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Poly (3, 4-ethylenedioxythiophene)-ionic liquid coating improves neural recording and stimulation functionality of MEAs

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Abstract

In vivo multi-electrode arrays (MEAs) can sense electrical signals from a small set of neurons or modulate neural activity through micro-stimulation. Electrode's geometric surface area (GSA) and impedance are important for both unit recording and neural stimulation. Smaller GSA is preferred due to enhanced selectivity of neural signal, but it tends to increase electrode impedance. Higher impedance leads to increased electrical noise and signal loss in single unit neural recording. It also yields a smaller charge injection window for safe neural stimulation. To address these issues, poly (3, 4-ethylenedioxythiophene) - ionic liquid (PEDOT-IL) conducting polymers were electrochemically polymerized on the surface of the microelectrodes. The PEDOT-IL coating reduced the electrode impedance modulus by over 35 times at 1 kHz. It also exhibited compelling nanostructure in surface morphology and significant impedance reduction in other physiologically relevant range (100Hz-1000Hz). PEDOT-IL coated electrodes exhibited a Charge Storage Capacity (CSC) that was about 20 times larger than that of bare electrodes. The neural recording performance of PEDOT-IL coated electrodes was also compared with uncoated electrodes and PEDOT-poly (styrenesulfonate) (PSS) coated electrodes in rat barrel cortex (SI). Spontaneous neural activity and sensory evoked neural response were utilized for characterizing the electrode performance. The PEDOT-IL electrodes exhibited a higher unit yield and signal-to-noise ratio (SNR) in vivo. The local field potential recording was benefited from the low impedance PEDOT-IL coating in noise and artifact reduction as well. Moreover, cell culture on PEDOT-IL coating demonstrated that the material is safe for neural tissue and reduces astrocyte fouling. Taken together, PEDOT-IL coating has the potential to benefit neural recording and stimulation electrodes, especially when integrated with novel small GSA electrode arrays designed for high recording density, minimal insertion damage and alleviated tissue reaction.

Introduction

Neural activity recorded on in vivo MEAs from adjacent neurons provides important information for understanding the neural basis of cognition and for developing brain computer interfaces (BCI) ^{1, 2}. Neural stimulation delivered through MEAs can also restore lost sensation ^{3, 4} or treat neurological disease such as Parkinson's disease and epilepsy ^{5, 6}. However, chronic MEA neural recording and stimulation suffer from performance instability and longevity issues ^{1, 7-9}. The signal degradation of chronically implanted MEA as a result of tissue response to foreign body prevents the study of same neural tissue over long periods of time. Among the factors impairing the performance of MEAs, elevated impedance may correlate with deterioration of the SNR of MEAs ^{10, 11}.

Recently, ultra-small MEAs have gained popularity because they have the potential to alleviate chronic tissue damage caused by the neural implants ¹²⁻¹⁴. Smaller implantations reduce the chance of severing the axon connections and pushing aside neural tissue next to the recording sites. And the foreign body response to implantation can also be significantly reduced when the overall device size is smaller than the dimensions of cell bodies. For small MEAs, smaller GSA electrodes are preferred since it allows for the reduction of overall device size. Additionally, smaller GSA provides outstanding selectivity of neural signal and enable recording of neural activity from densely packed, heavily interconnected populations of neurons simultaneously ¹⁵. However, the performance of the conductor-electrolyte interface for ultra-small GSA is usually hindered by the high impedance. Altering the charge transfer mechanism by depositing metal oxide (e.g. IrO₂) or fuzzy metal (e.g. Pt-Black) provides a possible solution, but poses the threat of releasing heavy metal ions or even particles into the surrounding tissue ¹⁰. Notable for its electrical conductivity, tunable morphology and good biocompatibility, electrochemically polymerized PEDOT composites are ideal for lowering the impedance of ultra-small GSA devices ^{13, 14}.

Biofouling of protein and glial cells is another critical challenge for chronic neural implants. Extracellular recording performance can be compromised by proteins and non-neuronal cells fouling on the electrode surface easily ^{16, 17}. Even a monolayer of glial cell encapsulating neural electrodes has been shown to be sufficient to dramatically increase the electrode impedance in vitro ¹⁸.

The neural stimulation specificity and longevity are also closely related to the impedance of electrodes. A low impedance stimulation can increase both efficacy and safety of neural stimulation by reducing faradaic reactions on electrode surface that could endanger the safety of surrounding tissue. For a specific amount of current injected through a microelectrode-electrolyte interface, the resulting voltage amplitude determines whether irreversible electrochemical reactions will occur at the interface ^{19, 20}. Irreversible chemical reactions may include electrolysis and possible dissolution of the electrode metal material. The amount of irreversible charge transfer correlates with the magnitude of tissue damage ²¹. Even when the impedance of stimulation electrodes is increased due to the glial scar, an initial low impedance can potentially negate the negative impact of this and provide a stable stimulation performance. Moreover, a smaller GSA of neural stimulation electrode can increase the neural stimulation specificity. Examples of current neural stimulation

application include deep brain stimulation (DBS), vagus nerve stimulation, limb prostheses, bladder prostheses, cortical visual prostheses and cochlear implants¹⁰ and PEDOT coating can potentially benefit any class of these stimulation electrodes.

Various conducting polymer coatings have been developed for microelectrodes^{16, 22, 23}, in order to improve the electrode impedance and biocompatibility. Among the conductive polymers, the polystyrenesulfonate (PSS) doped PEDOT has demonstrated stable chronic recording performance²⁴ and other beneficial properties. Additionally, biomolecules can be incorporated into PEDOT to improve tissue integration of the neural implant²⁵. High performance and stable long term neural stimulation of PEDOT polymer was also successfully performed in various neural tissues²⁶⁻²⁸. Furthermore, the surface morphology of PEDOT composites can be controlled by different polymerization conditions, so they can be adjusted to best satisfy various goals²⁹⁻³². Lately, PEDOT has been successfully polymerized around biological tissue³³, possibly allowing seamless integration of electronic device and host tissue.

