Chronic intracortical neural recordings using microelectrode arrays coated with PEDOT-TFB

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Abstract

Microelectrode arrays have been extensively utilized to record extracellular neuronal activity for brain-machine interface applications. Modifying the microelectrodes with conductive polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT) has been reported to be advantageous because it increases the effective surface area of the microelectrodes, thereby decreasing impedance and enhancing charge transfer capacity. However, the long term stability and integrity of such coatings for chronic recordings remains unclear. Previously, our group has demonstrated that use of the smaller counter ion tetrafluoroborate (TFB) during electrodeposition increased the stability of the PEDOT coatings *in vitro* compared to the commonly used counter ion poly(styrenesulfonate) (PSS). In the current work, we examined the long-term *in vivo* performance of PEDOT-TFB coated microelectrodes. To do so, we selectively modified half of the microelectrodes on Neuronexus single shank probes with PEDOT-TFB while the other half of the microelectrodes were modified with gold as a control. The modified probes were then implanted into the primary motor cortex of rats. Single unit recordings were observed on both PEDOT-TFB and gold control microelectrodes for more than 12 weeks. Compared to the gold-coated microelectrodes, the PEDOT-TFB coated microelectrodes exhibited an overall significantly lower impedance and higher number of units per microelectrode specifically for first four weeks. The majority of PEDOT-TFB microelectrodes with activity had an impedance magnitude lower than 400 k Ω at 1 kHz. Our equivalent circuit modeling of the impedance data suggests stability in the polymer-related parameters for the duration of the study. In addition, when comparing PEDOT-TFB microelectrodes with and without long-term activity, we observed a distinction in certain circuit parameters for these microelectrodes derived from equivalent circuit modeling prior to implantation. This observation may prove useful in qualifying PEDOT-

TFB microelectrodes with a greater likelihood of registering long-term activity. Overall, our findings confirm that PEDOT-TFB is a chronically stable coating for microelectrodes to enable neural recording.

Keywords: neural recording, brain computer interface, microelectrode arrays, conductive polymers, PEDOT, bio-impedance modeling

1. Introduction

Implantable microelectrode arrays (MEAs) are miniature devices that record electrical activity of single or multiple neurons in the brain. Compared to other modalities such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG), implantable MEAs enable neural recordings that have higher temporal and spatial resolution, which make them suitable candidates for applications such as brain-machine interfaces [1-3]. Although MEAs are fabricated in different shapes and designs, small microelectrodes facilitate spatial selectivity and high microelectrode density on a probe. However, reduced microelectrode size is associated with an increase in impedance, which elevates thermal noise levels [4, 5] and contributes to poor electrical coupling between the microelectrode and tissue [6]. As a result, high impedance microelectrodes can result in low signal-to-noise ratio (SNR) and loss of neuronal signals at the interface. Therefore, it is desirable to increase the effective surface area of the microelectrodes while maintaining their geometrical surface area.

Conductive polymers (CPs) such as poly(3,4-ethylenedioxythiophene) (PEDOT) and polypyrrole (PPy) have frequently been utilized over the last decade as coatings for neural probes [7-10]. CPs

can be readily electrodeposited on microelectrodes to create a porous and large electrochemical surface area resulting in coatings with excellent intrinsic electrical conductivity [11-13]. Among the CPs, PEDOT in particular has gained interest because of its unique electrochemical characteristics including a low oxidation voltage for the monomer [14] and high electrical conductivity due to the conjugated double bonds in the polymer backbone [15]. However, the long-term stability of the PEDOT film, especially for chronic neural applications, is unclear. Ludwig et al. [16] reported that PEDOT-coated microelectrodes on silicon shank probes were able to register activity only up to 8 days, whereas, latter work with PEDOT doped with carbon nanotubes demonstrated more than 12 weeks recording of the evoked potentials in visual cortex [17]. Based on the prior studies, it is difficult to ascertain the longevity of neuronal recordings with PEDOT coated microelectrodes. While prior work does not directly point out any major stability issues with PEDOT-PSS, the recent work show longer lifetimes with alternative PEDOT formulations [17]. Therefore it is necessary to examine the long-term utility of any PEDOT-coated probes for neural applications.

Electrochemical polymerization of EDOT requires a supporting counter ion, which is incorporated into the polymer film and plays an important role in the stability, electrical characteristics, and macroscopic features of the deposited polymer [18, 19]. The most common counter ion for PEDOT electrochemical polymerization is poly(styrenesulfonate) (PSS) [4, 13, 20, 21]. However, recent reports suggest that replacing PSS with a smaller molecule could enhance the electrical properties of the PEDOT as well as its long term stability [19, 22]. Prior work has shown that neural probes coated with PEDOT polymers doped with perchlorate ions, which are smaller than PSS and monoanionic, showed unit activity up to six weeks post implant

[9]. Recently, our group demonstrated improvements in the *in vitro* long-term PEDOT stability through use of an alternative smaller counter ion, tetrafluoroborate (TFB) [23]. PEDOT-TFB microelectrodes exhibited smaller impedance and longer lifetime in accelerated aging studies when compared with PEDOT-PSS or PEDOT-PSS blended with carbon nanotubes [23, 24].

