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Conductive Polymer "Molecular Wires" Increase Electrical Conductance Across Artificial Cell Membranes

Alik S. Widge*, Malika Jeffries-El‡, and Yoky Matsuoka*
*The Robotics Institute and ‡Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA

***Abstract*— Highly intimate contact between an electrode and a living neuron is strongly desired by both basic neuroscientists and engineers seeking to develop more effective neural prostheses. The net resistance between electrode and cell must be decreased in order to improve the quality of recordings and deliver the minimum necessary stimulating current specifically to the target cell. The ideal situation would be to establish chronic intracellular contact, bypassing the resistance of the cell membrane and the surrounding tissue. We present here evidence that regioregular polythiophene conductive polymers increase the electrical conductance of an artifical lipid bilayer that simulates a cell membrane. Our initial data on its behavior suggest that the polymer is freely diffusing within the lipid phase. This implies that these polymers, if tethered to a larger microelectrode, could permit long-term sustainable intracellular stimulation and recording. We therefore believe that this new molecule, when further developed, has the potential to significantly improve the performance of existing chronic electrode systems and possibly to enable new types of biosensors.**

***Index Terms*— Biomembranes, biomedical electrodes, biomedical transducers, brain-machine interface, molecular electronics, nanotechnology, neural prosthesis**

I. Introduction

The central nervous system does not effectively regenerate after injury, and the peripheral nervous system generally does not regenerate back to complete functionality. There has therefore been ongoing interest in electronic devices to replace and repair damaged parts of the nervous system. A wide variety of electrodes have been developed for this application, and encouraging results have been demonstrated in auditory prostheses [1] and cortical control of external actuators [2]–[4]. However, all existing electrode systems are plagued by difficulties in establishing a lasting and low-impedance contact between neuron and electrode. In many cases, a physical separation occurs due to formation of a glial scar or migration of neurons away from the electrode [5], [6]. Even when close physical contact is maintained, the larger currents used by existing stimulating electrodes often cause a decrease in neuronal excitability over time [7], [8]. Combined, these factors lead to a slow decline in performance of chronically implanted neural electrodes, with up to one-third of electrode sites becoming non-functional over a period of several weeks [9].

Conductive polymer coatings have been proposed as a way to improve the intimacy of the cell-electrode connection. A nanostructured "fuzzy" polymer coat increases electrode surface area, lowering the electrical impedance [10]. Polymer coatings can also contain biomolecules to attract neurons and promote their adherence to the electrode [11]. Because they are softer and more compliant than metal or silicon, it has been suggested that these coatings will reduce inflammation caused by stiffness mismatch between tissue and electrode.

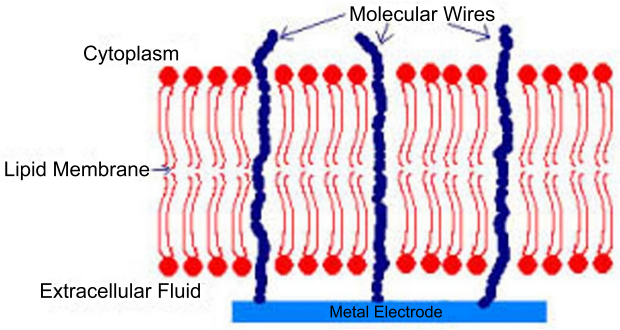

Fig. 1. Conceptual sketch of conductive polymer "molecular wires" penetrating a lipid bilayer. Because the wires are small, lipophilic molecules, the bilayer seals around them and does not permit the cytoplasmic leak seen with patch clamps. Intercalation of the wires into the membrane permits chronic intracellular stimulation and recording from living cells.