Ionic liquids are organic salts that are in liquid form at room temperature. They are non-volatile, highly polarized solvents that can dissolve various organic and inorganic compounds. In recent years, ILs have gained tremendous popularity in electrochemistry, due to their exceptional properties compared to regular solvents. As such, this novel class of materials is widely investigated for improving or replacing current solvent materials in all essential industrial and academic applications, including catalysts³⁴, sensors³⁵⁻³⁸, electrochemical actuators³⁹, and clean energy⁴⁰. One readily available IL, 1-Ethyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide (EMIIM) has shown numerous beneficial properties including low melting temperature, low viscosity, high electrical conductivity and broad electrochemical window. During the electrochemical synthesis of PEDOT polymer in aqueous solution, the low solubility of EDOT monomer in water (0.01M) limits the polymerization rate and efficiency. However, due to the similarity of the organic composition of EDOT and EMIIM, the solubility of EDOT monomer is increased more than 10 fold compared to water. Outstanding long-term stability³⁹ and electrical conductivity^{41, 42} have been observed in IL-based conducting polymer materials⁴³. Furthermore, numerous RTIL molecules had been tested for neural toxicity and our candidate EMIIM demonstrate extremely low toxicity for rat IPC-81 cell line⁴⁴ and relatively low toxicity to human Hela cells⁴⁵ and acetylcholinesterase (AChE), an important nervous system enzyme and primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides.

In this work, ultra-low impedance PEDOT-IL polymer with greatly enhanced surface nano structure compare to electrochemically synthesized²⁵ or solution casted^{46, 47} PEDOT-PSS was synthesized. The Charge Injection Limit (CIL) of PEDOT-IL for neural stimulations was improved as well. PEDOT-IL coated MEAs demonstrated improved acute neural recording performance. In vitro cell culture test showed that PEDOT-IL coating present minimum neuronal toxicity while inhibit non-specific glial cell attachments. Glial cells play a major role in scar tissue encapsulation that cause chronic neural recording/stimulation degradation. Taken together, PEDOT-IL shows great potential for benefiting BCI applications and neuroscience research by improving the interface between microelectrodes

and neural tissue. Our work of PEDOT-IL is one of the earliest research⁴⁸ to introduce the beneficial properties of RTIL to the field of BCI.

Results and Discussion

Electrodeposition and surface characterization

To evaluate the performance of PEDOT-IL modified electrodes, PEDOT-IL was deposited onto gold microelectrodes and the electrode characteristics were compared to the uncoated gold as well as PEDOT-PSS coated electrodes. PEDOT-PSS is a well characterized PEDOT coating for high performance neural recording^{24, 25} and stimulation^{26, 27}, and was chosen as a control. The surface characteristics of PEDOT-IL were very different from PEDOT-PSS as revealed by SEM (Fig. 1). PEDOT-IL has a very porous surface and fine textures with nanometer-diameter thin filaments (Fig.1B). The nanofiber-rich surface texture was not observed on PEDOT-PSS polymer, which showed a cauliflower-like morphology as shown in Fig 1A. The unique nano-texture of PEDOT-IL thus created a larger electrochemical surface area and further decreased resistance at the interface compare to PEDOT-PSS. The nano- texture of conducting polymers are essential for various applications. Importantly, PEDOT-IL and PEDOT-PSS deposition did not dramatically enlarge the original area of the gold electrode, instead, the polymer increased the surface roughness and effective electrochemical surface area of the electrode. For PEDOT based sensors, nano texture can increase the polymer surface leading to increased signal amplitude and sensitivity²⁹. For PEDOT based electrochemical drug release devices, nano structures can increase the amount of drug loading and release efficiency⁴⁹. Furthermore, the wettability of polymer surface is altered by various nano scale morphology features, which can be tailored to control cell adherence and growth^{31, 50}. Numerous studies utilized nano fabrication techniques to achieve different nano structures with doped PEDOT to accomplish these needs. The novel electrochemically synthesized PEDOT-IL in this study was easily fabricated on conductive electrode surface and can potentially satisfy the multiple needs mentioned above.

The additional surface texture without increased GSA also benefit neural recording performance. The signal quality from neurons near the electrode was improved by the reduction of electrode surface impedance, but the neural recording selectivity in a localized region was still maintained by the small GSA. The fine fibres and small pores of PEDOT-IL polymer were on the scale of 20 nm to 200 nm, similar in size to proteins and biological nanoparticles. Therefore, more intimate cell-material interactions might occur on PEDOT-IL polymers. As a result of the hollow structure of PEDOT-IL polymer, the electrolyte can access not only the outmost layers of the polymer, but also the interior, beneficial over the PEDOT-PSS polymer.

Electrochemical characterization

An impedance reduction across all frequencies measured was observed with the presence of PEDOT-IL polymer. Gold microelectrode impedance at 1000 Hz was reduced around 30 fold after PEDOT-IL deposition (Fig. 2A and Table 1). Comparing with the PEDOT-PSS coating, PEDOT-IL coating demonstrated more impedance amplitude reduction at 200 Hz. Impedance reduction at lower frequencies is important because this frequency range is

relevant to LFP recording. To further investigate the mechanism of the different impedance behaviour of PEDOT-IL and PEDOT-PSS, an equivalent circuit model for the polymer coated electrodes was built⁵¹. Custom written MATLAB script based on the LEVM program⁵² was utilized for the impedance modelling. The equivalent circuit model is shown in Fig. 2C and is composed of a diffusive impedance Z_D , an adsorption resistance R_{AD} and a double-layer impedance Z_{B-CPE} (top panel). Distributed element Z_D is further elaborated in bottom panel. Z_{B-CPE} is controlled by model parameters Q_{CPE} and α and Z_D is controlled by model parameters R_1 , R_3 and Q_3 and β . Model parameters R_1 , R_3 and Q_3 are mathematically derived from the distributed parameters r_1 , r_3 and q_3 in the bottom panel of Fig 2C with the equations of previous research⁵³, with all terms denominated exactly the same. Excellent fit quality was achieved for all three types of electrodes shown in Fig. 2B. All model parameters relevant to neural record performance are reported in Table 1. The parameter that demonstrates the largest difference between the two polymers was adsorption resistance R_3 . R_3 is related to the difficulty of electron transfer at the liquid-polymer interface⁵³. The low R_3 of the PEDOT-IL coating may be a result of the nano feature of PEDOT-IL since it increased the effective electron transfer area within a limited 3D space. Another reason may be that the large PSS molecules generated a non-conductive barrier for electron transfer.

