing on some of the sections from both sides of the cortex (and not limited to the surgical sites), no additional changes occurred that could have been attributed to the implantation of the polymers (i.e. no evidence of necrosis, infection or inflammation).

3.2. Neuronal toxicity

The median NeuN-IHC scores were 0 (0 to 3) (average [± standard error] 0.60 ± 0.05) and 0 (0 to 2) (average score [± standard error] 0.52 ± 0.05) for the sham and implanted polymer sides of the cortexes respectively (**Figure 3**). Overall, there were subtle changes in neuronal density in the boundary layer and the surrounding brain tissues in the two sides of the cortexes and with the sham surgery sides being more affected (*p=0.005*).

3.3. Glial reaction

The median GFAP-IHC scores were 0 (0 to 2) (average [± standard error] 0.61 ± 0.04) and 0 (0 to 2) (average score [± standard error] 0.44 ± 0.04) for the sham and implanted polymer sides of the cortexes respectively (**Figure 3**). Overall, the increases in GFAP-IHC staining observed were subtle and affected significantly more the sham surgery sides of the cortexes (*p=0.002*).

3.4. Presence of macrophages

The median ED1-IHC scores were 0 (0 to 3) (average [± standard error] 0.45 ± 0.05) and 0 (0

to 2) (average score [± standard error] 0.40 ± 0.04) for the sham and implanted polymer sides of the cortexes respectively (**Figure 3**). Overall, although ED1-IHC showed significant amounts of macrophages in the positive control brain tissue, only very few macrophages were present in rats implanted with PPy sheets. No difference was observed between the 2 sides of the cortexes (*p=0.248*).

PPys were implanted subdurally for a period of 7 weeks in adult female GAERS and using the histological and immunohistological methods (i.e. H&E staining, and NeuN, GFAP and ED1-IHC staining) described above we demonstrated in-vivo histobiocompatibility of the PPy with central nervous system (CNS) cells. These results confirmed previously published in-vitro work.[15]

All rats recovered well and rapidly following the surgery. They all gain significant weight during the 7 weeks of the implantation period showing very little impact of the PPys on the growth and vital functions of GAERS.

To be declared histobiocompatible, an implant must: 1) not be toxic to surrounding tissues and 2) not be recognized as foreign material by the immune system.[7] In the presence of acute toxicity, neurons will appear karyopyknotic and in the presence of chronic toxicity, macrophages/monocytes and astrocytes will be activated by necrotic cellular debris resulting from neurons necrosis.[19] Furthermore, as fibroblasts are absent from within the CNS, the scar tissue will originate from the astrocytes forming gliotic scarring.[7] This scarring will also be seen if the implant is recognized as a foreign material. In this case, astrocytes will undergo gliosis and microglia and monocytes/macrophages will initiate a local inflammatory reaction

resulting in the formation of multinucleate giant cells (i.e. fusions of multiple macrophages).[7, 20]

The Stratum 1 (or microgyric layer) of the cortex was missing from some of the sections. This variability in brain implant outline was attributable to mechanical procedures during sample preparation. Also, no difference between the sheets implantation side or the sham surgery side of the cortex were seen, the loss of the stratum 1 represents a limitation of the study. Loss of portions of the boundary layer adhering to the implant during its removal from the brain for histological procedures prevents precise examination of interface tissues. This problem could be avoided by keeping the implant in place during all stages of tissue preparation.[16, 21] Nevertheless, on the slices where the stratum 1 is present no major changes were observed (e.g. **Figure 2 A, B, C, D**).

The IHC scores observed on the PPy implanted side of the cortexes strongly favor the histobiocompatibility of the PPy. The median IHC scores (0 for all three IHC staining) shows that there were no evidence of neuronal degeneration, glial reaction or of the presence of macrophages in the majority of the slices rated. However, a previous study reported middle to moderate acute inflammatory reactions after the subcutaneous implantation of PPy in rats followed by a resolution of the inflammatory response.[22] Our study did not analyze the brain tissue immediately after implantation and may have missed the opportunity to observe an acute inflammatory reaction.