We have previously developed methods for self-assembling monolayers of the conductive polymer poly-(3-(2-ethylhexyl)-thiophene) (EHPT) and rendering those monolayers biocompatible through incorporation of neural cell adhesion molecules [12]. This polymer is optimized for high solubility in a variety of organic phases, including phospholipid bilayers, vesicles, and micelles. We propose that such a polymer, when tethered in a monolayer at an electrode surface, may be capable of providing a large number of parallel electrical connections through a cell membrane. A sketch of this device concept is shown in Figure 1. Essentially, individual polymer chains provide current pathways through the membrane much as an ion channel would. The key difference between this proposed technique and existing intracellular electrophysiology methods such as patch clamping is long-term sustainability. Because the individual nanowires are small, the membrane should be able to easily seal around them just as it does around integral membrane proteins. This would prevent the cytoplasmic leakage seen when the membrane is perforated by a micropipette, and thus prevent the cell death that invariably accompanies patch clamp techniques.

Despite EHPT's lipophilicity, it is unknown whether this polymer would actually be capable of inserting into a lipid membrane in the manner we propose. We therefore chose to evaluate the polymer in a simple and well-defined testbed that could simulate the membrane of a living cell. Methods to create artificial lipid bilayers separating two aqueous compartments have been known for decades, providing a convenient initial method of assessing the feasibility of our approach [13]. In this paper, we present initial results from testing EHPT's ability to increase current flow across such an artificial bilayer.

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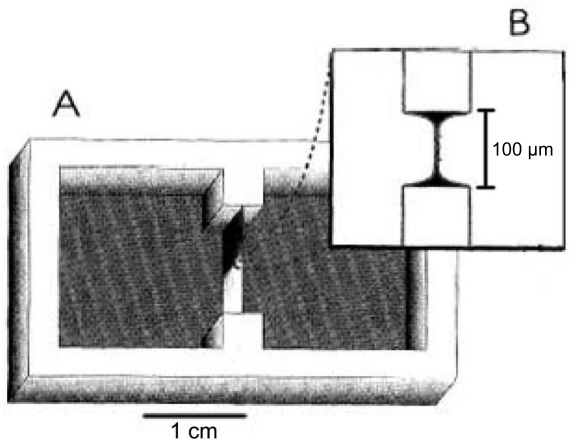

Fig. 2. Schematic diagram of the experimental chamber. (**A**), PTFE chamber partitioned by a thin septum. (**B**), close-up of septum showing microscale hole containing solvent-free artificial lipid bilayer. Holes used in these experiments ranged from 80 to 100 μm. Figure adapted from [14].

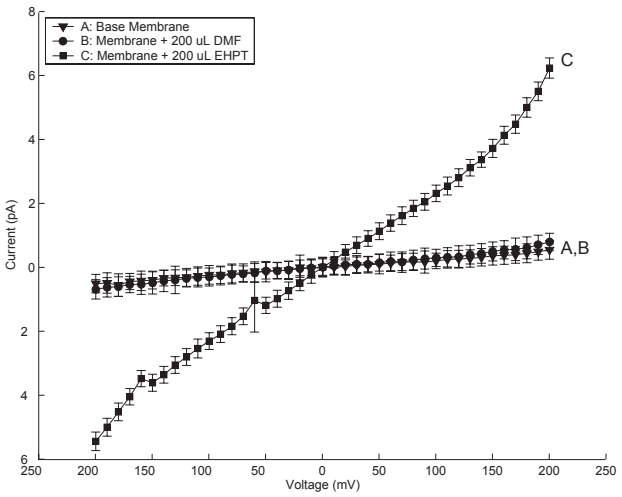

Fig. 3. Conductance changes in a DiPhyPC BLM after injection of pure solvent and polymer solution. (**A**), I-V characteristic of the base BLM. (**B**), BLM after injection of 200 μL of the solvent DMF. Essentially zero current is passed except at the highest available voltages in both **A** and **B**. (**C**), BLM after further injection of 200 μL of a saturated solution of EHPT in DMF. Conductance has increased by at least one order of magnitude, with supralinear increases at the extreme voltages.