The direct relationship between impedance and in vivo neural recording is yet to be fully determined. Different studies suggest different trends of correlation or no correlation^{54, 55}. Most of these studies employ 1 kHz impedance amplitude for the correlation study, which oversimplifies the various factors that could affect the electrode-electrolyte interface characteristics. Our study demonstrated the benefit of full impedance spectrum in understanding a porous charge transfer interface properties for neural recording. It revealed more information than solely using 1000 Hz impedance amplitude. Furthermore, the equivalent circuit modelling to accurately dissociate multiple components of a liquid-solid charge transfer interface is only feasible from a full impedance spectrum. This method could provide insights into elucidating the change of electrode properties⁵⁶ and tissue encapsulation⁵⁷ during chronic in vivo implantations.

Electrical stimulation properties

The charge storage capacity (CSC) and CIL of PEDOT-IL, PEDOT-PSS and bare gold electrodes were compared to evaluate the neural stimulation performance of microelectrodes. The CSC was measured by integrating the current of a cyclic voltammetry curve for all three types of electrodes. On the CV curve of PEDOT-IL, the redox peaks were found around -0.7V and the waveform closely resembles PEDOT-PSS. The CSC of PEDOT-IL coated electrode can be more than 20 times higher than the uncoated electrodes (Table 1). Although CSC demonstrate electrochemical redox activity of the polymer, much of the charge transfer under slow scan of the CV cannot be accessed under the much faster neurostimulation conditions. To further study the neurostimulation performance of this polymer, physiologically relevant electrical current stimulations were passed through the electrodes and the voltage waveform on the electrode surface was measured. The CIL was calculated by integrating the recorded current waveform at -0.6V because -0.6V is often considered the safe potential beyond which significant amount of irreversible chemical reactions could occur, such as dissolution of metal and water electrolysis. For neural

stimulation electrodes, many surface modifications have been developed, e.g. platinum black, iridium oxide and conducting polymer/carbon nanotubes to increase CIL of electrode ¹⁰. Our results indicate PEDOT-IL coating has a CIL of 1.2 mC/cm² which is increased from that of the gold electrode, 0.4 mC/cm² (Table 1). High CIL material is highly desired for chronic micro-stimulation applications such as retinal implants and cortical somatosensory prosthesis where very small electrodes are patterned in high density for high resolution ⁵⁸.

In vivo acute recording performance

The acute neural recording performance PEDOT-IL and PEDOT-PSS coating was compared to uncoated gold electrodes in Fig 3 and Table 1. The recording was performed in layer IV of primary sensory cortex (SI), or barrel cortex. Rat barrel cortex topographically and reliably represents mechanical stimulations from the facial whiskers. The sensory information transmitted from thalamus mainly activates the layer VI neurons of this cortical area in a well characterized and quantifiable manner when the corresponding facial whisker is displaced. Neural response was shown in the filtered neural data stream (Fig 3A). Very few action potentials were observed on the unmodified gold microelectrodes, and those that were recorded had extremely low signal amplitude (Fig 3A, B). Another drawback of the high impedance gold electrodes was the high noise (Table 1). The collective outcome of the two aspects resulted in the low SNR of gold electrode (Table 1). In comparison, both PEDOT-IL and PEDOT-PSS showed very clear extracellular action potentials (Fig 3A) with high SNR and readily distinguishable waveforms and higher number of automatically sortable units on each channel (Fig 3B, Table 1). The impedance of electrodes at 1 kHz is thought to affect the SNR of action potential recordings because action potential waveforms are usually around this frequency. With the similarly low 1 kHz impedance for both types of polymers, the electrodes performed similarly for unit recordings (Fig 3A).

Another important type of extracellular recording is LFP. LFP is the low-frequency oscillation originated from the summation of dendritic electrical potentials from a local area. It usually represents the average activity level of a type of synaptic transmission in a certain area. It is useful for the study of the cognitive states of a brain region or for determining disease state such as epilepsy. More recently, LFP recording via Electrocortical Graph (EcoG) has also been shown to provide sufficient information for brain computer interface control ⁵⁹. Great improvement was also observed in LFP recording on the polymer coated electrodes. 60Hz electrical noise from power supplies can strongly impede LFP waveform recording (Fig 4A **Left**, Table 1); and the noise is especially prominent when nearby electronic devices are not well grounded. This interference was unavoidable with high impedance electrodes even when the grounding of devices was done very carefully. To decrease the amplitude of 60 Hz noise notch filters can be applied, but this would irreversibly and significantly distort original physiological signals in frequency bands near 60Hz. This frequency band of LFP is referred to as “Gamma Oscillation” and is often involved in important brain functions like sensory processing, working memory and other high order brain functions ⁶⁰.

LFP recording during sensory evoked trials were examined. In PBS, strong artifacts from mechanical whisker stimulator was observed across all frequencies on high impedance electrodes (Fig 4A **Middle**). The power increase centered at 60 Hz was not prominent due to the fact that preamplifier was already saturated in these frequency bands as shown in Fig 4A **Left**. In vivo recording was also affected in similar manners as shown in the power spectrum difference between evoked LFP and spontaneous LFP in Fig 4A **Right**. The polymer coated electrodes successfully recorded clean LFP signals without the artifact contamination thanks to the low impedance in this frequency band, while the gold electrodes exhibit increases of oscillation power across all important LFP frequencies. In this figure, the physiologically evoked LFP power was clearly weaker than artifacts and was evenly distributed across most frequencies. The detailed power spectrogram in Fig 4B further demonstrates the in vivo evoked LFP recording performance. The spontaneous LFP signal before sensory stimuli on bare gold electrodes (Fig 4B, **Left**) was weaker compare to the PEDOT coated electrodes (Fig 4B, **Middle** and **Right**) as emphasized by the white circles in Fig 4B. The sensory stimuli trigger a nearly identical high amplitude response with each of the 3 stimuli on the gold electrode (Fig 4B, **Left**). The extreme similarity of 3 triggered LFP response indicate the majority of the LFP response was contaminated by electrical artifact because sensory evoked LFP signal should be weakened over stimuli due to adaptation, as depicted in Fig 4B **Middle** and **Right**. In summary, PEDOT-IL coated electrode significantly reduced the noise artifact of LFP recording.

