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In Vivo Polymerization of Poly(3,4-ethylenedioxythiophene) (PEDOT) in Rodent Cerebral Cortex

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***Abstract*—Maintaining a reliable neural interface is a well-known challenge with implanted neural prostheses. Here we evaluate a method of forming an integrated neural interface through polymerization of PEDOT *in vivo*. Polymerization resulted in lower impedance and improved recording quality of local field potentials on implanted electrodes in the rat cerebral cortex. Histological analysis by optical microscopy confirmed successful integration of the PEDOT within tissue surrounding implanted electrodes. This technique offers a unique neural interfacing approach with potential to improve the long-term functionality of neural prostheses.**

I. Introduction

Neural prostheses offer treatment and scientific understanding of physical and mental neurological disorders through interfacing with neural tissue. While an extremely versatile tool, long-term functionality problems arise due to an unreliable neural interface. During the implant time course the composition of the neural interface changes as a reactive tissue response encapsulates and isolates the implanted device. The response is a complex cellular process typically characterized by an encapsulation layer of glial cells [1] and often neuronal cell death and migration away from the device [2]. As a result, interfacial impedance increases and performance diminishes for recording and stimulation purposes.

Clinical uses of advanced implantable electrode systems require a reliable, high-performance neural interface which has yet to be realized. Strategies on improving the reliability of the neural interface include modifying device geometry [3], employing bioactive coatings [4]–[6], and applying voltage pulses [7], [8]. Such techniques have demonstrated limited success in improving reliability, demonstrating the difficulty in suppressing the reactive tissue response.

Forming a biocompatible, integrated neural interface after implantation, and potentially after stabilization of the reactive tissue response, would be ideal in maintaining chronic reliability. Such an approach is possible with conductive polymer technology. PEDOT can be polymerized in and around living neural tissue, creating a conductive polymer network in direct contact with the surrounding tissue [9], [10]. Further, PEDOT has shown success as a biocompatible material [11] as well as a stable electrode material [12], [13].

In this study, we extend *in vitro* and *in situ* PEDOT polymerization studies [9], [10] by investigating *in vivo* polymerization of PEDOT in brain tissue. Using an electrode-cannula system, we successfully delivered monomer solution locally and polymerized PEDOT *in vivo* in rodent cerebral cortex. We observed that interfacial impedance dropped and recording quality improved. Further, imaging of the tissue revealed dense PEDOT within 100 µm around the tip of the insertion site demonstrating a potential approach to better interfacing healthy neurons outside the zone of influence of the reactive tissue response.

II. Materials and Methods

A. Electrochemical Polymerization

In order to quantify the relationship between deposition time and the size of the PEDOT "cloud", *in vitro* polymerization was first analyzed in agarose gel (1 wt% in 1X phosphate buffered saline, PBS). Dual role electrode-guide devices (C315G-MS303/2/SPC, PlasticsOne, Roanoke, VA) consisting of a cannula guide (26 gauge) between two stainless steel wires (200 µm diameter) were inserted in agarose gel. Prior to polymerization, 6 µl of 0.01M monomer 3,4-ethylenedioxythiophene (EDOT) (483028, Sigma-Aldrich) solution prepared in 1X PBS was delivered at a rate of 0.2 µl/min. Once the EDOT injection was complete, constant voltage (potentiostatic) polymerization was performed at 2 V using an Autolab potentiostat/galvanostat (PGSTAT12, Eco Chemie, Utrecht,

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Netherlands) with deposition times between 0.5-45 minutes. A large-area platinum wire was used as the current return.

In vivo polymerization was performed by inserting the electrode-cannula device into the rodent cerebral cortex. 6 µl of 0.01M EDOT was injected at 0.2 µl/min followed by electrochemical polymerization at 2 V. The *in vivo* deposition time was determined from the *in vitro* results.

B. Surgery

Three male Sprague-Dawley rats were anesthetized via intraperitoneal injection of a cocktail of ketamine and xylazine followed by a ketamine bolus of 0.15-0.2 ml when necessary to maintain anesthesia level. The head was shaved and placed in a stereotaxic frame. A pulse oximeter was attached to the foot while oxygen was delivered. The area above the skull was numbed via injections of 1 ml lidocaine. The skull was then exposed and a craniometry approximately 2.5 mm in diameter was created either above the barrel cortex (n=1) or the primary motor cortex (n=2). The dura was removed and sterile saline was administered when necessary to prevent dehydration.