Here, we report for the first time the use of highly stable PEDOT-TFB coated microelectrodes for long-term neural recordings. We show that PEDOT-TFB microelectrodes on average register more units compared to control gold (Au) microelectrodes for at least first four weeks post implantation. In addition, we demonstrate that the impedance of PEDOT-TFB microelectrodes remains lower than Au microelectrodes over at least 12 weeks. Moreover, our equivalent circuit model of the *in vivo* EIS data suggests that the polymer-related components of the circuit model remained stable for the duration of the study. In addition, we observed that coated microelectrodes that appeared more likely to yield long term recordings were associated with increased double layer capacitance assessed by equivalent circuit modelling of the electrochemical impedance spectra prior to implantation.

2. Material and Methods

2.1. Electrochemical modification of neural probes

Single-shank silicon-based chronic probes were purchased from Neuronexus (Neuronexus Inc., Ann Arbor, MI). Each probe was 5 mm long with the thickness of 15 μm . It consisted of 16 iridium microelectrodes with surface area of 177 μm^2 and 100 μm inter-microelectrode distance. Electrochemical modification and characterization was carried out using a CHI 660D

1 electrochemical workstation (CH Instruments Inc., Austin, TX). Similar to the process described
2 in earlier reports [24], initial impedance profiles of the microelectrodes were measured by
3 running electrochemical impedance spectroscopy (EIS) in Phosphate Buffered Saline (PBS) over
4 the frequency range 0.1 Hz to 100 kHz using a 20 mV sine wave. A thin layer of Au was
5 electrochemically deposited on the microelectrodes using an aqueous solution of 5 mM
6 chloroauric acid (HAuCl_4) in 0.1 M NaClO_4 as the supporting electrolyte. Chloroauric acid was
7 employed to avoid use of the highly toxic HAu(CN)_2 reagent and possible incorporation of
8 cyanide in the Au layer. The deposition was performed by running fast voltage sweeps between -
9 1.5 and 0 V at a rate of 1 V/s for ~6 cycles. Subsequently, PEDOT-TFB was deposited on
10 alternating blocks of 4 consecutive microelectrodes in each probe (8 total), the order of which
11 was counterbalanced across probes to control for the position of PEDOT-TFB modified
12 microelectrodes within the cortical layers of the brain. The scheme for the probe modification
13 and an optical image of a modified probe are shown in Fig. 1A & 1B. The solution for the
14 conductive polymer deposition contained 10 mM EDOT, the PEDOT monomer, in 0.1 M
15 tetrabutylammonium tetrafluoroborate in acetonitrile. The deposition process was performed by
16 cyclic voltammetry between 0 to 1.2 V with a scan rate of 1 V/s for 50 cycles. The total
17 deposition charge was 390 mC/cm^2 and the polymer thickness was around $1.7 \mu\text{m}$. To maintain
18 consistency, the deposition parameters such as voltage window, number of cycles, and the
19 deposition solution were kept consistent for all the microelectrodes. The microelectrodes were
20 then cycled between 0 and 0.5V in PBS, which exchanges the loosely attached TFB anions.
21 Although there is little information available on the toxicological effects of TFB other than a
22 general study carried out by BG RCI [25], this step was seen as a precaution, to minimize
23 possible cytotoxic effects, since exchange of small anions into/out of conducting polymers has

been shown to be relatively facile compared to polymeric anions such as polystyrene sulfonate [26]. Successful deposition of Au and PEDOT-TFB was confirmed with EIS measurements after each of the above deposition steps. All the pre-implantation EIS measurements were performed using a 2-electrode setup in PBS at room temperature. A pilot study revealed that the polymer coated microelectrodes showed less variation in impedance if they were kept moist. As such, the probes were prepared a few days before the implantation and stored in deionized water until they were packaged for sterilization. The probes were then air dried and sterilized by exposure to ethylene oxide gas for 24 hours.

2.2. Surgical procedure

Five adult female Long Evans rats were implanted with the modified probes in the motor cortex using a similar procedure similar to that described previously [23]. Briefly, anesthesia was induced by applying 5% isoflurane oxygen mixture at a rate of 1 L/min. The animal was secured and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Puralube eye ointment (PharmaDerm, Florham Park, NJ) was applied. Six skull screws were placed in burr holes drilled with a micro drill. After locating bregma, the craniotomy window was prepared at -1.5 to +1.5 mm anterior/posterior and 0.5 to 2.5 mm medial/lateral. The probe was placed in a holder attached to the stereotaxic arm and positioned over the craniotomy, avoiding any surface vasculature. The ground and reference wires of the probe were secured to separate skull screws and coated with conductive silver paint (West Chester, PA). A hole was made in dura just below the tip of the probe and then the probe was lowered to the surface of the cortex. The probe was inserted using a micropositioner and advanced every 40 seconds at the following increments: 100 μm steps for the first 400 μm , then 50 μm steps to a depth of 1500 μm , and finally 25 μm steps to