Neuronal toxicity and glial cell adhesion

To verify the safety of PEDOT-IL for neurons, the key cell types to record from and stimulate, MTT assay was carried out in primary neuronal cultures grown in the presence of PEDOT- IL film and the result was compared to PEDOT-PSS, which is known to exhibits excellent in vitro and in vivo biocompatibility⁶¹. This assay determines the metabolic activity levels of cells to indicate the viability. The polymer coated macroelectrodes were placed in cell culture inserts with neuronal cultures grown on the bottom of wells coated with Laminin. The inserts were taken out during the spectrum measurement of MTT assay. The PEDOT-IL electrodes exhibited an equivalent level of neuron viability compared to PEDOT-PSS (Table 1). The MTT assay substantiates the safety of PEDOT-IL polymer for the neural tissue surrounding implanted MEA.

As glial cell adhesion and encapsulation is one of the major failure mode for neural recording, it is important to assess how glial cells respond to the polymer. The assessment of glial cell adhesion on PEDOT-IL polymers was examined by astrocyte culture seeded directly on half PEDOT-IL coated gold macroelectrodes. Astrocyte grew well on the gold surface (Fig. 5A) without adhesion promoter but was completely repelled by the PEDOT-IL surface (Fig. 5B). On the contrary, PEDOT-PSS was highly permissive to astrocyte attachment. Astrocyte adhesion is particularly interesting due to its role in chronic neural tissue response. Immediately post implantation, glial cells adhere on the MEA¹. These activated glial cells generate a “kill zone” around the implant, characterized by the absence of neurons, which directly compromise the recording quality. The contrasting behavior between PEDOT-PSS and PEDOT-IL on cell adhesion may be a result of low serum protein adsorption on PEDOT-IL or surface topography difference⁶². Further investigations on the

exact mechanisms will be performed to shed light on design strategies on non-fouling electrode materials. Electrode surface modification like PEDOT-IL that repels glial cells could potentially benefit spike recording by reducing the “kill zone” of glial cells and bringing neurons closer to the recording sites.

Experimental

Materials

Ionic liquid 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide (EMIIM) was acquired from Covalent Associates, Inc. (OR). All other chemicals were obtained from Sigma-Aldrich (MO). 22 mm plastic coverslips (Fisher, PA) were cut to uniform dimension of 7×22 mm, cleaned with 8N HNO₃ for 30 min, washed in deionized H₂O and stored in ethanol. Coverslips were then sputter coated with 35nm of gold, using a Cressington Sputter Coater (Cressington, UK). 32 channel gold site microelectrode arrays for in vivo neural recording were generously provided by Science and Biomedical Microsystems (MD). The GSA of electrode sites on the probe was 155 μm^2 . Each of the 3 shanks that were 150 μm apart had 10 sites arranged in an arrowhead formation; with one site at the tip of the shank and the rest in two parallel columns. The middle shank also has 2 sites at least 200 μm away from the rest. They were not included in the neurophysiology experiments. The adjacent sites center to center distance is 25 μm and the center to center distance of the furthest sites was 135 μm . The densely packed sites ensured all recording were performed within the same layer of cortex.

Electrochemical deposition and characterization of the film

PEDOT-IL films were electrochemically deposited onto electrodes in a solution of 0.03M 3, 4-ethylenedioxythiophene (EDOT) monomer dissolved in EMIIM IL. The deposition solution for PEDOT-PSS consisted of 0.01 M EDOT and 0.1M PSS dissolved in deionized water. The concentration of EDOT was reduced due to its poor solubility in water. Both PEDOT- IL and PEDOT-PSS were galvanostatically polymerized in a three-electrode cell consisting of a platinum sheet counter electrode and Ag/AgCl reference electrode using a FAS 2 Femtostat (Gamry, PA). On microelectrodes, 2 nA of current was passed for 200 s to form the film, and the charge density for deposition was 0.26 C/cm². For macroelectrode, chronocoulometry was used to deposit a total charge of 100 mC at 1.1 V. Half of the gold macroelectrode was coated with PEDOT-IL and the rest was gold surface. The impedance spectrum from 10 Hz to 32 kHz and cyclic voltammetry measurements with a scan rate of 1 V/s and a scan range of -0.9 V to 0.6 V were obtained with an Autolab N128 (Metrohm Autolab B.V., The Netherlands) in 10 mM phosphate buffered solution (PBS). Scanning Electron Microscopy (SEM) images of polymer deposition on microelectrodes were taken with a JSM 6330F SEM (Joel, Japan). For neural probes, 3.5 nm of Pd was sputtered onto the electrode surface with a Cressington Sputter Coater.

Charge injection limit measurement

A cathodic leading square wave current was generated by Digidata digitizer output (Model 1322a, Molecular Devices, CA) and a linear voltage-current conversion isolator (Model 2200, A-M systems, WA). This current signal mimics deep brain stimulation (DBS)

parameters. The stimulation began with a 200 μ s cathodic current, immediately followed by a 400 μ s charge balanced current with half amplitude and reversed polarity. Various current amplitudes were delivered through the microelectrode surface and voltage profiles were recorded with the digitizer at a sample rate of 500 kHz. Linear regression was applied to voltage-current relationship and a voltage threshold of -0.6 V was set as safe charge injection window for stimulation¹⁹⁻²¹. The CIL was calculated based on the amount of charge at -0.6 V per GSA.

Acute in vivo neural recording

All animal work was performed under the guidelines of the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC). A Sprague-Dawley (SD) rat was anesthetized under 3% Isoflurane and head-fixed in a SR-6R stereotaxic frame (Narishige, NY). A 2 mm by 2 mm craniotomy above the somatosensory barrel cortex (SI) of the right hemisphere was opened on the skull, centered at coordinates of 2.5 mm posterior to Bregma and 5.5 mm lateral to midline⁶³. Dura mater was recessed and the neural probe was inserted into the cortex with a micromanipulator at a slow speed to 950 μ m beneath the cortical surface. On the same implanted neural recording probe, adjacent gold sites were functionalized with polymer or left unfunctionalized to achieve direct comparison of Au/PEDOT-PSS/PEDOT-IL. The neural signal was amplified with a 16 channel Medusa preamplifier and recorded with an RX5 processor at 25 kHz sampling rate (Tucker-Davis Technologies, FL). Neural signal was subsequently imported into MATLAB with custom scripts for analysis. Sensory stimulation to contralateral facial whisker was delivered by a custom made whisker stimulator based on the motion of a mechanical relay, controlled and synchronized by 5 V transistor-transistor logic (TTL) signals from RX5 digital output⁶⁴. Each whisker stimulation is consisted of 3 single TTL triggers of 100 ms duration delivered at 4 Hz as indicated by 3 black lines in each panel of Fig 4B.

