In Situ Electrochemical Polymerization of Poly(3,4-ethylenedioxythiophene) (PEDOT) for Peripheral Nerve Interfaces

Jamie M. Murbach[1], Adrienne Widener[1], Yuxin Tong[2], Shrirang Chhatre[3], Vivek Subramanian[3], David C. Martin[3], Blake N. Johnson[2], Kevin J. Otto[1,4,5,6]

[1]*Department of Materials Science and Engineering, University of Florida, Gainesville, FL, 32611, USA*

[2] *Department of Industrial and Systems Engineering, Virginia Tech, Blacksburg, VA 24061, USA*

[3] *Department of Materials Science and Engineering, University of Delaware, Newark, DE 19716, USA*

[4]*J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, 32611, USA*

[5]*Department of Neuroscience, University of Florida, Gainesville, FL, 32611, USA*

[6]*Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL, 32611, USA*

Abstract

The goal of this study was to perform *in situ* electrochemical polymerization of poly(3,4-ethylenedioxythiophene) (PEDOT) in peripheral nerves to create a soft, precisely located injectable conductive polymer electrode for bi-directional communication. Intraneural PEDOT polymerization was performed to target both outer and inner fascicles via custom fabricated 3D printed cuff electrodes and monomer injection strategies using a combination electrode-cannula system. Electrochemistry, histology, and laser light sheet microscopy revealed the presence of PEDOT at specified locations inside of peripheral nerve. This work demonstrates the potential for using *in situ* PEDOT electrodeposition as an injectable electrode for recording and stimulation of peripheral nerves.

Introduction

Neural prostheses provide a means to understand and influence neurological injury and disease through bi-directional communication. Peripheral nerve injuries have a detrimental impact on military personnel and veterans as the greatest source of long-term disability including limb loss from combat-related injuries. Specifically, peripheral nerve recording and stimulation have the potential to influence neurological injury through bi-directional communication using peripheral nerve devices. Extraneural peripheral nerve electrodes are commonly used for peripheral nerve injury; unfortunately, extraneural devices have poor selectivity and signal resolution[1]. This trade-off motivates the use of intraneural devices. However, long-term performance of intraneural devices is limited. An immunological foreign body response (FBR) to implanted devices limits efficacy of chronic stimulation and recording capabilities over time due to the acute response of macrophages and subsequent glial sheath formation leading to encapsulation around the implant which blocks signal from nervous tissue to device electrodes.[2–4] There have been a variety of approaches to mitigate the FBR and enhance device function including the implementation of intrinsically conducting polymers (CPs) at the device-tissue interface to improve recording and stimulation of neural devices. An intriguing option for deployment of the CPs is *in situ* polymerization of the conductive polymer. Specifically, here we demonstrate that poly(3,4-ethylenedioxythiophene) (PEDOT) interfaces can be created *in situ*, resulting in a less invasive intraneural electrode for intrafascicular targeting.

Previously, PEDOT has been studied for neural interfacing applications for microelectrodes and biomedical devices.[5–10] PEDOT exhibits high chemical stability and conductivity and significantly improves electrochemical properties of devices, specifically decreasing impedance magnitude over all frequencies.[11,12] PEDOT has been applied to neural devices in the central nervous system (CNS) and has been shown to enhance signal quality, charge storage capacity (CSC), and charge transport for electrical stimulation and recording.[9,13–15,11,16] PEDOT has also been implemented for other biomedical applications due to its ease of synthesis, ability to entrap and release bioactive molecules and drugs[17–19], and ability to be modified and tailored for specific applications.[20–22]

In situ polymerization of PEDOT has shown great potential *in vitro*, in tissue slice culture, and *in vivo* for recording and stimulation.[12,23–25] There have been few studies using PEDOT in the peripheral nervous system (PNS)[26,27] and none known by the authors using *in situ* polymerization methods of PEDOT in the PNS. This work demonstrates a method of *in situ* polymerization of an injectable PEDOT electrode for peripheral nerve interfacing to develop a less invasive ionic and electron charge transport pathway between the device and surrounding neural tissue to improve long-term functionality and performance of bi-directional interfaces.

Experimental Methods:

In Situ Electrochemical polymerization:

Polymerization was quantified initially in an agarose gel and subsequently in excised rat sciatic nerves to assess deposition. A monomer solution was made by mixing 0.01 M 3,4-ethylenedioxythiophene (EDOT) (97%, Sigma Aldrich, St. Louis, MO) in 1X phosphate buffered saline (PBS) (pH 7.4) with 0.1 wt% poly(4-sodium styrene sulfonate) (PSS, M_n ~ 70,000g/mol, Sigma Aldrich, St. Louis, MO). *In situ* polymerization was initially performed in a 0.5 wt% agarose gel (0.5 wt% agarose in 0.01 M EDOT:PSS) to determine the relationship between deposition time and PEDOT size. Intraneural PEDOT polymerization was performed in excised rat sciatic nerves using 100 µm diameter platinum iridium (PtIr) microwire to assess electrodeposition with small diameter electrodes. Polymerization was also assessed using a custom designed Plastics One guide-electrode device (C316I-MS303/2/ GDE/ELECT 31GA, Plastics One, Roanoke, VA) consisting of a 31-gauge cannula with two 200 µm diameter stainless steel (SS) electrodes. Figure 1A depicts the insertion and injection of monomer for electrochemical polymerization. The epineurium was separated from the nerve for facile insertion of the electrode into nerve and the cuff array was subsequently placed around the nerve shown in Figure 1B-C.[28] Once inserted into the nerve, 6-10 µl of EDOT:PSS was injected at a constant rate of 0.2 µl/min using a Quintessential Stereotaxic Injector (Stoelting QSI™, Wood Dale, IL). While injecting the monomer solution, constant voltage (potentiostatic) polymerization was performed using a three-electrode cell configuration using an Autolab PGSTAT128N (Metrohm Autolab B.V., Utrecht, The Netherlands) at 2V with the PtIr microwire or Plastics One SS device as the working electrode (WE), SS as the counter electrode (CE), and an Ag|AgCl glass body electrode (Thermo Fisher Scientific) as the reference electrode (RE).

Electrochemical Impedance Spectroscopy (EIS):

Electrochemical impedance spectroscopy (EIS) measurements were recorded prior to and after polymerization of PEDOT using an Autolab PGSTAT128N with a three-electrode cell configuration. EIS is commonly performed to assess the device-tissue interface. EIS measurements were collected by applying a voltage sine wave between the WE and Ag|AgCl RE while measuring current magnitude and phase over a 0.01-10 kHz frequency range, using a 15-sine 10 mV RMS excitation voltage. Pt wire was used as the CE.

Cyclic Voltammetry Measurements:

Cyclic voltammetry (CV) measurements were performed using an Autolab PGSTAT128N by sweeping the voltage of the WE at a constant rate to assess the resultant current flow between the WE and CE. The voltage range applied for SS was -0.6 to +0.8 V and for PEDOT was -0.8 to

+0.6 V to remain within the limit to prevent hydrolysis reduction and oxidation reactions. Fast (5 V/s) sweep rates were performed to assess static equilibrium and determine the charge storage capacity of the bare devices and devices with PEDOT. From the measurements, the peaks represent the reduction and oxidation reactions occurring at the interface, and the peak amplitudes represent the number of charge carriers present. The cathodal charge storage capacity (Q_{cap}) was determined by integrating the cathodal current density and dividing by the sweep rate and surface area of the electrode.

Tissue Processing, Histology, and Microscopy:

After polymerization, nerves were fixed in 4% paraformaldehyde (PFA) (95%, Sigma Aldrich, St. Louis, MO) for 24 hours, washed with 1X PBS for 24 hours, and cryoprotected in 30% sucrose for at least 24 hours. To analyze polymerization in tissue, nerves were frozen in 2-methyl butane at -40°C for 30 seconds before slicing. Tissue was embedded with Optimal Cutting Temperature solution (OCT) (Sakura Finetek, Alphen aan den Rijn, The Netherlands) and horizontally sectioned into 20 µm thick slices (Leica CM 1950, Buffalo Grove, IL). Tissue sections were then washed with 1X PBS, allowed to dry, and stained with 4',6-diamidino-2-phenylindole (DAPI) (H-100, Vector Lab, Burlingame, CA) for cell nuclei. Stained tissue slices were imaged using fluorescence and light microscopy (Leica DMi 8, Buffalo Grove, IL) at 10x magnification using a 488 nm laser.

Select samples were whole-mount collected and prepared for imaging via CLARITY to evaluate PEDOT in whole nerve samples and complement the classic histology techniques.[29] CLARITY removes opaque lipids from tissue, resulting in a clear or transparent sample ideal for imaging by laser light sheet microscopy. Nerves were fixed in 4% PFA for 24 hours, incubated in a hydrogel solution at 4°C for 3 days, and embedded in the hydrogel at 37°C for 3 hours to crosslink the hydrogel. The sample was then placed in a clearing solution containing sodium dodecyl sulfate (SDS) (>85%, Tokyo Chemical Industry, Tokyo, Japan) for 3 months to clear lipids from the tissue. Once optically clear, the tissue was incubated in a series of 2,2'-thiodiethanol (TDE, Sigma Aldrich, St. Louis, MO) solutions for 3.5 hours at 37°C with agitation for refractive index correction. Prior to imaging, nerves were washed with 1X PBS to remove remaining hydrogel. Laser light sheet microscopy (Zeiss Z.1 Light sheet, Germany) was used to analyzed large CLARITY cleared samples three-dimensionally for assessment of polymerization of PEDOT in nerve. PEDOT fluorescence was imaged using 405 and 488 nm lasers.