the final depth of 1800-2000 μm . This was done to monitor neuronal spiking throughout insertion and locate a position where activity could be recorded on at least half of the microelectrodes. The craniotomy was filled with silicone elastomer (Kwik-Cast, World Precision Instruments, Sarasota, FL) and allowed to dry. To secure the probe to the skull and screws, a layer of Loctite prism 454 adhesive (Electron Microscopy Science, Hatfield, PA) was applied and dried instantly with Loctite accelerant 7452 (Newark Element 14, Chicago, IL). Dental cement (Lang Dental Manufactures, Wheeling, IL) was used to cover the screws and adhesive to create a robust head cap. To close the surgery site, skin was attached to the dental cement using GLUture (World Precision Instruments, Sarasota, FL), and a non-prescription tri-antibiotic cream (Target Corporation, Minneapolis, MN) was applied. 3 mL 0.9% saline was administered subcutaneously at the end of the surgery. Ketoprofen was administered subcutaneously at 5 mg/kg and continued twice daily for three days. Gentamicin was also injected subcutaneously at 8 mg/kg and continued once a day for one week. All animal procedures comply with the National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee at George Mason University, Fairfax, VA.

2.3. Data collection

At days 2 and 7 post implant, neural recordings and impedance measurements were performed for each animal. Data were collected weekly thereafter for the remainder of the study. The duration of the study was intended for 12 weeks, but data collection continued for two of the animals until weeks 19 and 23. Animals were freely moving during the neural recording sessions. However, to reduce the background signal, animals were anesthetized during the EIS data collections.

2.4. Neural recordings and data analysis

Neural signals were acquired using a Cerebus system (Blackrock Microsystems, Salt Lake City, UT) in 10-minute recording sessions. The data were sampled at 30 kHz and high-passed filtered at 250 Hz. To remove any movement artifacts, a common average reference was subtracted from each channel [27] using a custom routine in MATLAB (Mathworks, Natick, MA). For spike detection, a threshold was set at six standard deviations from the mean noise level for each individual channel. The captured spikes were then sorted for each channel into well resolved units using the Scanning K-means method in Offline Sorter V.3 (Plexon, Inc, Dallas, TX) and confirmed by visual inspection. Consistent with prior work [9, 28], the signal-to-noise (SNR) ratio for a unit was defined as:

$$SNR = \frac{\text{peak} - \text{to} - \text{peak amplitude of the mean waveform}}{2 \times \text{RMS noise of the recording site}}$$

A unit was considered valid if it had SNR greater than two [9, 16] and showed at least 30 spikes in the 10 minute recording period.

2.5. In vivo impedance measurements and circuit modeling

In vivo EIS measurements were made using a CHI 604E potentiostat by applying a 20 mV sine wave between 0.1 Hz to 100 kHz with frequencies logarithmically spaced in every decade. The measurements were performed in a two-electrode configuration using a stainless steel skull screw as the reference electrode. The sedated animals were placed in a small Faraday cage to provide electrical shielding and lower the ambient electrical noise.

The collected EIS data were fit to equivalent circuit models discussed in 3.6 using ZSimpWin (Princeton Applied Research, Oak Ridge, TN). A good fit was determined based on Chi-square (χ^2) value and percent error for every component of the model [10]. The acceptable χ^2 value for

the models was chosen to be 10^{-2} or below and the percent error values for every circuit component were less than 10%.

2.6. Data analysis

Data are reported as the mean $\pm$ standard error of the mean. Statistical analyses for the average units per microelectrode, 1 kHz impedance over time, and circuit parameters over time were performed using repeated measures ANOVA. In the ANOVA analysis, the within-subjects variables were time points and between-subject factors were the coatings (PEDOT-TFB and Au). Following the ANOVA analysis to explore the differences at each time point, Student's t-test was utilized. The number of PEDOT-TFB microelectrodes with two or more units and PEDOT-TFB units with SNR greater than 6 were statistically analyzed using the Z-test for proportions [24]. In all statistical tests, $p < 0.05$ was considered significant. All statistical analysis was performed in either MATLAB (Mathworks Inc.) or IBM SPSS Statistics (SPSS Inc.).

3. Results

3.1. Probe modification

As shown in Fig. 1C, the deposition of a thin Au layer on the Ir microelectrodes resulted in reduction in the impedance for frequencies between 0.1 – 1 kHz. The electroplated Au provides a rough surface which contributes to the lower impedance values compared to bare Ir [29]. In addition, the rough Au surface improves adhesion between the metallic microelectrode and the conductive polymer [23]. The deposition of PEDOT-TFB resulted in much greater reduction in impedance throughout the entire spectrum likely due to formation of the porous polymer film. The magnitude of the impedance at 1 kHz frequency, the most relevant frequency in multiunit