Neural data analysis

The raw data was filtered through a second order band pass Butterworth filter of 300 Hz-3000 Hz to yield the spike stream. A 1 Hz to 300 Hz band pass filter was applied to yield local field potential (LFP) stream. Because the whisker stimulator and animal movement caused baseline fluctuations in spike data stream, the algorithm called Subtraction of Artifacts by Local Polynomial Approximation (SALPA) was utilized to correct for these artifacts⁶⁵. The offline spike detection threshold is set at 3.5 standard deviations of spike data stream²⁴. Following spike detection, a K-means method was applied to pre-cluster the threshold-crossing events on a single channel to a large number of plausible units, and eventually the small clusters were aggregated based on a mixture of multivariate t-distribution algorithm⁶⁶. The peak-to-peak amplitude of mean waveform was considered the signal amplitude of a sorted unit, and 2 times standard deviation of spike stream, excluding all threshold crossing events (TCEs) was considered noise amplitude⁶⁷. SNR definition is shown in Eq. 1 where $\bar{W}$ represents the mean waveform of a cluster:

$$SNR = (\max(\bar{W}) - \min(\bar{W})) / (2 * SD(Stream)) \quad (1)$$

The software package Chronux was utilized to generate the power spectrum and spectrogram of LFP signals^{68, 69}. 60 Hz electrical noise from the power supply was omitted in the power spectrum because the signal saturated the preamplifier on the non-modified gold microelectrodes. The whisker stimulator artifact was characterized by measuring electrical signal in a 0.01 M PBS in order to separate the real neural response from electrical artifacts. The power spectrum increase of whisker stimulator in Fig 4A Middle and Right was calculated by subtracting the spontaneous LFP power between 0 and 1 seconds of Fig 4B from the evoked LFP power between 1 and 2 seconds of Fig 4B while black vertical lines indicate the whisker stimulations. Each recording session included 15 whisker stimulations delivered at frequency of 0.3 Hz, thus the 15 power spectrum subtraction results are averaged to achieve an optimal estimation. The power spectrograms examples of a single whisker on each type of recording electrode in Fig 4B was calculated using a moving window of 50 ms with a step size of 5 ms, a multi-taper method of Chronux toolbox was utilized to improve the quality of the spectrograms⁶⁹. In order to fit a general trend of LFP power spectrum and estimate the strong noise on gold electrode at 60 Hz, the LFP power spectrum below 10 Hz, between 45 Hz and 75 Hz, as well as that between 110 Hz and 130 Hz was removed before an exponential decay function was fitted to the spectrum. 120 Hz was excluded due to the harmonic power contamination of 60 Hz noise. The power change versus fitted function at 60 Hz was reported.

Cell culture

Primary neuron and astrocyte cells were isolated from E18 Sprague-Dawley rat hippocampal tissue. All cell culture and staining reagents were acquired from Invitrogen, CA unless indicated otherwise. The tissue was digested using a buffer solution consisting of trypsin (0.025%, Sigma-Aldrich, MO), NaCl (137 mM), KCl (5 mM), Na₂HPO₄ (7 mM), and HEPES (25 mM). Neuron cultures were maintained in Neurobasal medium supplemented with B27 (2%), GlutaMax (1%), and penicillin/streptomycin (1%). Astrocyte cultures were maintained in Dulbecco's Modified Eagle Medium (Sigma-Aldrich, MO) supplemented with fetal bovine serum (10%, Sigma-Aldrich, MO) and penicillin/streptomycin (1%).

To determine the toxicity level of PEDOT-IL, neuron culture was selected because of its relevance in neural recording as well as its high sensitivity to insults. The macroelectrodes with PEDOT-IL (n=3) and PEDOT-PSS (n=3) coating were placed in culture well inserts (Fisher, PA) to prevent direct contact of neuron with polymer, Neurons were seeded in 24 well plates at a density of 50,000 cells/cm² and grown for 2 days, surfaces were treated with poly-l-lysine (100 µg/mL) in PBS for 2 h and washed several times with PBS prior to culture. Vybrant MTT Cell Proliferation Assay (Invitrogen, CA) was utilized to quantitatively assess the polymer neural toxicity. The cells were cultured in the presence of different materials for the entire duration. The culture insert with different materials were only taken out before MTT assay, after the whole culture period. Each well containing 2 mL of culture media was exposed to 22.8 mm² of film. Culture media with no cells (n=3) treated with MTT reagent served as a blank baseline for the assay. The relative neuron viability was calculated as Eq. 2:

$$V_{sample} = (OD_{sample} - \overline{OD_{PBS}}) / (\overline{OD_{PSS}} - \overline{OD_{PBS}}) \quad (2)$$

The polymer coated macroelectrodes was further tested for astrocyte adhesion due to the extreme importance of this process in the glial scar formation and degradation of chronic recording signal for neural implants⁷⁰. For astrocyte culture, surfaces were not pre-treated with any adhesion molecules. Astrocytes were seeded on the PEDOT-IL (n=3) and PEDOT-PSS (n=3) coated surfaces at a density of 25,000 cells/ cm² and were grown for 7 d.

Immunocytochemistry

Cultures were fixed in 4% paraformaldehyde in PBS for 30 min, then washed several times in PBS. Samples were treated with a blocking buffer (5% goat serum/0.2% triton-X in PBS) for 30 min, then incubated with primary antibody for 2 h at room temperature. Antibodies used was astrocyte-specific glial fibrillary acidic protein (GFAP, rabbit polyclonal, 1:500, Dako, CA). Samples were gently washed in PBS and then incubated in secondary antibody (goat anti-rabbit AlexaFluor 568, 1:1000) for 45 min at room temperature.