The low average IHC scores (0.40 to 0.52) observed demonstrated that when some IHC staining changes occurred, they were of minimal extent and of lower or similar amplitude

than the one observed on the sham surgery sides of the cortexes. The statistically significant differences in IHC scores observed between the PPy implantation sites demonstrate that it was not the lack of statistical power that allowed the conclusion that the PPy does not induce more IHC staining changes compared to a sham surgery. The subjectivity of scale used for grading IHC related changes was taken into consideration during the planning of the study and it was decided that at least 3 evaluators were to grade every brain section. For each of the 6 rats and for each of the 3 IHC staining, 3 series of 15 sections were graded twice (PPy and sham surgery sides) by 3 investigators making a total 1620 IHC related data points that allowed establishment of statistical significances. The absence of a control group (rat not undergoing craniotomy) prevented comparison with normal brain tissue however all evaluator were trained and experimented in IHC evaluation allowing them to have reasonable assumption on what a normal brain tissue should look like.

Any surgery will induce tissue trauma resulting in cell death and tissue scarring. The comparison of NeuN-IHC and GFAP-IHC scores reveals that the amount of neuronal death and gliosis was significantly less in the PPy side than the sham surgery side of the cortexes (p values of 0.005 and 0.002 respectively) implying that the application of PPy could protect the CNS tissue after surgery. In the present experiment, neither the dura nor the skull flaps were replaced. In a pilot study (unpublished data) we found that during the craniotomy the careful dissection of the dura to maintain a flap intact was performed at the detriment of the brain tissue situated just below. In consequence the dura was peeled away and not replaced. The brain tissue also presented some edema resulting in an expansion of the cortex partially protruding through the craniotomy and in consequence the skull window was not replaced to avoid more mechanical damages.

Although the PPy may have just been an additional layer of protection, the potential neuro-

protective effects of PPys need to be studied further. Several publications have demonstrated the benefice of the use of PPy to allow tissue proliferation/regeneration. Indeed PPy is an advantageous substrate to enhance nerve cell interaction with potential application as nerve guidance channels to bridge the gap between severed nerve ends.[23] Furthermore, using an ischemia-reperfusion rat myocardial infarction model researchers demonstrated that PPy enhanced infiltration of myofibroblasts into the infarct area highlighting the potential clinical benefit of using this PPy as an injectable scaffold to repair ischemic myocardium after myocardial infarction.[24]

The softness of the polymer has probably also contributed to the apparent reduction in local reactive response but the absence of animals implanted with a relatively stiff implant as a negative control prevents firm conclusion. The PPy's friable nature prevented appropriate retrieval of the implant for adequate explant analysis. Also, very little fibrin was visually detected, on a macroscopic scale, around the implant during brain dissection. The absence of explant analysis prevents to exclude the possibility of a formation of a fibrin capsule which could prevent the polymer to release drug into the central nervous system.

Due to the absence of evidence of toxic injury or immune mediated inflammation, the authors conclude that PPys offer good histocompatibility with CNS cells and that PPy sheets could potentially be used as intracranial AED delivery implant.

Acknowledgements

The authors gratefully acknowledge the sponsorship of the Victorian Government through its Science Technology and Innovation Initiative administered by the

Department of Industry, Innovation and Regional Development. The authors wish to acknowledge funding from the Australian Research Council (ARC) Centre of Excellence Scheme (CE140100012), the use of facilities at the University of Wollongong Electron Microscopy Centre, support of the Australian National Fabrication Facility (ANFF) – Materials Node. Professor Gordon Wallace acknowledges the support of the ARC through an ARC Laureate Fellowship (FL110100196).

Keywords: cerebral cortex implants, conductive polymers, toxicity, biocompatibility