neural activity, was reduced from $287.8 \pm 17.6 \text{ k}\Omega$ to $158.4 \pm 18.1 \text{ k}\Omega$ ($n = 74$ microelectrodes for 5 probes) after the Au deposition and subsequently to $40.0 \pm 1.8 \text{ k}\Omega$ ($n = 36$ microelectrodes for 5 probes) after coating with PEDOT-TFB. The phase of the impedance became more positive around 1 kHz, suggesting that increased effective surface area after the PEDOT-TFB deposition induced high conductivity of the film [30]. As shown by our group [23], PEDOT-TFB coated microelectrodes showed lower overall impedance levels compared to those coated with PEDOT-PSS. Such a difference could be explained by the role of the smaller counter ion which results in a more porous surface and also is suggested to be a result of improved crystallinity of the polymer [22, 31]. Such a variation in crystallinity with counter ion has been reported by Breiby [32] where methyl-substituted PEDOT was polymerized the presence of PSS vs perchlorate. As shown in Fig. S1, the grazing incidence powder x-ray diffraction data for PEDOT-TFB compared to the other variants of PEDOT has a sharper peak suggesting more crystallinity in the deposited film. This finding is in agreement with prior results for PEDOT-TFB on larger microelectrodes [22]. Overall, in the 5 probes, 6 microelectrodes were determined to be defective because they showed high impedance values before any electrochemical deposition and their impedance did not change by either Au or PEDOT deposition. Therefore, these 6 microelectrodes were excluded from the dataset.

3.2. Neural recordings

Both Au and PEDOT-TFB microelectrodes registered well-resolved units over time (Fig. 2A), demonstrating that PEDOT-TFB microelectrodes can record neuronal activity in chronic studies. Overall, 78% of the PEDOT microelectrodes ($n = 36$ microelectrodes) and 52% of the Au microelectrodes ($n = 38$ microelectrodes) showed unit activity 2 days after implantation. After 12 weeks post implantation, the percentage of microelectrodes with unit activity reduced to 39%

and 33% for PEDOT-TFB and Au microelectrodes, respectively. We observed an overall higher number of units per microelectrode for PEDOT-TFB compared to Au (Fig. 2B, ANOVA, $p < 0.01$). A follow-up analysis was performed to compare the average number of units at every time point for PEDOT-TFB and Au microelectrodes. It was determined that PEDOT-TFB microelectrodes registered predominantly higher number of units for the first four weeks of recording. As shown in Fig. 3A, the number of PEDOT-TFB microelectrodes with two or more units was significantly greater for the first four weeks. For the PEDOT-TFB microelectrodes with unit activity, 36% had two or more active units during weeks 1-2 and weeks 3-4. Whereas for the Au microelectrodes with unit activity, these ratios were only 16% and 19%. The SNR values spanned a wide range between 3 – 25 for both PEDOT-TFB and Au. However, PEDOT-TFB microelectrodes had significantly more units with SNR greater than six for the first two weeks (Fig. 3B). The higher number of units per microelectrode and better SNR values for the first few weeks suggest the improved recording capability with PEDOT-TFB microelectrodes at least for a limited time. It has been suggested that brain-tissue response and the glial scar formation around the probe would impact the chronic recordings [33]. Although PEDOT-TFB microelectrodes had lower impedance compared to Au microelectrodes, the root mean square (RMS) noise was similar in both Au and PEDOT-TFB microelectrodes. This observation is consistent with other reports that the dominant noise in unit recordings is not necessarily due to the thermal noise of the microelectrodes [34].

3.3. Impedance profiles in vivo

As demonstrated in Fig. 4, over the first week of implantation, the magnitude of the impedance at 1 kHz increased in both Au and PEDOT-TFB microelectrodes. Such a trend is consistent with prior work reporting an increase in impedance values for intracortical microelectrode arrays after implantation [16, 35, 36]. However, compared to Au microelectrodes, PEDOT-TFB microelectrodes had consistently lower impedance values at 1 kHz over time ($p < 0.05$). Fig. 5 shows the Bode plots for the EIS measurements from both PEDOT-TFB and Au microelectrodes prior to implantation, as well as 2, 14, and 70 days post implantation. The impedance values at 1 kHz for PEDOT-TFB microelectrodes initially increased to $143.5 \pm 20.4 \text{ k}\Omega$ and then to $1.2 \pm 0.1 \text{ M}\Omega$ after 2 and 14 days post implantation, respectively ($n = 36$ microelectrodes). However, as shown in Fig. 5A, between days 14 to 70, the EIS did not show any marked differences throughout the spectrum suggesting the stability of the PEDOT-TFB film over time. For the Au microelectrodes, the 1 kHz impedance increased from $520.4 \pm 80.3 \text{ k}\Omega$ prior to implantation to $1.8 \pm 0.1 \text{ M}\Omega$ and $2.2 \pm 0.1 \text{ M}\Omega$ at days 2 and 14 post implantation, respectively ($n = 38$ microelectrodes). Although the 1 kHz impedance remained almost unchanged following implantation, it can be seen in Fig. 5B that impedance values at low frequencies, e.g. below 10 Hz, showed consistent reduction over time. Such a reduction in impedance at low frequencies might be due to delamination of the insulator around the edges of the metal microelectrodes [37].