Conclusions

In conclusion, electrochemical deposition of PEDOT-IL polymer on the surface of neural microelectrodes greatly improves the neural recording performance and stimulation by lowering the electrical impedance. The origin of impedance reduction was studied by equivalent circuit modeling and may be partially explained by the distinct surface texture that lowers the surface adsorption resistance. The nano structure of this polymer along with the low toxicity and anti-glial cell fouling capability demonstrates great potential for applications in the field of neural interface and neural tissue engineering.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Schwartz AB, Cui XT, Weber DJ, Moran DW. *Neuron*. 2006; 52:205–220. [PubMed: 17015237]
2. Wolpaw JR, Birbaumer N, McFarland DJ, Pfurtscheller G, Vaughan TM. *Clin Neurophysiol*. 2002; 113:767–791. [PubMed: 12048038]
3. Veraart C, Raftopoulos C, Mortimer JT, Delbeke J, Pins D, Michaux G, Vanlierde A, Parrini S, Wanet-Defalque MC. *Brain Res*. 1998; 813:181–186. [PubMed: 9824694]
4. Kolarcik CL, Catt K, Rost E, Albrecht IN, Bourbeau D, Du Z, Kozai TD, Luo X, Weber DJ, Tracy Cui X. *J Neural Eng*. 2015; 12:016008. [PubMed: 25485675]
5. Loddenkemper T, Pan A, Neme S, Baker KB, Rezai AR, Dinner DS, Montgomery EB Jr, Luders HO. *Journal of clinical neurophysiology* : official publication of the American Electroencephalographic Society. 2001; 18:514–532. [PubMed: 11779965]

6. Obeso JA, Olanow CW, Rodriguez-Oroz MC, Krack P, Kumar R, E Lang A. The New England Journal of Medicine. 2001; 345:956–963. [PubMed: 11575287]
7. Williams JC, Rennaker RL, Kipke DR. Brain research Brain research protocols. 1999; 4:303–313. [PubMed: 10592339]
8. Kipke DR, Vetter RJ, Williams JC, Hetke JF. IEEE Trans Neural Syst Rehabil Eng. 2003; 11:151–155. [PubMed: 12899260]
9. Kozai TD, Du Z, Gugel ZV, Smith MA, Chase SM, Bodily LM, Caparosa EM, Friedlander RM, Cui XT. J Neurosci Methods. 2014; 242C:15–40. [PubMed: 25542351]
10. Cogan SF. Annual Review of Biomedical Engineering. 2008; 10:275–309.
11. Suner S, Fellows MR, Vargas-Irwin C, Nakata GK, Donoghue JP. IEEE Trans Neural Syst Rehabil Eng. 2005; 13:524–541. [PubMed: 16425835]
12. Skousen JL, Merriam SM, Srivannavit O, Perlin G, Wise KD, Tresco PA. Prog Brain Res. 2011; 194:167–180. [PubMed: 21867802]
13. Ludwig KA, Langhals NB, Joseph MD, Richardson-Burns SM, Hendricks JL, Kipke DR. J Neural Eng. 2011; 8:014001. [PubMed: 21245527]
14. Kozai TD, Langhals NB, Patel PR, Deng X, Zhang H, Smith KL, Lahann J, Kotov NA, Kipke DR. Nat Mater. 2012; 11:1065–1073. [PubMed: 23142839]
15. Rebrik SP, Wright BD, Emondi AA, Miller KD. Neurocomputing. 1999; 26–27:1033–1038.
16. Cui X, Wiler J, Dzaman M, Altschuler RA, Martin DC. Biomaterials. 2003; 24:777–787. [PubMed: 12485796]
17. Krishnan S, Weinman CJ, Ober CK. Journal of Materials Chemistry. 2008; 18:3405–3413.
18. Merrill DR, Tresco PA. IEEE Trans Biomed Eng. 2005; 52:1960–1965. [PubMed: 16285400]
19. McCreery DB, Agnew WF, Yuen TGH, Bullara L. Biomedical Engineering, IEEE Transactions on. 1990; 37:996–1001.
20. Merrill DR, Bikson M, Jefferys JG. J Neurosci Methods. 2005; 141:171–198. [PubMed: 15661300]
21. Shannon RV. Biomedical Engineering, IEEE Transactions on. 1992; 39:424–426.
22. Cui X, Lee VA, Raphael Y, Wiler JA, Hetke JF, Anderson DJ, Martin DC. J Biomed Mater Res. 2001; 56:261–272. [PubMed: 11340598]
23. Cui XY, Hetke JF, Wiler JA, Anderson DJ, Martin DC. Sensors and Actuators a-Physical. 2001; 93:8–18.
24. Ludwig KA, Uram JD, Yang J, Martin DC, Kipke DR. J Neural Eng. 2006; 3:59–70. [PubMed: 16510943]
25. Cui XY, Martin DC. Sensors and Actuators B-Chemical. 2003; 89:92–102.
26. Luo X, Weaver CL, Zhou DD, Greenberg R, Cui XT. Biomaterials. 2011; 32:5551–5557. [PubMed: 21601278]
27. Cui XT, Zhou DD. IEEE Trans Neural Syst Rehabil Eng. 2007; 15:502–508. [PubMed: 18198707]
28. Zhou H, Cheng X, Rao L, Li T, Duan YY. Acta Biomater. 2013; 9:6439–6449. [PubMed: 23402765]
29. Jang J, Chang M, Yoon H. Adv Mater. 2005; 17:1616–1620.
30. Damlin P, Kvarnström C, Ivaska A. Journal of Electroanalytical Chemistry. 2004; 570:113–122.
31. Lee W, Jin MK, Yoo WC, Lee JK. Langmuir. 2004; 20:7665–7669. [PubMed: 15323517]
32. Mathiyarasu J, Senthilkumar S, Phani KL, Yegnaraman V. J Nanosci Nanotechnol. 2007; 7:2206–2210. [PubMed: 17655019]
33. Wilks SJ, Woolley AJ, Ouyang L, Martin DC, Otto KJ. Conf Proc IEEE Eng Med Biol Soc. 2011; 2011:5412–5415. [PubMed: 22255561]
34. Giernoth R. Angewandte Chemie International Edition. 2010; 49:2834–2839.
35. Di W, IVASKA A. Chemia analityczna. 2006; 51:839–852.
36. Musameh MM, Kachosangi RT, Xiao L, Russell A, Compton RG. Biosens Bioelectron. 2008; 24:87–92. [PubMed: 18457943]
37. Gopalan AI, Lee KP, Ragupathy D. Biosens Bioelectron. 2009; 24:2211–2217. [PubMed: 19167880]