References

[1] S. H. Bauquier, A. Lai, J. L. Jiang, M. J. cook, *Neuroscience Bulletin* **2015**.
[2] J. W. Sander, *Current opinion in neurology* **2003**, *16*, 165.
[3] S. D. Shorvon, *Epilepsia* **1996**, *37 Suppl 2*, S1.
[4] A. J. Halliday, T. E. Campbell, T. S. Nelson, K. J. McLean, G. G. Wallace, M. J. Cook, *J Clin Neurosci* **2013**, *20*, 148.
[5] A. J. Halliday, S. E. Moulton, G. G. Wallace, M. J. Cook, *Adv Drug Deliv Rev* **2012**, *64*, 953.
[6] A. J. Halliday, M. J. Cook, *CNS Neurol Disord Drug Targets* **2009**, *8*, 205.
[7] A. J. Halliday, T. E. Campbell, J. M. Razal, K. J. McLean, T. S. Nelson, M. J. Cook, G. G. Wallace, *J Biomed Mater Res A* **2011**.
[8] M. J. Cook, T. J. O'Brien, S. F. Berkovic, M. Murphy, A. Morokoff, G. Fabinyi, W. D'Souza, R. Yerra, J. Archer, L. Litewka, S. Hosking, P. Lightfoot, V. Ruedebusch, W. D. Sheffield, D. Snyder, K. Leyde, D. Himes, *Lancet neurology* **2013**, *12*, 563.
[9] G. G. Wallace, G. G. Wallace, "*Conductive electroactive polymers : intelligent materials systems*", 2nd edition, CRC Press, Boca Raton, FL, 2003, p. 237 p.
[10] A. S. Hutchison, T. W. Lewis, S. E. Moulton, G. M. Spinks, G. G. Wallace, *Synthetic Met* **2000**, *113*, 121.
[11] T. W. Lewis, S. E. Moulton, G. M. Spinks, G. G. Wallace, *Synthetic Met* **1997**, *85*, 1419.
[12] S. E. Moulton, M. D. Imisides, R. L. Shepherd, G. G. Wallace, *J Mater Chem* **2008**, *18*, 3608.
[13] Y. Zhong, R. V. Bellamkonda, *J Control Release* **2005**, *106*, 309.
[14] R. Muller, Z. Yue, S. Ahmadi, W. Ng, W. M. Grosse, M. J. Cook, G. G. Wallace, S. E. Moulton, *Sensors and Actuators B: Chemical* **2016**, *236*, 732.
[15] P. M. George, A. W. Lyckman, D. A. LaVan, A. Hegde, Y. Leung, R. Avasare, C. Testa, P. M. Alexander, R. Langer, M. Sur, *Biomaterials* **2005**, *26*, 3511.
[16] J. Yang, A. C. Charif, J. E. Puskas, H. Phillips, K. J. Shanahan, J. Garsed, A. Fleischman, K. Goldman, M. T. Luebbers, S. M. Dombrowski, M. G. Luciano, *Journal of the mechanical behavior of biomedical materials* **2015**, *45*, 83.
[17] L. F. Eng, *Journal of neuroimmunology* **1985**, *8*, 203.
[18] V. S. Polikov, P. A. Tresco, W. M. Reichert, *Journal of neuroscience methods* **2005**, *148*, 1.
[19] V. Kumar, A. K. Abbas, J. C. Aster, "*Robbins and Cotran pathologic basis of disease*", Ninth edition. edition, Elsevier/Saunders, Philadelphia, PA, 2015, p. xvi.
[20] E. Fournier, C. Passirani, C. N. Montero-Menei, J. P. Benoit, *Biomaterials* **2003**, *24*, 3311.
[21] S. S. Stensaas, L. J. Stensaas, *Acta neuropathologica* **1978**, *41*, 145.
[22] Z. Wang, C. Roberge, L. H. Dao, Y. Wan, G. Shi, M. Rouabhia, R. Guidoin, Z. Zhang, *J Biomed Mater Res A* **2004**, *70*, 28.
[23] C. E. Schmidt, V. R. Shastri, J. P. Vacanti, R. Langer, *Proceedings of the National Academy of Sciences of the United States of America* **1997**, *94*, 8948.
[24] S. S. Mihardja, R. E. Sievers, R. J. Lee, *Biomaterials* **2008**, *29*, 4205.

TABLE

Table 1 - Health monitoring chart. For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss, mobility and grooming twice a day. A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, mildly affected, moderately affected, severely affected by the surgery, respectively.

Post-operative health monitoring chart

	Scores
Percentage of weight loss	
No weight loss	0
Less than 10% weight loss	1
Weight loss between 10 and 20%	2
Weight loss of more than 20%	3
Mobility Score	
Normal mobility	0
Rat ataxic moving at a normal speed	1
Rat ataxic and moving slowly	2
Recumbent rat	3
Grooming Score	
Normal grooming activity	0
Grooming activity mildly decreased	1
Grooming activity significantly decreased	2
No grooming activity	3
Debilitation score	0-9

Figures

Figure 1 - Scanning Electron Microscope (SEM) images of the polypyrrole thick film doped with dextran sulfate (top surface A and B, cross-section C).