Interestingly, the 1 kHz impedance for the PEDOT-TFB microelectrodes, which registered unit activity, was significantly lower compared to those without activity ($p < 0.05$). Fig. 6 shows a histogram of 1 kHz impedance for PEDOT-TFB microelectrodes based on activity. It can be seen from the figure that majority of the PEDOT-TFB microelectrodes with activity had 1 kHz impedance between 100 – 400 k Ω . However, we did not find a similar trend for the Au

microelectrodes (Fig. S2). In fact, the PEDOT microelectrodes with no activity exhibited an impedance very similar to that of bare Au, while PEDOT microelectrodes showing active units continued to exhibit low impedance throughout the study.

3.4. Equivalent circuit modeling

To interpret the EIS results and gain insight about the changes at the microelectrode-tissue interface, we fit the data according to previously well-established models for both the microelectrodes and the tissue [7, 19, 38]. The equivalent circuit models, which include separate compartments for the microelectrode and the tissue, for the microelectrode-tissue interface for both PEDOT and Au microelectrodes are shown in Fig. 7. The model for the tissue compartment included a tissue resistor (R_t), a tissue capacitor (C_t), and a resistor for the encapsulation layer around the device (R_{en}). C_t represents the membrane capacitance of the cells in the surrounding tissue and R_t represents the resistive extracellular pathways between the cells [39, 40]. For the PEDOT-TFB microelectrodes, the polymer film was characterized by a resistor (R_{poly}), describing the charge transfer resistance of the polymer, in series with a constant phase element (Q_{poly}) [41] representing the diffusion-related effects mainly due to the porous surface of the polymer [13, 19]. In parallel with R_{poly} and Q_{poly} , there is a double layer capacitance (C_{dl}) for the entire polymer surface [19, 42]. The model for the Au microelectrodes consists of a constant phase element (Q_{gold}), representing the rough surface of the Au, in parallel with a resistor (R_g), representing the impurities trapped in the deposited Au [29].

The results for both PEDOT-TFB and Au microelectrode-tissue interfaces for selected time points are summarized in tables 1 and 2, respectively. For sake of simplicity, in tables 1 and 2,

we only report the modeling results for a few time points to highlight the temporal changes in certain parameters. For both microelectrode types, the R_{en} and R_t increase from day 2 to 7. However, the values for R_{en} and R_t fluctuate over subsequent measurements. Such variations could be an indicator, at least in part, of dynamic changes around the probe over time. As shown previously [40, 43, 44], the inflammatory response to implanted intracortical microelectrodes is more likely to be multiphasic. The expression of activated microglia and astrocytes around the microelectrodes also has been found to fluctuate with time [40, 43]. In addition, it has been suggested the neuronal density in vicinity of the probe varies largely over 16 weeks [43]. It should be noted that the R_t values for PEDOT-TFB microelectrodes are much smaller than the values for Au microelectrodes, implying a possible differential response for the two interfaces. The C_t in the Au microelectrode-tissue drops ~ 3.5 -fold from day 2 to 7 which could be due, at least in part, to cell migration from the Au microelectrodes [45]. The polymer-related parameters exhibit large changes in the first week post implantation mainly due to the interaction between the tissue and the polymer film. Possible protein absorption or cell attachment to the surface of the PEDOT coated microelectrode has been shown to affect the microstructure of the polymer surface [35, 46]. However, as shown in Fig. 8B after the first week, both R_{poly} and Q_{poly} show stable trends suggesting the stability of the polymer and lack of degradation of the PEDOT or delamination from the underlying Au-coated substrate. As shown in Fig. 8A, the tissue resistance (R_t) for the PEDOT microelectrodes is highest between weeks 1-3, and then decreases gradually for 4 consecutive weeks. This observation is consistent with prior histology findings on acute tissue response after cortical implants [47, 48]. We did not observe a similar effect for the Au microelectrodes, perhaps due to the fact that the R_t was already so high for the Au-tissue interface that any subtle changes could not be detected.

We also used the circuit model to investigate the basis for differences in the impedance between the PEDOT-TFB microelectrodes with and without long-term activity reported in section 3.3 and shown in Fig. 6. It was determined that both C_{dl} and R_{poly} were significantly different between the two groups. As shown in Fig. 9, the C_{dl} was much higher for PEDOT-TFB microelectrodes that registered activity, suggesting a larger functional interface for these recording sites. In addition, the PEDOT-TFB microelectrodes with activity had lower R_{poly} , which suggests that the morphology of the polymer film had much larger changes for those PEDOT-TFB microelectrodes that did not register any neuronal activity. We then asked whether or not there were features in pre-implantation EIS of those microelectrodes that were associated with long term recording performance. Such features, if present, could aid in controlling the deposition process for PEDOT-TFB-coated microelectrodes or facilitate the qualification of coated microelectrodes for implantation. Fig. 10 shows the pre-implantation R_{poly} vs C_{dl} parameters for PEDOT-TFB microelectrodes with and without long-term activity *in vivo*. In this figure, we considered a PEDOT-TFB microelectrode with long-term activity if it registered units during the last 4 weeks of the study i.e. weeks 9 - 12. Likewise, microelectrodes without long-term activity were those that did not detect any units during the same period. As seen in the figure, there exists a clear distinction in R_{poly} vs C_{dl} for PEDOT-TFB microelectrodes with and without long-term activity.