Statistics:

Mean 1 kHz impedance magnitude, Q_{cap}, and binned frequency ranges were evaluated for statistical significance using Student's t-tests ($p < 0.01$). Error bars for all data were calculated using the standard error of the mean from $n = 4$ bare devices and devices with PEDOT for impedance and phase and $n = 6$ bare devices and devices with PEDOT for cyclic voltammetry measurements. The approximate area of each electrode was 31,400 μm^2.

Results, Analysis, and Discussion:

Initially, custom-made 3D printed cuff electrodes were used to polymerize PEDOT in an attempt to direct polymerization through nerve.[28] Due to the robust and heterogeneous epineurium, this method proved difficult to electrodeposit PEDOT through nerve at low current thresholds. Therefore, the *in situ* deposition method through syringe injection was employed to simultaneously inject and polymerize a PEDOT electrode within peripheral nerve in addition to placement of 3D printed cuff electrodes around the nerve. Impedance magnitude, phase angle, cyclic voltammetry measurements, and histology of nerve slices were analyzed before and after polymerization using both 3D printed cuff arrays and injectable *in situ* PEDOT methods. Using the injectable *in situ* polymerization method, intraneural polymerization was observed in excised rat sciatic nerves through electrochemical impedance and phase measurements, fluorescence microscopy, and laser light sheet microscopy.

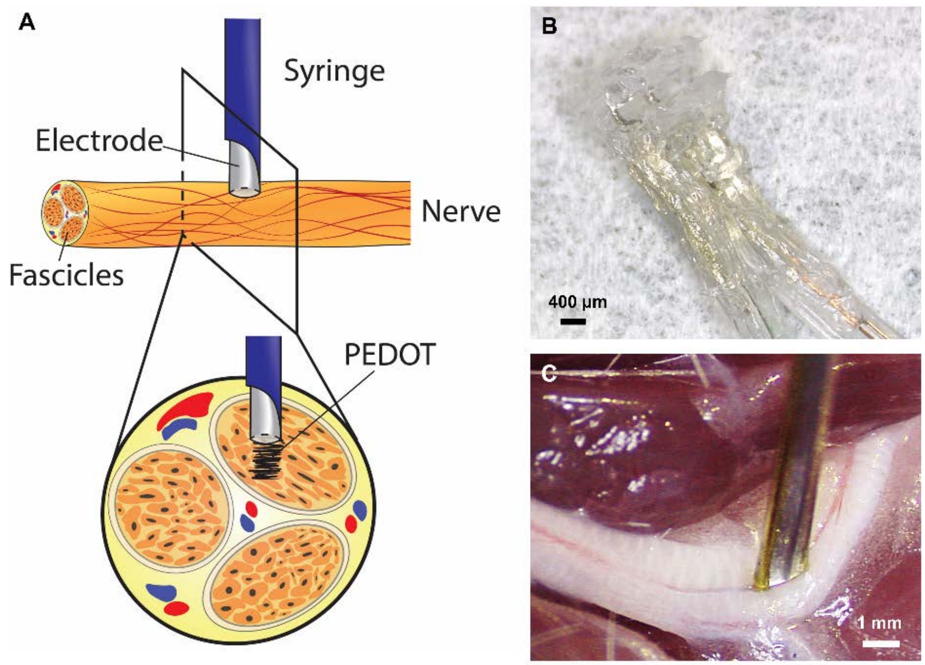

Figure 1. A) Schematic of delivery and *in situ* polymerization of PEDOT with a Plastics One guide-electrode device in peripheral nerve. B) 3D printed silicone cuff electrode arrays. C) A Plastics One guide-cannula device with an injection port and 200 μm SS electrodes for simultaneous injection and polymerization of PEDOT.

Electrochemical Impedance Spectroscopy:

After polymerization of PEDOT in nerve, the impedance magnitude decreased over all frequencies measured as compared to bare PtIr electrodes as shown in Figure 2A. The drop in impedance is associated with the increase in electrochemical surface area and porosity of PEDOT that can likely increase detection sensitivity to neural activity. Lower impedance magnitude is associated with better signal quality, higher signal-to-noise ratios for improved recordings, and lower power required for stimulation. Phase values near 0 are indicative of a highly resistive interface, while phase values near -90 represent a largely capacitive interface. Above 100 Hz, PEDOT possessed a lower phase than bare electrodes (Figure 2B), characteristic of a more resistive interface. Impedance and phase measurements were filtered using a Savitsky-Golay filter to smooth noise, especially evident around 60 Hz. At 1 kHz frequency, injectable PEDOT electrodes had a significantly lower mean impedance magnitude of 17.8 kΩ than the 1kHz impedance magnitude of 179.7 kΩ for bare electrodes, as shown in Figure 3F. The 1 kHz impedance magnitude was analyzed for recording of neural devices because this value has relevance in biological systems as the fundamental frequency of an action potential. Frequency ranges were binned and assessed for stimulation applications demonstrated in Figure 3A-E. This data exhibited a significant decrease in all frequency ranges for electrodes with PEDOT. Significantly lower impedance magnitude over all frequencies measured demonstrates lower power required for stimulation at lower current densities with PEDOT, which has been shown previously.[9,12]