38. Shan C, Yang H, Han D, Zhang Q, Ivaska A, Niu L. Biosens Bioelectron. 2010; 25:1504–1508. [PubMed: 20007014]
39. Lu W, Fadeev AG, Qi B, Smela E, Mattes BR, Ding J, Spinks GM, Mazurkiewicz J, Zhou D, Wallace GG, MacFarlane DR, Forsyth SA, Forsyth M. Science. 2002; 297:983–987. [PubMed: 12098704]
40. Armand M, Endres F, MacFarlane DR, Ohno H, Scrosati B. Nat Mater. 2009; 8:621–629. [PubMed: 19629083]
41. Kannan B, Williams DE, Laslau C, Travas-Sejdic J. Journal of Materials Chemistry. 2012; 22:18132–18135.
42. Kubisa P. Progress in Polymer Science. 2004; 29:3–12.
43. Luo X, Cui XT. Acta Biomater. 2011; 7:441–446. [PubMed: 20832505]
44. Ranke J, Stolte S, Stormann R, Arning J, Jastorff B. Chem Rev. 2007; 107:2183–2206. [PubMed: 17564479]
45. Wang X, Ohlin CA, Lu Q, Fei Z, Hu J, Dyson PJ. Green Chemistry. 2007; 9:1191–1197.
46. Kim, DH.; Richardson-Burns, S.; Povlich, L.; Abidian, MR.; Spanninga, S.; Hendricks, JL.; Martin, DC. Indwelling Neural Implants: Strategies for Contending with the In Vivo Environment. Reichert, WM., editor. Boca Raton (FL): 2008.
47. Leleux P, Badier JM, Rivnay J, Benar C, Herve T, Chauvel P, Malliaras GG. Advanced healthcare materials. 2014; 3:490–493. [PubMed: 24106008]
48. Leleux P, Johnson C, Strakosas X, Rivnay J, Herve T, Owens RM, Malliaras GG. Advanced healthcare materials. 2014; 3:1377–1380. [PubMed: 24591460]
49. Abidian MR, Kim DH, Martin DC. Adv Mater. 2006; 18:405–409. [PubMed: 21552389]
50. Arima Y, Iwata H. Biomaterials. 2007; 28:3074–3082. [PubMed: 17428532]
51. Bisquert J, Belmonte GG, Santiago FF, Ferriols NS, Yamashita M, Pereira EC. Electrochemistry Communications. 2000; 2:601–605.
52. Macdonald JR. Electrochimica Acta. 1990; 35:1483–1492.
53. Duan YY, Clark GM, Cowan RS. Biomaterials. 2004; 25:3813–3828. [PubMed: 15020157]
54. Barrese JC, Rao N, Paroo K, Triebwasser C, Vargas-Irwin C, Franquemont L, Donoghue JP. J Neural Eng. 2013; 10:066014. [PubMed: 24216311]
55. Prasad A, Xue QS, Sankar V, Nishida T, Shaw G, Streit WJ, Sanchez JC. J Neural Eng. 2012; 9:056015. [PubMed: 23010756]
56. Kozai TDY, Catt K, Li X, Gugel ZV, Olafsson VT, Vazquez AL, Cui XT. Biomaterials. in press.
57. Williams JC, Hippensteel JA, Dilgen J, Shain W, Kipke DR. J Neural Eng. 2007; 4:410–423. [PubMed: 18057508]
58. Eckhorn R, Wilms M, Schanze T, Eger M, Hesse L, Eysel UT, Kisvarday ZF, Zrenner E, Gekeler F, Schwahn H, Shinoda K, Sachs H, Walter P. Vision Res. 2006; 46:2675–2690. [PubMed: 16571357]
59. Wang W, Collinger JL, Degenhart AD, Tyler-Kabara EC, Schwartz AB, Moran DW, Weber DJ, Wodlinger B, Vinjamuri RK, Ashmore RC, Kelly JW, Boninger ML. PLoS One. 2013; 8:e55344. [PubMed: 23405137]
60. Bragin A, Jando G, Nadasy Z, Hetke J, Wise K, Buzsaki G. J Neurosci. 1995; 15:47–60. [PubMed: 7823151]
61. Xiao Y, Martin DC, Cui X, Shenai M. Appl Biochem Biotechnol. 2006; 128:117–130. [PubMed: 16484721]
62. Moxon KA, Kalkhoran NM, Markert M, Sambito MA, McKenzie JL, Webster JT. IEEE Trans Biomed Eng. 2004; 51:881–889. [PubMed: 15188854]
63. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; London, UK: 2007.
64. Krupa DJ, Brisben AJ, Nicolelis MA. J Neurosci Methods. 2001; 104:199–208. [PubMed: 11164246]
65. Wagenaar DA, Potter SM. J Neurosci Methods. 2002; 120:113–120. [PubMed: 12385761]

66. Shoham S, Fellows MR, Normann RA. J Neurosci Methods. 2003; 127:111–122. [PubMed: 12906941]
67. Kozai TD, Li X, Bodily LM, Caparosa EM, Zenonos GA, Carlisle DL, Friedlander RM, Cui XT. Biomaterials. 2014; 35:9620–9634. [PubMed: 25176060]
68. Mitra, P.; Bokil, H. Observed Brain Dynamics. Oxford University Press; 2008.
69. Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. Journal of Neuroscience Methods. 2010; 192:146–151. [PubMed: 20637804]
70. Turner JN, Shain W, Szarowski DH, Andersen M, Martins S, Isaacson M, Craighead H. Exp Neurol. 1999; 156:33–49. [PubMed: 10192775]

Fig. 1. SEM images of electrochemically deposited PEDOT-PSS and PEDOT-IL on gold microelectrodes. (A) Surface morphology of PEDOT-PSS. The polymer shows a cauliflower surface morphology. (B) PEDOT-IL surface shows nanostructures.