4. Discussion

In this work, we demonstrated stable long-term unit recordings with PEDOT polymer doped with a small counter ion, TFB. The PEDOT-TFB coating showed marked lower impedance and higher number of units per microelectrode for the initial 4 weeks after implantation. While most

of the prior work with PEDOT-coated neural probes reported recordings for up to 6-7 weeks [9, 16, 21, 35, 49], our recordings lasted for at least 12 weeks. Such long-term recordings suggest that this new variant of PEDOT may be suitable for chronic neural applications. While prior work considered only 1 kHz impedance, we collected the impedance data over a wide frequency spectrum and developed an equivalent circuit model which helped us determine certain parameters to distinguish between PEDOT-TFB microelectrodes with and without long-term activity.

A major concern with any new material for invasive applications is the possible toxic effects that could impede its utility, especially for brain implants. Consistent with the lack of *in vitro* functional toxicity of the material reported by our group previously [23], our long lasting neural recordings with PEDOT-TFB microelectrodes suggest the suitability of this material *in vivo* for at least the duration of the study.

The choice of TFB originally was based on an earlier study by the Robinson group using larger surface area electrodes that compared PEDOT prepared in the presence of PSS, TFB and hexafluorophosphate as well as other conducting polymers [31]. This study showed that similar behavior is found in purely impedance-based comparisons in 0.1 M KCl for several polymer-anion combinations. In particular, for PEDOT the small anions produce very different behavior from either PSS or toluene sulfonate which is essentially the monomer of PSS [23, 31]. Other studies have suggested perchlorate (ClO_4) as an alternative counter ion to PSS [9, 50]. However, it was shown that the polymer produced in the presence of ClO_4 had brittle structure [51], a characteristic that did not seem likely to produce films that would withstand extended use.

1
2 We observed a large increase in the impedance after implantation throughout the whole
3 frequency spectrum in both Au and PEDOT-TFB microelectrodes. Similarly, an increase in
4 impedance was also reported previously [36], suggesting that this effect was due to the change in
5 the microelectrode-electrolyte interface and not degradation of the microelectrode material. We
6 also observed a steady increase in impedance for the first week, which reached a plateau
7 thereafter. As suggested by Ludwig and colleagues [9], the acute brain-tissue response to the
8 implant begins after implantation and lasts for 1-2 weeks. In this period, swelling around the
9 probe may occur such that microglia and reactive astrocytes begin to accumulate around the
10 probe. Our findings show the number of microelectrodes with two or more units was higher for
11 PEDOT-TFB microelectrodes (compared to Au), but only for the first 4 weeks. Similarly, in
12 comparison to the Au coated microelectrodes, the PEDOT-TFB microelectrodes had more units
13 with high SNR, but only for the first two weeks. These observations suggests that although low
14 impedance microelectrodes could potentially improve neural recordings acutely, long-term
15 applications may involve other factors such as the brain-tissue response that could mask the
16 benefits of microelectrode coating.

17
18 To examine the post implantation changes in the impedance for different variants of PEDOT, we
19 performed a comparison of 1 kHz impedance between prior work and ours. Table 3 shows the 1
20 kHz *in vivo* impedance of PEDOT microelectrodes taken from 5 studies (including ours). The
21 impedance values are normalized by the surface area of the microelectrodes utilized in each
22 study. The normalized impedance values either largely increase shortly after implantation (week
23 1) or later post implantation (weeks 6-7). This observation suggests that increase in the

1 impedance post implantation is less likely due to the counter ion. However, any comparison
2 beyond 6-7 week is still elusive because of the short duration in most of the prior work. It should
3 be noted that different factors other than the anion identity have a direct impact on the nature of
4 the films. These factors include fabrication/deposition procedure, storage, sterilization
5 conditions, implant procedures, etc.

6
7 Kozai and colleagues [17] utilized neural probes coated with PEDOT-PSS-CNT and observed an
8 increasing trend in the impedance of the microelectrodes over time. Yet, such an increase in the
9 impedance, compared to their PEDOT-PSS controls, did not affect neuronal recording measures
10 such as number of units, SNR, and spike amplitude. Another study [50] reported microelectrodes
11 with very low impedance ($3 \Omega/\mu\text{m}^2$) prior to implantation but large increase in the impedance
12 later post implantation. These findings indicate that 1 kHz impedance by itself could not be a
13 true indicator of long-term stability for PEDOT microelectrodes. Equivalent circuit modeling
14 which takes into account wide spectrum impedance data seems to be a more suitable approach to
15 examine the changes at the electrode-tissue interface.

16
17 Recently there has been an interest to utilize polymer-based approaches to develop neural probes
18 with minimal brain tissue response [52-54]. Examples include neural probes covered with
19 biodegradable polymers or polymer-based probes with mechanical properties similar to the brain
20 tissue [55, 56]. Conductive polymers such as PEDOT-TFB could be well integrated in such new
21 designs as suitable materials for the microelectrodes.