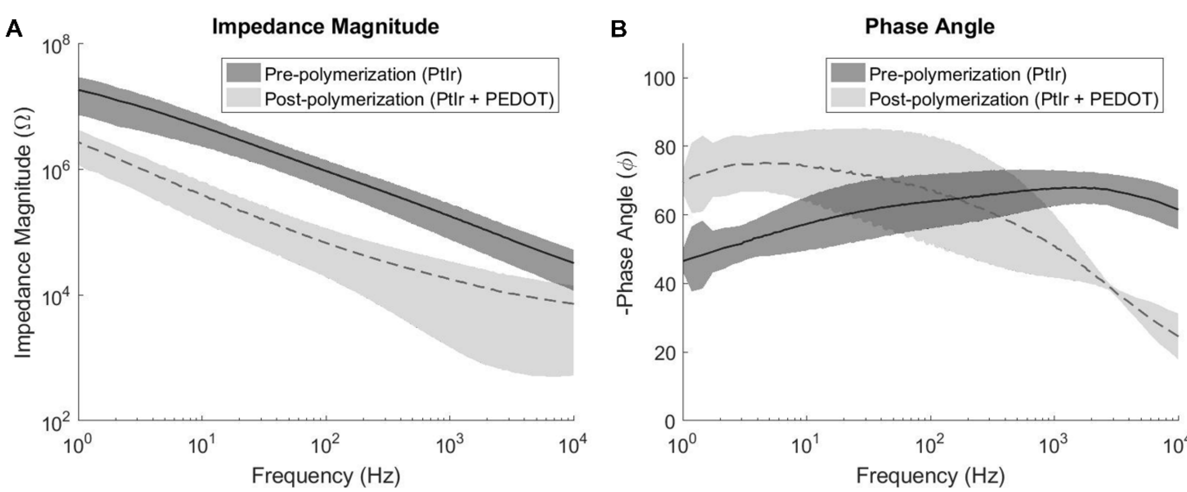

Figure 2. Electrochemical impedance spectroscopy (EIS) measurements. A) Impedance magnitude pre-polymerization (PtIr) and post-polymerization (PtIr + PEDOT) between 1 and 10,000 Hz. B) Pre-polymerization (PtIr) and post-polymerization (PtIr + PEDOT) phase angle measurements.

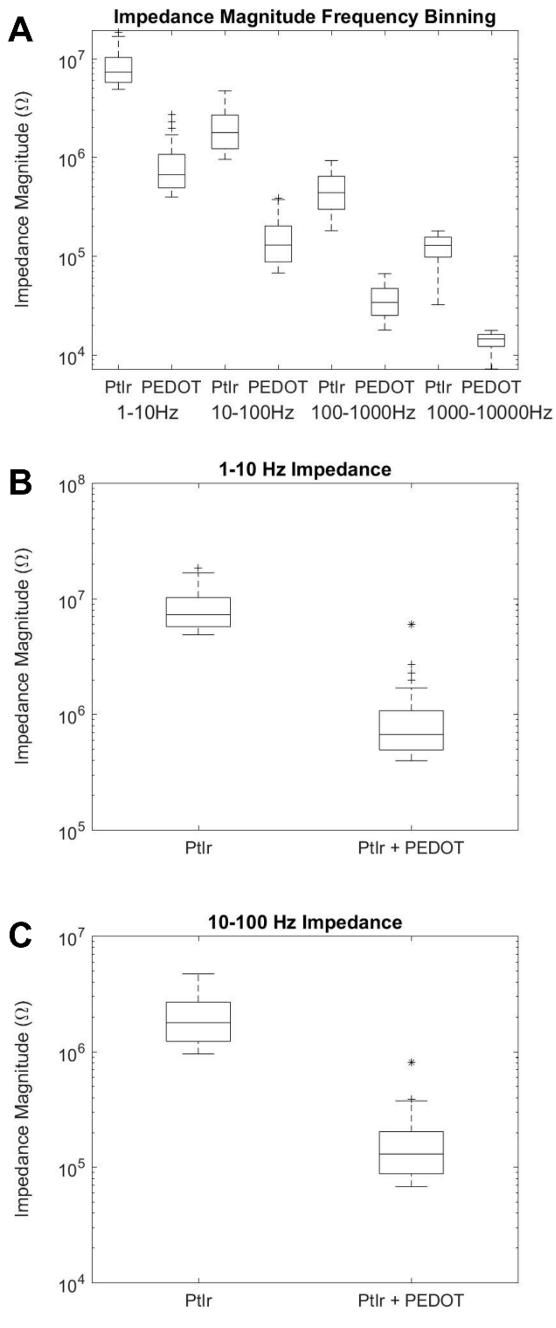

Figure 3. A) PtIr electrodes and PtIr + PEDOT electrodes mean impedance magnitude of binned frequency ranges scaled logarithmically. B) 1-10 Hz, C) 10-100 Hz, D) 100-1000 Hz, and E) 1000-10000 Hz binned frequency range impedance magnitudes of bare PtIr electrodes pre-polymerization compared to PtIr + PEDOT electrodes post-polymerization. Electrodeposited PEDOT impedances are significantly lower than the impedance magnitude of the bare electrodes across all frequency ranges. F) 1 kHz impedance magnitude of PtIr and PtIr + PEDOT demonstrates a significant decrease in impedance of PEDOT electrodes.