II. Methodology

A. Creation of Artificial Lipid Bilayers

Artificial bilayer lipid membranes (BLMs) were constructed in a polytetrafluoroethylene (PTFE) experimental chamber as described in [14] and shown in Figure 2. Partitions made of PTFE film 0.025 mm thick with hole diameters that varied from 80 μm to 100 μm were used to divide the chamber into two halves. During an experiment, one half chamber (chosen arbitrarily) faces the experimenter and is termed the "front". The lipid for forming the membrane, lyophilized 1,2-diphytanoyl-sn-Glycero-3-Phosphocholine (DiPhyPC, Avanti Polar Lipids, Alabaster, AL) was dissolved in pentane at 10 mg lipid per 1 mL pentane. Lipid bilayers were formed across the hole using a technique modified from [13]. Initially, saline solution (1M NaCl in these experiments) partially fills each half of the chamber (volume approximately 6 mL). Approximately 20 μL of lipid in pentane is applied to the surface of the aqueous phase with the liquid just below the hole in the partition. To facilitate membrane formation, the rim of the hole is conditioned with hexadecane using a solution of hexadecane in pentane (1:100 v/v). After allowing the pentane to evaporate, the fluid levels are slowly raised in sequence to a point just above the hole to form the lipid bilayer. The additional saline is introduced into the chamber via PTFE tubes connected between the chamber and syringes filled with saline, and the process is monitored visually by observing the fluid levels through a microscope. During the raising of the fluid levels, an AC voltage is applied across the partition. Membrane formation is detected by a sudden step increase in the capacitively coupled current.

A pair of Ag/AgCl electrodes connected to an Axon Instruments headstage and amplifier were used to apply computer-controlled voltages to the membrane and to measure currents through the membrane. Applied voltages ranged from 200 mV to -200 mV in steps of 10 mV. Currents were recorded after the application of each voltage and averaged over a 200 ms window. Recorded signals were lowpass filtered with a 1 kHz cutoff before being digitized for recording. The PTFE chamber and electrodes are located inside a mu-metal box on a vibration isolation table to eliminate pickup of environmental signals.

B. Introduction of EHPT to Artificial Bilayers

Regioregular head-to-tail EHPT of average molecular weight 3000 g/mol (synthesized as described in [15]) was dissolved in N,N-dimethylformamide (DMF) to saturation. Bilayers were formed as described above, and the current-voltage (I-V) characteristic of the bilayer was recorded. The EHPT solution and/or pure DMF were then injected in aliquots of 100 or 200 μL into the aqueous subphase of one half chamber under continual stirring. The system was allowed to settle and equilibrate for under five minutes, after which time another I-V characteristic was recorded. The capacitively coupled current was re-checked after each injection to ensure that the bilayer itself was not compromised. In the event that a bilayer was weakened or disrupted, we were often able to re-establish it immediately by lowering and raising the saline level in one or both chambers. Reformation did not appear to affect the conductance behavior of the BLMs described in this paper. In order to verify that observed effects were due to EHPT's lipophilic properties, we repeated the same experiment with poly-(3-hexylthiophene) (HPT). HPT was synthesized analogously to EHPT and was also roughly 3000 g/mol.

III. Results

A. The Conductive Polymer EHPT Increases the Conductance of an Artificial Membrane

Figure 3 shows the results of adding DMF with and without EHPT to an artificial lipid bilayer. Introduction of pure DMF into the aqueous phase that contacts one side of the BLM has no effect on the average conductance, as shown by curve B