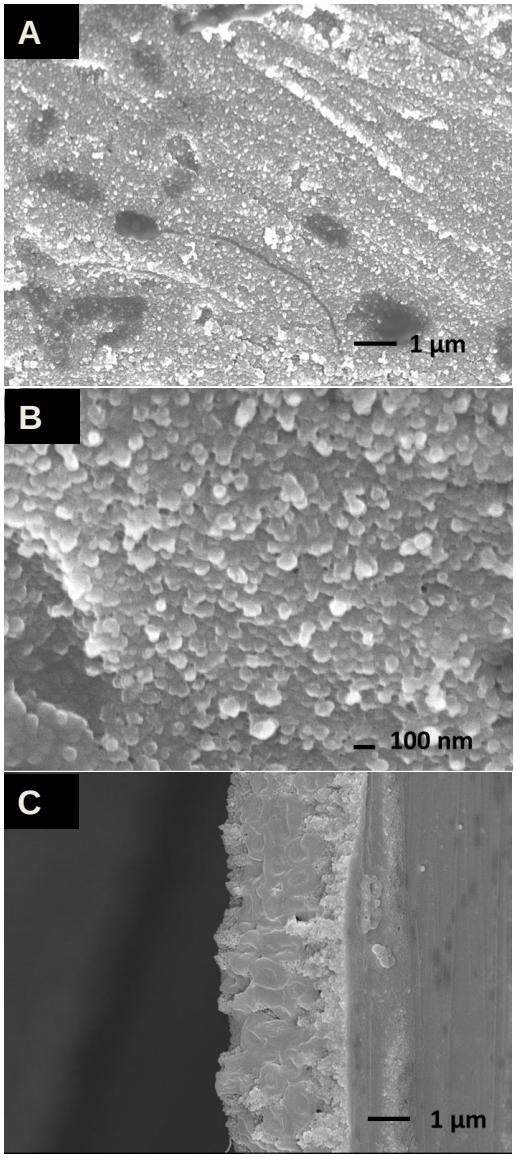


Figure 2 - Samples of parallel series of coronal sections that were cut throughout the cortex of a rat with PPy sheets implanted subdurally for a period of 7 weeks. The rat had a polymer implanted on one side of the cortex (A, B, C, D) and a sham craniotomy performed on the other side (A', B', C', D'). Those samples are representative of the changes seen after H&E (A, A'), NeuN (B, B'), GFAP (C, C') and ED1 (red)/DAPI (blue) (D, D') immunohistochemistry (IHC) staining. A brain slice from a rat with a confirmed cortical abscess was also ED1 (red)/DAPI (blue) stained (D") and used as a positive control for ED1 staining. The stratum one is missing from the sham surgery side of the cortex and changes in IHC staining were minor on both sides of the cortex.