Cyclic Voltammetry measurements:

Cyclic voltammetry measurements were performed using Plastics One SS electrodes and Plastics One SS electrodes with electrodeposited PEDOT to provide information on the charge storage capacity of devices alone and devices with PEDOT. A fast sweep rate (5 V/s) was applied to assess information at the electrode tip, whereas slower sweep rates provide information of the electrode tip and shaft with electrolyte penetration underneath the insulation.[30] PEDOT electrodes have a significantly greater mean Q_{cap} of 2.53 $\mu C/\mu m^2$ compared to bare SS electrodes with a mean Q_{cap} of 0.53 $\mu C/\mu m^2$ represented in Figure 4A-B for fast sweep rates (5 V/s) demonstrating the increased charge available. The greater Q_{cap} of PEDOT electrodes may be useful for physiologically relevant electrical stimulation compared to bare electrodes. Lower power applied for stimulation minimizes the potential formation of reactive species in tissue and enhances device longevity. While Plastics One devices facilitate intraneural polymerization, they are not ideal for electrophysiological stimulation. Combined use of Plastics One devices and 3D printed cuff arrays for polymerization and stimulation will be assessed in future studies as a chronic neural interface.

Previously, the 3D printed cuff arrays have been fabricated with electrodeposited PEDOT designed for peripheral nerve stimulation.[28]

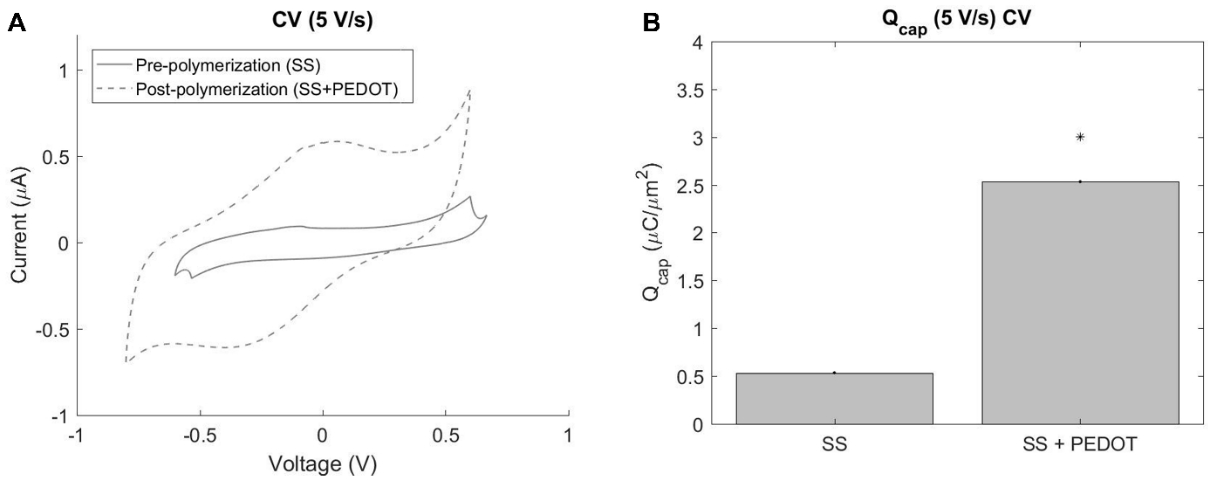

Figure 4. Cyclic voltammetry measurements of SS devices and devices with electrodeposited PEDOT. A) Fast (5 V/s) sweep rates of PEDOT electrodes demonstrate increased cathodal charge storage capacity (Q_{cap}) compared to SS devices. B) Mean Q_{cap} calculations show significantly greater charge available at the device-tissue interface with PEDOT electrodes ($p < 0.01$).

Tissue Processing, Histology, and Microscopy:

Polymerization was assessed in 2D sectioned nerve slices. Figure 5A represents a control sciatic nerve stained with DAPI for cell nuclei without polymerization of PEDOT, and Figure 5B depicts a nerve slice containing PEDOT using 3D printed nerve cuffs alone for shallow intraneural polymerization. PEDOT was imaged by fluorescence spectroscopy due to its intrinsic fluorescence with a 488 nm laser.[12] Precise intraneural polymerization of PEDOT deep within fascicles was achieved by *in situ* injection and polymerization methods demonstrated by the decreased impedance magnitude post-polymerization in Figures 2A, 3A-F as well as fluorescence spectroscopy in 2D nerve slices shown in Figure 5C. The nerve in Figure 5C expresses green fluorescent protein (GFP) background due to its acquisition from a transgenic rat expressing GFP ubiquitously.