Prior to implantation, the cannula guide was plugged with a dummy cannula (stainless steel wire). Each rat was implanted with a single electrode-cannula device. Dental cement was used to secure the device to the skull in order to connect to the electrodes and insert a cannula without causing electrode movement within the tissue. A stainless steel needle pierced subcutaneously through the rat's hind neck skin acted as current return.

C. Impedance and Recording Measurement

Impedance and electrophysiology data were collected before and after polymerization. Electrochemical impedance spectroscopy (EIS) measurements were done by configuring the potentiostat to sequentially inject two 25 mV_{RMS} waveforms, one consisting of 15 frequencies between 0.01-1 kHz and the other consisting of 15 frequencies between 0.1-10 kHz. Each frequency sweep was taken in triplicates and the mean values were analyzed.

Extracellular neural recordings were acquired using Tucker-Davis Technologies equipment (RX7 and Medusa PreAmp, TDT, Alachua, FL). Neural electrophysiological data were amplified and low-pass filtered at 300 Hz to isolate local field potentials (LFPs).

D. Histology

Following *in vivo* data collection animals were sacrificed and their heads immediately removed and placed into a 4% buffered formaldehyde fixative at 4°C for 24 hours. The brain was then removed from skull and washed three times in 1X PBS. Using a razorblade, the cerebellum was removed and the hemispheres were separated. Tissue sections 400 µm thick were collected using a vibratome (Model VT100s, Leica Microsystems GmbH), and placed in coverglass-bottomed petri dishes for imaging. Slices were imaged using a Zeiss LSM10 inverted laser scanning confocal microscope (Carl Zeiss MicroImaging, Jena, Germany). Native PEDOT fluorescence was imaged using 405 and 488 nm lasers while a 633 nm laser simultaneously contributed to the collected transmission light image.

III. Results and Discussion

A. Electrochemical Polymerization

In vitro polymerization in agarose gel resulted in uniform, spherical PEDOT clouds as seen in Fig. 1a. Additionally, a "ringing" effect was observed at the outer edge of the PEDOT clouds, especially apparent with longer deposition times. This is possibly an effect of the potentiostatic deposition method. PEDOT can also be deposited under constant current (galvanostatic) modes which result in electrode coatings with a more uniform morphology than potentiostatically grown PEDOT [14]. It is also a possible effect of the changing EDOT concentration gradient as the polymerization reaction persists.

As seen in Fig. 1b, the outer diameter of the PEDOT cloud displayed a linear dependence with deposition time. Using this relationship, 30, 60 and 120 second deposition times were chosen for *in vivo* polymerization to control the PEDOT growth within 50-200 µm from the electrode tip. Each deposition time was tested in a separate rat subject. The resultant current profile started at approximately 20 µA and grew logarithmically to levels ranging from 45-90 µA, indicating decreased impedance.

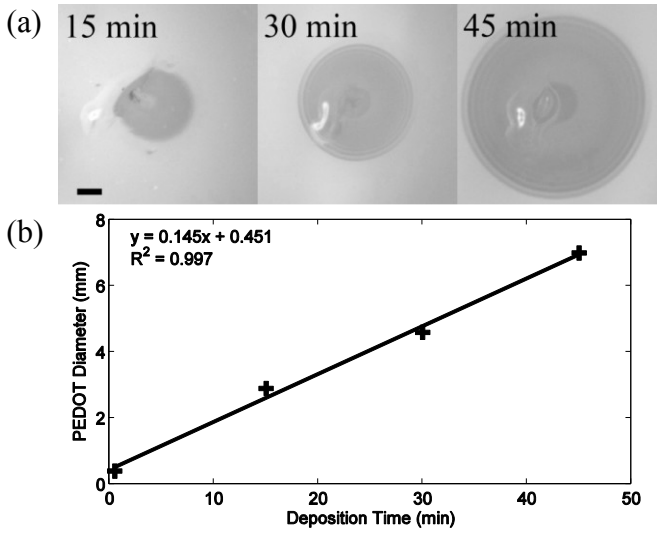

Fig. 1. PEDOT was polymerized in agarose gel to test the effect of deposition time on the diameter of the PEDOT cloud. (a) Uniform spherical clouds formed with a "ringing" effect visible at the outer edges (scale bar 1 mm). (b) The PEDOT cloud diameter was linearly proportional with deposition time.