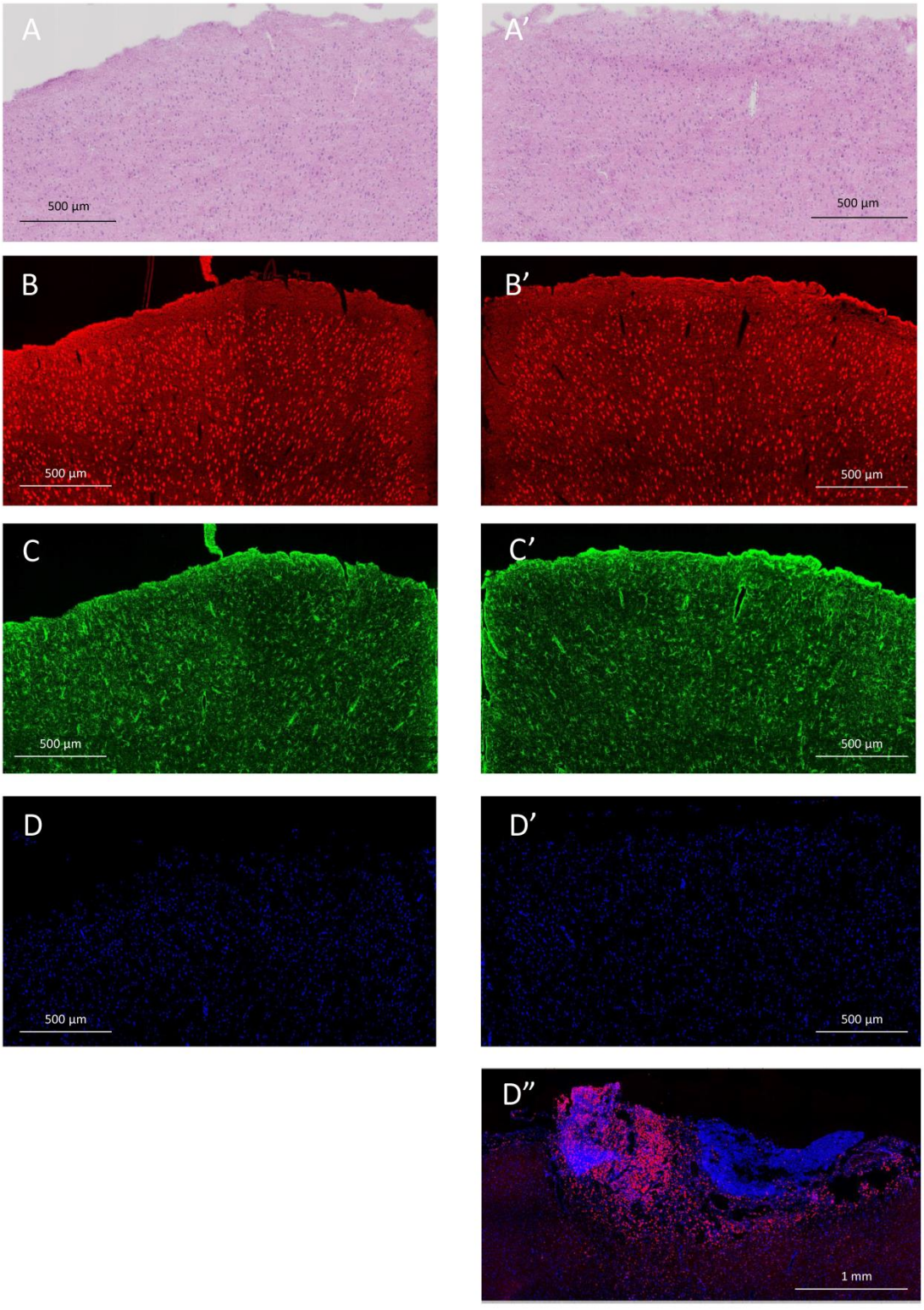
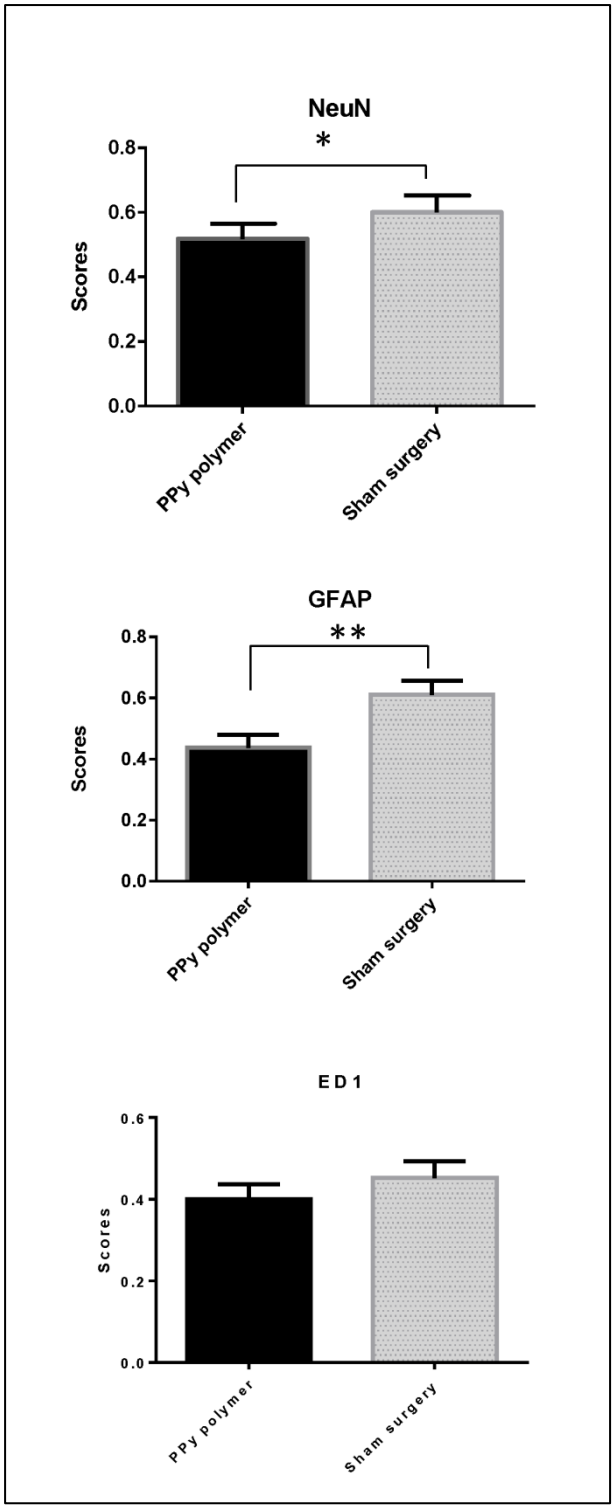
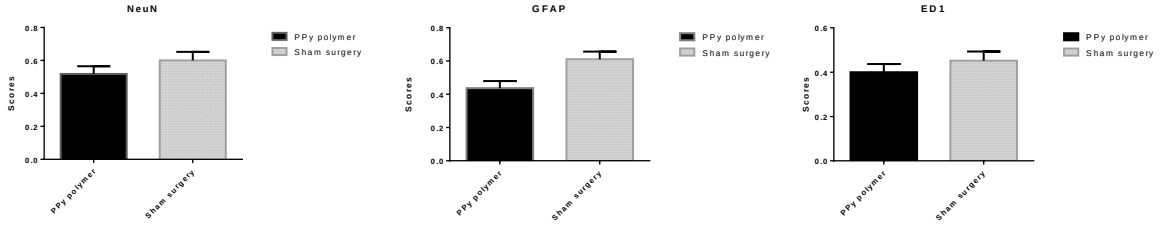