Laser light sheet microscopy reveals intraneural PEDOT polymerization in whole tissue samples shown in Figure 6A-B. Figure 6A represents a bright field image of PEDOT polymerized in rat sciatic nerve, and the same location of PEDOT fluorescence was imaged with 405 and 488 nm lasers demonstrating intraneural PEDOT polymerization in Figure 6B. PEDOT deposition is depicted by white arrows in Figure 6A-B. Rat sciatic nerves imaged by laser light sheet microscopy were fixed and cleared prior to polymerization due to tissue damage of PEDOT nerves caused by active clearing techniques. Polymerization in fixed tissue is believed to have different mechanical and structural properties than fresh tissue, which may affect diffusion and deposition of PEDOT in nerve. The mechanical integrity of *in situ* deposited PEDOT must be studied and characterized further to assess chronic stability in peripheral nerve.

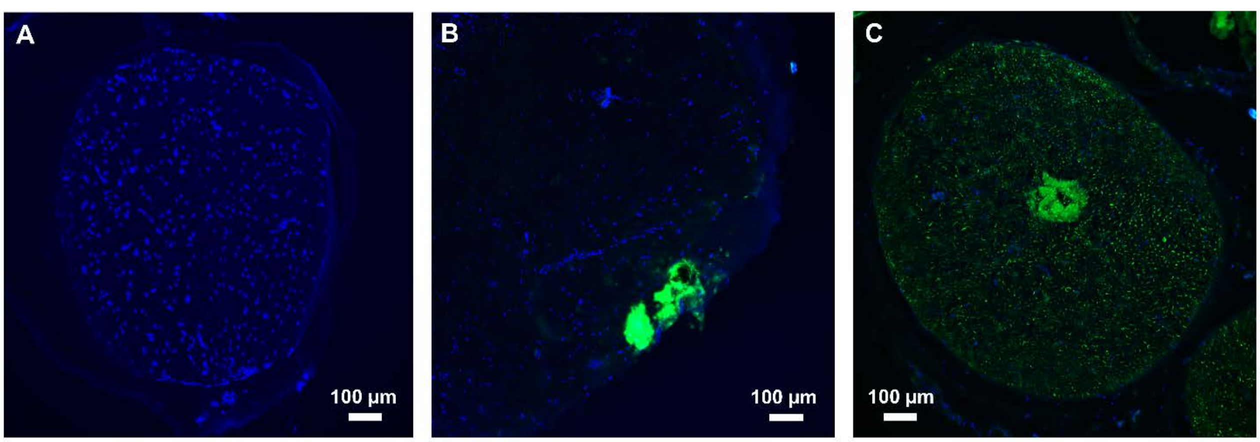

Figure 5. Histology and microscopy of tissue slices with A) a control nerve without PEDOT, B) intraneural PEDOT polymerization using 3D printed cuff arrays, and C) intraneural PEDOT

polymerization by *in situ* injection and polymerization method with a 3D printed cuff array. Scale bars are 100 µm.

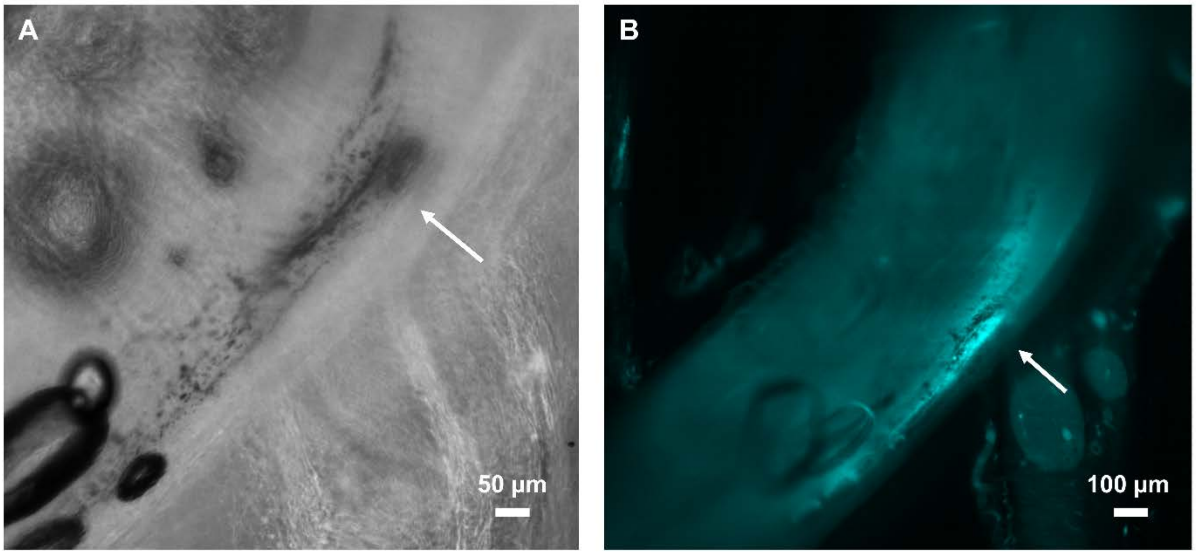

Figure 6. Laser light sheet microscopy images of whole peripheral nerve samples demonstrating intraneural *in situ* PEDOT polymerization. A) Bright field image of PEDOT polymerized in rat sciatic nerve. B) PEDOT fluorescence in nerve. Precise location of electrodeposition is depicted by white arrows.