Fig. 2.

Electrochemical characterization of PEDOT-IL on gold microelectrodes. (A) Impedance reduction of gold microelectrode by PEDOT-PSS and PEDOT-IL coating. PEDOT-IL further reduces electrode impedance compared to PEDOT-PSS. The most significant reduction of PEDOT-IL compared to PEDOT-PSS occurs at 100 Hz – 1000 Hz. (B) Example equivalent circuit modelling of different microelectrode surfaces. All model parameters demonstrate very good fit. (C) Equivalent circuit diagram. Bottom panel shows the infinite ladder grid composition of the distributed element Z_D shown in the top panel circuit diagram. (D) Cyclic voltammetry profile of bare gold electrodes ($n=3$), PEDOT-PSS ($n=4$) and PEDOT-IL ($n=3$) coated electrodes. The redox peaks of PEDOT-PSS and PEDOT-IL occur at the same potential. PEDOT-PSS exhibits a higher CSC. The semi-transparent shaded area with corresponding color indicates the standard deviation of CV measurements for each electrode group.

Fig. 3.

Acute spike recording performance of PEDOT modified microelectrodes. (A) Spike data band pass filtered at 300 Hz – 3000 Hz. Red dotted line indicates triggers of whisker sensory stimulations. Left: Data stream on bare gold microelectrode. A substantial electrical artifact is observed. The peak of the electrical artifact is truncated due to the extremely high amplitude of the artifact. Few spikes are observed on this channel. Middle: Spike recording on a PEDOT-PSS electrode. The data stream includes a significant amount of threshold crossing spike waveforms for automatic classification algorithm. Right: Spike recording on a PEDOT-IL electrode. Similarly large amount of spike waveforms are observed compared to the PEDOT-PSS electrode. (B) Representative results of the unsupervised spike sorting algorithm on different electrodes. Left: Only one low amplitude cluster is observed on the bare gold electrode. According to the low SNR of the signal, the waveforms are likely attributed to random drift of background noise or multi-units. Middle: Example recording with three different units observed on a PEDOT-PSS electrode. Besides the low amplitude unit labelled in blue, two more clusters with high SNR are observed on the PEDOT-PSS channel. Right: Example units on a PEDOT-IL electrode. The signal amplitude of the three units resembles units on the PEDOT-PSS electrode.

Fig. 4.

LFP recording of PEDOT modified electrodes. (A) Power spectrum of three types of microelectrodes recording LFP in PBS and in vivo. Left: Lines indicate power spectrum of baseline LFP recordings in PBS, 60 Hz noise from the power line is omitted in the LFP power spectrum because the gold microelectrode saturated the amplifier signal at 60 Hz. Middle: The power increase of LFP on each type of electrode in PBS when whisker stimulator is turned on adjacent to the PBS solution, mimicking the in vivo whisker stimulation paradigm. The dramatic increase in LFP power on an uncoated gold electrode demonstrates the detrimental electronic interference for recording LFP due to high impedance. Both PEDOT-IL and PEDOT-PSS modified electrodes exhibit very little power increase when whisker stimulator is turned on. Right: Power spectrum increase triggered by sensory stimulation during an in vivo recording experiment. Baseline LFP power constituted of spontaneous LFP signal before whisker stimulation was subtracted from the evoked LFP power after whisker stimulation. (B) Example power spectrograms of sensory evoked LFP recording on gold (Left), PEDOT-PSS (Middle) and PEDOT-IL (Right). Every heat map was the average of 15 identical sensory stimulation trials, and each sensory stimulation consisted of 3 bursts of whisker stimuli. The black lines correspond to onset of sensory stimuli. Black arrows on top of each panel indicate whisker stimulation evoked artifact mixed with sensory response, while in **Left** panel the self-repeating artifacts dominates the signal while in **Middle** and **Right** panel the variable whisker response consisted the majority of LFP. The white circle in each panel emphasizes a spontaneous burst of LFP before whisker stimulation.

Fig. 5. Cell culture on PEDOT-IL polymer. Representative astrocyte growth on the (A) gold area and (B) PEDOT-IL area of a gold macroelectrode that was partially modified with PEDOT-IL. (C) Astrocyte growth on a PEDOT-PSS coated gold macroelectrode. Blue color is DAPI and red is GFAP.

Table 1

Comparison of electrode properties between gold, PEDOT-PSS and PEDOT-IL.

	Bare Gold	PEDOT-PSS	PEDOT-IL
1kHz Impedance ($k\Omega$)	1449 $\pm$ 49	51.8 $\pm$ 0.5	38.7 $\pm$ 1.0
R_1 ($k\Omega$)		39.7 $\pm$ 2.7	48 $\pm$ 8.5
Q_3 (nF)		135 $\pm$ 38	244 $\pm$ 107
R_3 ($k\Omega$)		130 $\pm$ 30	17 $\pm$ 1
β		0.65 $\pm$ 0.04	0.56 $\pm$ 0.06
R_s($k\Omega$)	20.3 $\pm$ 2.1	17.8 $\pm$ 1.0	11.9 $\pm$ 3.2
Q_{cpe} (nF)	0.1 $\pm$ 0.0	28.4 $\pm$ 5.1	14.8 $\pm$ 2.2
α	0.96 $\pm$ 0.03	0.98 $\pm$ 0.02	0.99 $\pm$ 0.00
CSC(mC/cm²)	0.4 $\pm$ 0.1	9.6 $\pm$ 0.2	8.7 $\pm$ 2.5
CIL(mC/cm²)	0.4 $\pm$ 0.0	1.5 $\pm$ 0.0	1.2 $\pm$ 0.0
Noise (μV)	65.0 $\pm$ 12.0	16.6 $\pm$ 1.0	16.2 $\pm$ 0.8
SNR	2.9 $\pm$ 0.1	4.4 $\pm$ 1.1	4.4 $\pm$ 0.5
unit per channel	0.3	1.5	2
60Hz LFP power increase (dB)	0.5 $\pm$ 0.0	-0.2 $\pm$ 0.0	-0.0 $\pm$ 0.1
MTT assay		1.0 $\pm$ 0.1	1.1 $\pm$ 0.2