22

1 Interestingly, we observed that the impedance of the PEDOT-TFB microelectrodes at 1 kHz was
2 significantly smaller for those that showed neuronal activity compared to PEDOT-TFB coated
3 microelectrodes that did not. Our circuit modeling revealed that PEDOT-TFB microelectrodes
4 with minimal changes in their surface structure (R_{poly} and C_{dl}) had a higher chance of registering
5 units. In our circuit model, C_{dl} represents the effective surface area at the microelectrode-tissue
6 interface. The reduction in C_{dl} value points to the changes in the porous surface of the film.
7 Similarly, the increase in the R_{poly} for the PEDOT-TFB microelectrodes without activity could be
8 due to the absorption of non-conductive species, such as proteins, in the pores of the polymer
9 films [10, 24]. Examining the pre-implantation EIS, we found a distinction in R_{poly} vs C_{dl} for
10 those PEDOT-TFB microelectrodes that registered long-term activity *in vivo* compared to those
11 that did not. Such observation in pre-implantation EIS data may prove helpful in identifying
12 PEDOT-TFB microelectrodes with a greater likelihood of detecting long-term units. However, a
13 detailed analysis will be required to determine how well these parameters could predict whether
14 a microelectrode would register activity in the long term.

15
16 Our findings from the EIS measurements and the equivalent circuit modeling suggest an overall
17 stability for the PEDOT-TFB microelectrodes by maintaining lower impedance over time
18 compared to the control Au microelectrodes. Such long-term stability in the PEDOT-TFB
19 coatings could be attributed to the small TFB counter ions. The small counter ions result in high
20 order crystallinity in the PEDOT structure, which is mechanically more stable [57] .

21 22 **5. Conclusions**

Our findings suggest that PEDOT-TFB is a chronically stable coating for neural recording microelectrodes. Compared to Au microelectrodes, the coating provides a larger effective surface area and improved coupling between microelectrode and brain tissue. As such, PEDOT-TFB could facilitate chronic recordings with ultra-small and high-density neural arrays.

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Figure Captions:

Fig.1. The probe modification process and changes in impedance due to Au and PEDOT-TFB deposition. (A) The single shank 16-microelectrode probes were first electrodeposited with Au. The probe microelectrode sites were grouped in blocks of 4. In every other block, the microelectrodes were modified with PEDOT-TFB. (B) An optical image of the modified probe showing 10 microelectrodes. (C) EIS Magnitude (left) and phase (right) versus frequency for a single probe after modifications with Au and PEDOT-TFB. The solid lines are the mean and dotted lines are SEM ($n = 16$ microelectrodes for pre-deposition and Au, $n = 8$ for PEDOT-TFB).

Fig.2. Unit recordings from PEDOT-TFB and Au microelectrodes. (A) Representative well-resolved units registered by a PEDOT-TFB (top) and Au (bottom) microelectrodes over time. (B) The average number of units per microelectrode as a function of time and microelectrode coating. There was an overall significant effect of material for PEDOT-TFB (ANOVA, $p < 0.01$). A follow-up analysis with t-test determined that PEDOT-TFB microelectrodes registered predominantly higher number of units for four weeks (t-test, $p < 0.05$). * denotes the significance

Fig.3. Distribution of microelectrodes with multiple units (A) and high SNR recordings (B) for PEDOT-TFB and Au microelectrodes. (A) Compared to Au, the number of PEDOT-TFB microelectrodes with two or more units was significantly greater for the first four weeks ($p < 0.05$). (B) PEDOT-TFB microelectrodes showed more units with SNR greater than 6 only for the first two weeks.* denotes significance between the groups based on the proportion test ($p < 0.05$)

Fig.4. The impedance at 1 kHz for Au and PEDOT-TFB microelectrodes over time. The PEDOT-TFB microelectrodes maintained lower impedance for 12 weeks ($p < 0.05$). The error bars denote the SEM ($n = 36$ for PEDOT-TFB microelectrodes and $n = 38$ for Au microelectrodes).

Fig.5. Bode plots for the EIS measurements from both PEDOT-TFB (A) and Au (B) microelectrodes prior to implantation, as well as 2, 14, and 70 days post implantation. A large increase in the impedance magnitude and a shift in the phase occurred for both PEDOT-TFB and Au microelectrodes between pre-implant and day 2 post implant. Between days 14 – 70, the impedance remained stable throughout the whole frequency spectrum for PEDOT-TFB microelectrodes whereas the Au microelectrodes show reduction in the magnitude of the EIS for low frequencies. The solid lines are the mean and the dashed lines are the SEM.

Fig.6. The 1 kHz impedance for the PEDOT-TFB microelectrodes based on registering unit activity. Not only did the PEDOT-TFB microelectrodes with activity, compared to those without activity, have lower 1 kHz impedance, but also the majority of them showed 1 kHz impedance magnitude between 100 – 400 k Ω .

Fig.7. Equivalent circuit models to fit the EIS data for PEDOT-TFB and Au microelectrode-tissue interfaces.