Conclusions:

We have demonstrated that PEDOT can be locally electrodeposited within individual peripheral nerves using an electrode and cannula delivery system. The *in situ* electrochemical PEDOT polymerization resulted in lower impedance magnitude over all frequencies measured and significantly decreased impedance magnitude for all frequencies for stimulation and 1 kHz impedance for recording applications. PEDOT deposition resulted in greater Q_{cap} for stimulation compared to bare Plastics One SS electrodes. Histology and microscopy confirm intraneural polymerization from PEDOT autofluorescence in 2D nerve sections as well as imaging by laser light sheet microscopy in whole nerve samples. Long-term stability and *in vivo* assessment in the PNS is required for further investigation of injectable PEDOT electrodes for peripheral nerve stimulation and recording.

Acknowledgments:

This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) Targeted Neural Plasticity (TNT) program under the auspices of Drs. Doug Weber and Tristan McClure-Begley through the DARPA Contracts Management Office: No. HR0011-17-2-0019 and the National Science Foundation (NSF CMMI – 1739318).

References:

1. Grill, W. M. & Mortimer, J. T. Neural and connective tissue response to long-term implantation of multiple contact nerve cuff electrodes. *J. Biomed. Mater. Res.* **50,** 215–226 (2000).
2. Lago, N., Ceballos, D., J Rodríguez, F., Stieglitz, T. & Navarro, X. Long term assessment of axonal regeneration through polyimide regenerative electrodes to interface the peripheral nerve. *Biomaterials* **26,** 2021–2031 (2005).
3. Lago, N., Yoshida, K., Koch, K. P. & Navarro, X. Assessment of biocompatibility of chronically implanted polyimide and platinum intrafascicular electrodes. *IEEE Trans. Biomed. Eng.* **54,** 281–290 (2007).
4. de la Oliva, N., Navarro, X. & del Valle, J. Time course study of long-term biocompatibility and foreign body reaction to intraneural polyimide-based implants. *J. Biomed. Mater. Res. - Part A* **106,** 746–757 (2017).
5. Abidian, M. R. & Martin, D. C. Multifunctional Nanobiomaterials for Neural Interfaces. *Adv. Funct. Mater.* **19,** 573–585 (2009).
6. Asplund, M., Nyberg, T. & Inganäs, O. Electroactive polymers for neural interfaces. *Polym. Chem.* **1,** 1374–1391 (2010).
7. Cui, X. & Martin, D. C. Electrochemical deposition and characterization of poly(3,4-ethylenedioxythiophene) on neural microelectrode arrays. *Sensors Actuators B* **89,** 92–102 (2003).
8. Green, R. & Abidian, M. R. Conducting Polymers for Neural Prosthetic and Neural Interface Applications. *Adv. Mater.* **27,** 7620–7637 (2015).
9. Wilks, S. J., Richardson-Burns, S. M., Hendricks, J. L., Martin, D. C. & Otto, K. J.

Poly(3,4-ethylenedioxythiophene) as a Micro-Neural Interface Material for Electrostimulation. *Front. Neuroeng.* **2,** (2009).

10. Yang, J., Kim, D. H., Hendricks, J. L., Leach, M., Northey, R., & Martin, D. C. Ordered surfactant-templated poly(3,4-ethylenedioxythiophene) (PEDOT) conducting polymer on microfabricated neural probes. *Acta Biomater.* **1,** 125–136 (2005).

11. Ludwig, K. A., Uram, J. D., Yang, J., Martin, D. C. & Kipke, D. R. Chronic neural recordings using silicon microelectrode arrays electrochemically deposited with a poly (3 , 4-ethylenedioxythiophene) (PEDOT) film. *J. Neural Eng* **3,** 59–70 (2006).

12. Wilks, S. J., Woolley, A. J., Ouyang, L., Martin, D. C. & Otto, K. J. In Vivo Polymerization of Poly(3,4-ethylenedioxythiophene) (PEDOT) in Rodent Cerebral Cortex. *IEEE* (2011).

13. Cui, X. T. & Zhou, D. D. Poly (3,4-Ethylenedioxythiophene) for Chronic Neural Stimulation. *IEEE Trans. Neural Syst. Rehabil. Eng.* **15,** 502–508 (2007).