A
500 µm
A'
500 µm
B
500 µm
B'
500 µm
C
500 µm
C'
500 µm
D
500 µm
D'
500 µm
D"
1 mm

Figure 3 - Average (and standard error) scores attributed to cortical sections immunohistochemically (ICHIHC) stained with NeuN, GFAP and ED1. PPy mats were implanted subdurally in six adult female GAERS rats. Each rat had a polymer implanted on one side of the cortex and a sham craniotomy performed on the other side. After a period of 7 weeks the rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Fifteen sections per rat for each immunohistochemical stain (NeuN, GFAP and ED1), were subjectively rated from 0 to 3 (0 = no change in immunoreactivity, 1 = subtle changes in immunoreactivity, 2 = clear changes in immunoreactivity but only affecting a very limited part of the cortex, 3 = clear changes in immunoreactivity affecting an extended area of the cortex) by 3 investigators. Differences in immunoreactivity between the two sides of the cortex were evaluated using multilevel mixed-effects ordered logistic regression. *: p=*0.005*; **: *p=0.002*.


NeuN
*
Scores
0.8
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery
GFAP
**
Scores
0.8
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery
ED1
Scores
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery


NeuN
Scores
0.8
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery
PPy polymer
Sham surgery
GFAP
Scores
0.8
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery
PPy polymer
Sham surgery
ED1
Scores
0.6
0.4
0.2
0.0
PPy polymer
Sham surgery
PPy polymer
Sham surgery

Alternative therapies aiming at improving the availability of anti-epileptic drugs (AEDs) such as the intracranial implantation of polymer-based drug delivery systems have shown some promising results. This blinded controlled prospective randomized study investigated the biocompatibility of polypyrrole (PPy) polymer that will be used for intracranial triggered release AED delivery. We conclude the PPy used offer good histocompatibility with cortical cells.

Sebastien H. Bauquier*, Karen J. McLean, Jonathan L. Jiang, Ray C. Boston, Alan Lai, Zhilian Yue, Simon E. Moulton, Amy J. Halliday, Gordon Wallace, Mark J. Cook
Corresponding Author*

A brain slice from a rat with a confirmed cortical abscess