Fig.8. The tissue resistance for the PEDOT-TFB microelectrodes (A) and the polymer-related components, R_{poly} and Q_{poly} (B) over time. R_{poly} and Q_{poly} in the circuit model reflect the characteristics of the microscopic structures in the polymer film. R_t for PEDOT-TFB microelectrodes reaches its maximum value between weeks 1 and 2. Afterward, the tissue resistance decreases to a level approximately 75% of the maximum.

Fig.9. Double layer capacitance (A) and polymer charge transfer resistance (B) showed the largest differences for PEDOT-TFB microelectrodes with and without unit recordings.

Fig.10. The pre-implant parameters R_{poly} vs C_{dl} for the PEDOT-TFB microelectrodes with and without long-term activity. A microelectrode with long-term activity was defined if it showed

unit activity during weeks 9-12, the last 4 weeks of the study. The separation between the two groups are shown with a dotted lined obtained by linear discriminant analysis.

Table captions:

Table 1. The fitted parameters for the equivalent circuit model for PEDOT-TFB microelectrode-tissue interface. For the pre-implant, the R_{en} is the resistance of the electrolyte.

Table 2. The fitted parameters for the equivalent circuit model for Au microelectrode-tissue interface. For the pre-implant, the R_{en} is the resistance of the electrolyte.

Table 3. Comparison of *in vivo* 1 kHz impedance of PEDOT microelectrodes between different studies. The impedance values are normalized by the surface area of the microelectrodes utilized in each study.

Figure1

A

Electrode
material:

Iridium

Gold

PEDOT-TFB
& Gold

B

C

Figure2

Figure3

Figure4

Figure 5

Figure6

Figure7

PEDOT-TFB

Au

Figure8

Figure9

Figure10

Table1

	Pre-implant	Day 2	Day 7	Day 28	Day 56	Day 77
Q_{poly} (nF.cm ⁻² .s ⁿ⁻¹)	142.5 ± 20.4	37.6 ± 7.7	29.6 ± 3.8	26.6 ± 4.3	24.5 ± 3.6	23.0 ± 3.9
n (0<n<1)	0.87 ± 0.02	0.97 ± 0.02	0.91 ± 0.01	0.90 ± 0.01	0.93 ± 0.01	0.93 ± 0.01
R _{poly} (MΩ)	0.02	0.08 ± 0.01	1.6 ± 0.6	1.7 ± 0.4	1.5 ± 0.6	1.2 ± 0.3
C _{dl} (nF)	0.07 ± 0.01	4.2 ± 1.0	2.3 ± 0.6	0.9 ± 0.3	0.8 ± 0.2	1.0 ± 0.3
R _{en} (kΩ)	7.2 ± 0.3	11.5 ± 0.4	15.8 ± 0.7	13.2 ± 0.6	12.3 ± 0.6	12.1 ± 0.7
R _t (kΩ)	N/A	80.2 ± 7.9	252.5 ± 41.5	190.1 ± 28.7	188.2 ± 20.5	167.5 ± 13.5
C _t (pF)	N/A	49.3 ± 1.4	58.1 ± 3.3	59.6 ± 4.2	62.7 ± 4.8	55.8 ± 3.7

Table2

	Pre-Implant	Day 2	Day 7	Day 28	Day 56	Day 77
Q_{gold} (nF.cm-2.sn-1)	180.1 ± 54.9	1.1 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	2.5 ± 0.4	3.60 ± 0.7
n (0<n<1)	0.61 ± 0.04	0.79 ± 0.01	0.8 ± 0.01	0.78 ± 0.02	0.74 ± 0.01	0.73 ± 0.01
R_{gold} (MΩ)	204 ± 67	412 ± 76	480 ± 82	411 ± 77	430 ± 82	525 ± 86
R_{en} (kΩ)	10.4 ± 0.6	14.4 ± 1.3	16.4 ± 0.8	14.2 ± 0.9	17.0 ± 1.8	13.3 ± 2.1
R_t (MΩ)	N/A	1.6 ± 4.1	1.9 ± 3.6	2.7 ± 4.2	3.1 ± 0.7	1.9 ± 4.8
C_t (pF)	N/A	300.7 ± 56.1	82.9 ± 9.2	96.6 ± 17.1	103.8 ± 23.3	73.7 ± 36.2

Table3

	Pre-implantation ($\Omega/\mu\text{m}^2$)	Week 1 ($\Omega/\mu\text{m}^2$)	Week 2 ($\Omega/\mu\text{m}^2$)	Weeks 6-7 ($\Omega/\mu\text{m}^2$)
PEDOT-ClO ₄ [9]	85 ± 5	185 ± 85	1564 ± 1137	1152 ± 426
PEDOT-PSS [17]	388 ± 4	5600 ± 800	6100 ± 600	4000 ± 800
PEDOT-PSS–CNT [17]	361 ± 32	3600 ± 400	4200 ± 480	7200 ± 800
PEDOT-ClO ₄ nanotubes [50]	3 ± 1	800 ± 320	1100 ± 88	1040 ± 120
PEDOT–TFB	254 ± 10	807 ± 115	6892 ± 847	4520 ± 565

Neural probe modification

Chronic *in vivo* single unit neural recordings