14. Kozai, T. D. Y., Catt, K., Du, Z., Na, K., Srivannavit, O., Haque, R. M., Seymour, J., Wise, K. D., Yoon, E., & Cui, X. T. Chronic In Vivo evaluation of PEDOT/CNT for stable neural recordings. *IEEE Trans. Biomed. Eng.* **63,** 111–119 (2016).

15. Ludwig, K. A., Langhals, N. B., Joseph, M. D., Richardson-Burns, S. M., Hendricks, J. L., & Kipke, D. R. Poly(3,4-ethylenedioxythiophene) (PEDOT) polymer coatings facilitate smaller neural recording electrodes. *J. Neural Eng* **8,** (2011).

16. Venkatraman, S., Hendricks, J., King, Z. A., Sereno, A. J., Richardson-Burns, S., Martin, D., & Carmena, J. M. In Vitro and In Vivo Evaluation of PEDOT Microelectrodes for Neural Stimulation and Recording. *IEEE Trans. Neural Syst. Rehabil. Eng.* **19,** 307–316 (2011).

17. Alba, N. A., Du, Z. J., Catt, K. A., Kozai, T. D. Y. & Cui, X. T. In vivo electrochemical analysis of a PEDOT/MWCNT neural electrode coating. *Biosensors* **5,** 618–646 (2015).

18. Boehler, C., Kleber, C., Martini, N., Xie, Y., Dryg, I., Stieglitz, T., Hofmann, U. G., & Asplund, M. Actively controlled release of Dexamethasone from neural microelectrodes in a chronic in vivo study. *Biomaterials* **129,** 176–187 (2017).

19. Chikar, J. A., Hendricks, J. L., Richardson-Burns, S. M., Raphael, Y., Pfingst, B. E., & Martin, D. C. The use of a dual PEDOT and RGD-functionalized alginate hydrogel coating to provide sustained drug delivery and improved cochlear implant function. *Biomaterials* **33,** 1982–1990 (2012).

20. Bhagwat, N., Kiick, K. L. & Martin, D. C. Electrochemical deposition and characterization of carboxylic acid functionalized PEDOT copolymers. *J. Mater Res* **29,** 2835–2844 (2014).

21. Feldman, K. E. & Martin, D. C. Functional Conducting Polymers via Thiol-ene Chemistry. *Biosensors* **2,** 305–317 (2012).

22. Guimard, N. K., Gomez, N. & Schmidt, C. E. Conducting polymers in biomedical engineering. *Prog. Polym. Sci.* **32,** 876–921 (2007).

23. Ouyang, L., Shaw, C., Kuo, C., Griffin, A. & Martin, D. In vivo polymerization of poly (3 4-ethylenedioxythiophene) in the living rat hippocampus does not cause a significant loss of performance in a delayed alternation task. *J Neural Eng* **11,** (2014).

24. Richardson-burns, S. M., Hendricks, J. L., Foster, B., Povlich, L. K., Kim, D. H., & Martin, D. C. Polymerization of the conducting polymer around living neural cells. *Biomaterials* **28,** 1539–1552 (2007a).

25. Richardson-burns, S. M., Hendricks, J. L. & Martin, D. C. Electrochemical

polymerization of conducting polymers in living neural tissue. *J. Neural Eng* **4,** L6–L13 (2007b).

26. Egeland, B. M., Urbanchek, M. G., Peramo, A., Richardson-Burns, S. M., Martin, D. C., Kipke, D. R., Kuzon, W. M., & Cederna, P. S. In Vivo Electrical Conductivity across Critical Nerve Gaps Using Poly(3,4-ethylenedioxythiophene)-Coated Neural Interfaces. *Plast. Reconstr. Surg.* 1865–1873 (2010). doi:10.1097/PRS.0b013e3181f61848

27. Urbanchek, M. G., Kung, T. A., Frost, C. M., Martin, D. C., Larkin, L. M., Wollstein, A., & Cederna, P. A. Development of a Regenerative Peripheral Nerve Interface for Control of a Neuroprosthetic Limb. *Biomed Res. Int.* (2016).

28. Tong, Y., Murbach, J. M., Subramanian, V., Chhatre, S., Delgado, F., Martin, D. C., Otto, K. J., Romero-Ortega, M., & Johnson, B. N. A Hybrid 3D Printing and Robotic-assisted Embedding Approach for Design and Fabrication of Nerve Cuffs with Integrated Locking Mechanisms. *MRS Adv.* 1–8 (2018).

29. Chung, K., Wallace, J., Kim, S. Y., Kalyanasundaram, S., Andalman, A. S., Davidson, T. J., Mirzabekov, J. J., Zalocusky, K. A., Mattis, J., Denisin, A. K., Pak, S., Berstein, H., Ramakrishnan, C., Grosenick, L., Gradinaru, V., & Deisseroth, K. Structural and molecular interrogation of intact biological systems. *Nature* **497,** 332–337 (2013).

30. Cogan, S. F. Neural Stimulation and Recording Electrodes. *Annu. Rev. Biomed. Eng.* **10,** 275–309 (2008).