B. Impedance

In vivo PEDOT polymerization resulted in a lower impedance magnitude across the entire spectrum as seen in Fig. 2. Additionally, greater polymerization times resulted in lower impedances, especially at higher frequencies (0.5-10

kHz). The 1 kHz impedance dropped from a mean of 76.3 kΩ before polymerization to 24.9, 17.6, and 11.3 kΩ for PEDOT deposited at 30, 60, and 120 seconds. These results are likely due to the large increase in the geometrical and electrochemical surface area of the electrode interface. Increasing deposition time forms larger PEDOT clouds with greater surface area and lower impedance.

EIS is a common technique for *in vivo* assessment of the electrode-tissue interface and electrode recording performance. The interfacial impedance, which is proportional to recording noise level, typically shows increasing trends with time post-implantation as the reactive tissue response progresses [15]. The increasing impedance corresponding to increasing noise levels coupled with lower neural densities adjacent to the electrode results in poor recording quality in a relatively short amount of time after device implantation. Mechanisms to lower impedance *in vivo*, such as applying voltage pulses [8], have been successful at improving recording quality. Further, PEDOT coatings on chronically implanted electrodes have shown to improve performance in terms of impedance, signal-to-noise, and LFP recordings [16].

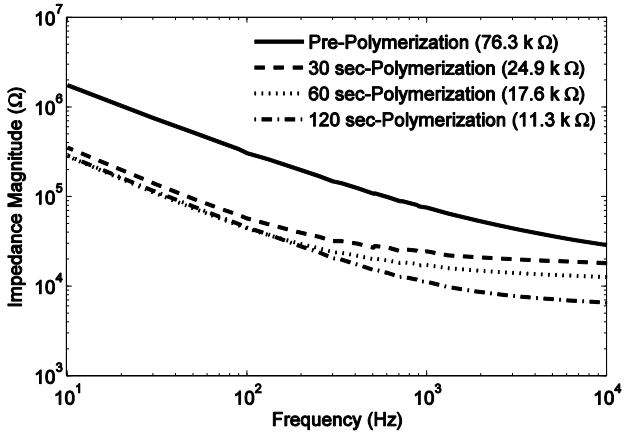

Fig. 2. *In vivo* EIS data were collected before and after polymerization for 30, 60, and 120 seconds. Each deposition time was tested in a separate electrode and rat. Impedance magnitude dropped after polymerization with the 1 kHz impedance magnitude, shown in parentheses, decreasing from 76.3 kΩ to 24.9, 17.6, and 11.3 kΩ for deposition times of 30, 60, and 120 seconds.

C. Recordings

Prior to *in vivo* polymerization, LFPs displayed a high root mean square (RMS) level as well as a large peak in the mean power spectrum corresponding to 60 Hz noise (Fig. 3a,b). *In vivo* PEDOT polymerization resulted in LFPs with a lower RMS level and a large decrease in power at 60 Hz (Fig. 3c,d). During polymerization, the overall area of the electrode in direct contact with tissue increases dramatically as PEDOT grows. The improved LFP recording quality is a likely result of the larger, lower-impedance electrode-tissue interface.

The electrodes used in this report are too large to distinguish single unit activity; however, the utility of this approach can be translated to smaller devices. Future microelectrode designs will attempt to minimize size in order to maximize number of channels and their sensitivity. However, as electrode size is minimized, high impedance becomes more problematic. Recently, PEDOT coated electrodes have shown to facilitate recordings from high impedance electrode sites 15 μm in diameter [17]. Similarly, *in vivo* polymerization could enable recordings from high-impedance electrodes on the order of tens of micrometers and potentially much smaller.

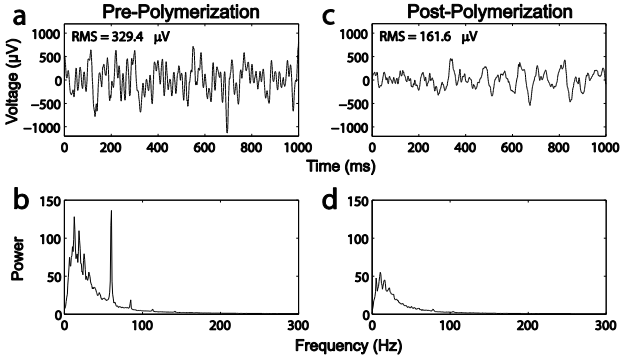

Fig. 3. (a) Pre-polymerization LFPs displayed a high RMS level and (b) a large peak in the mean power spectrum corresponding to 60 Hz noise. After *in vivo* polymerization for 30 seconds, (c) the RMS level of the LFPs dropped and (d) the power at 60 Hz was much lower.

D. Histology

Imaging of the tissue revealed successful *in vivo* PEDOT polymerization in the form of a fluorescent cloud surrounding the tip of the electrode insertion tract in the cortex (Fig. 4). Similar to that seen *in vitro*, the size of the PEDOT cloud *in vivo* was proportional to the deposition time. Fig. 5 displays sections cut through the insertion tract and PEDOT clouds deposited at 30, 60, and 120 seconds which resulted in PEDOT deposited approximately 130, 160, and 180 μm from the edge of the insertion tract. Once again, a "ringing" effect was observed *in vivo*. Two PEDOT rings

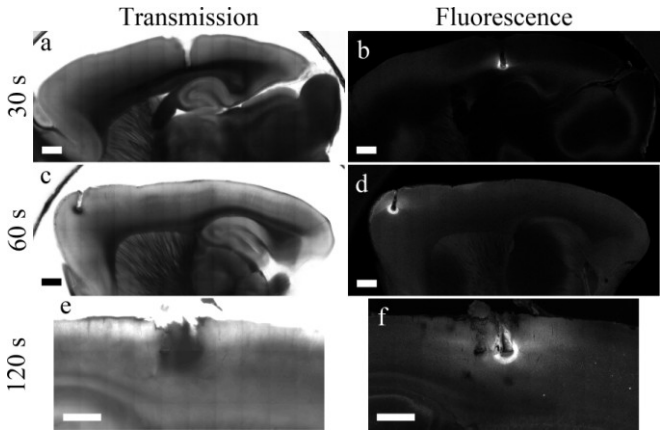

Fig. 4. Histology of PEDOT clouds in tissue. (a) Transmission light and (b) 488 nm fluorescence images of the whole brain slice revealed the location within the cortex of the PEDOT cloud deposited for 30 seconds, (c,d) PEDOT deposited for 60 seconds, and (e,f) PEDOT deposited for 120 seconds (scale bar 1 mm).

were visible in the fluorescence images of PEDOT clouds deposited at 60 seconds (Fig. 5b) and 120 seconds (Fig. 5c).

In contrast to the uniform PEDOT spheres formed in agarose gel, the PEDOT clouds formed *in vivo* displayed a nonuniform shape. One contributing factor is increased diffusion up the insertion tract evident by fluorescent PEDOT along the tract. In addition, the heterogeneity of brain tissue likely affected the EDOT diffusion and PEDOT cloud formation.

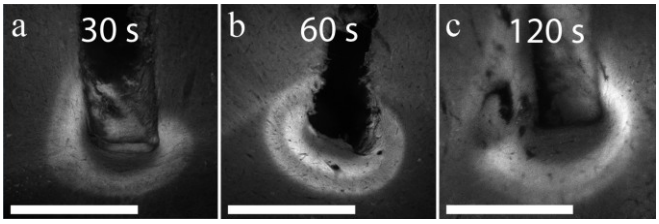

Fig. 5. Detailed imaging of intrinsic 488 nm fluorescence of PEDOT clouds in tissue. (a) 30 second deposition time resulted in a dense PEDOT cloud approximately 130 µm from the tip of the electrode tract. (b) PEDOT deposited for 60 seconds extended out 160 µm and (c) PEDOT deposited for 120 seconds extended out 180 µm. Two PEDOT rings are visible for the 60 and 120 second deposition times (scale bar 500 µm).

Characterization of the reactive tissue response has shown its greatest zone of influence is within a 100 µm radius of the implanted device. After 2-4 weeks of implantation, this zone exhibits approximately 40% decrease in neuronal cell body density [2] whereas the cellular composition typically appears normal beyond this region. As seen in Fig. 4, PEDOT can polymerize *in vivo* beyond this region and potentially directly interface with healthy neural tissue for improved recording quality and stimulation performance.

IV. Conclusion

Polymerization of PEDOT *in vivo* in rodent cerebral cortex demonstrates a unique approach in interfacing implanted electrodes with excitable tissue. The *in vivo* polymerization technique described here resulted in lower interfacial impedance and improved recording quality. Imaging the tissue revealed successful integration of a dense PEDOT network within the tissue extending more than 100 µm adjacent to the electrode demonstrating potential to interface with healthy neurons beyond the damaged tissue region associated with the reactive tissue response. This technique may be a valuable strategy in improving the long-term functionality and reliability of implanted neural prostheses.

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