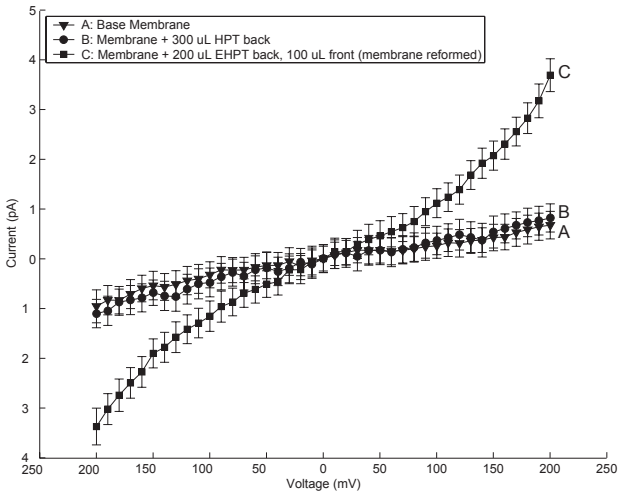

Fig. 4. Lack of conductance change when lipophilic EHPT is replaced by non-lipophilic HPT. (**A**), I-V characteristic of the base BLM. (**B**), BLM after injection of 300 μL of a saturated solution of HPT in DMF. No significant conductance change is seen. (**C**), BLM after further injection of 300 μL of saturated EHPT in DMF (200 μL back, 100 μL front) and reformation of both membrane leaflets. Conductance is now comparable to that seen in Figure 3.

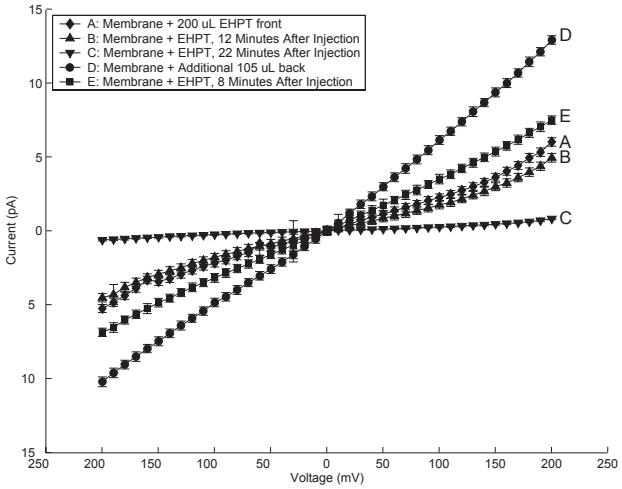

Fig. 5. Decay of increased BLM conductance over several minutes. These curves represent the same DiPhyPC BLM described in Figure 3. (**A**), I-V characteristic of membrane injection of 200 μL of EHPT in DMF to the front half-chamber. This repeats Curve C of Figure 3. (**B**), BLM after 12 minutes. No significant changes in conduction are seen. (**C**), BLM after 22 minutes since injection. The current has now returned to the value for an unmodified membrane. (**D**), BLM after adding 105 μL of EHPT to the back half-chamber. This further addition of polymer produces a larger current than that seen with the original 200 uL. (**E**), BLM after eight minutes of further equilibration. Conductance has now decayed significantly from its original value.

in Figure 3 (which essentially overlays A). Injection of an equivalent amount of the polymer solution in the same half-chamber, however, produces an elevenfold increase in conductance (ignoring the values around 0 V, which are larger). This increase is seen even though the majority of the injected polymer did not enter or associate with the bilayer. The small volumes of DMF that we used are readily miscible with the 6 mL of saline that fills each well of the chamber, and thus the DMF and polymer appear to mostly intermix with and remain in the aqueous phase. It should be noted that although no increase in average current response to a voltage pulse is seen in the presence of DMF alone, we did observe transient spike-like increases in current when constant voltages were applied over tens of seconds after application of DMF. These may represent transient membrane disruptions.

The increase in current flow appears to be dependent on the concentration of polymer present. Injection of only 100 μL did not produce a significant effect (not shown), whereas 200 μL did. We were unable to directly assess the effects of injecting a full 300 μL of EHPT into a single half chamber, as this volume of EHPT in DMF solution showed a marked tendency to destabilize and permanently disrupt the membrane. (Interestingly, the same tendency is not seen with 300 μL of HPT solution.) However, we were able to inject 200 μL into one half chamber and 105 μL into the other, which increased the current to nearly 13 pA (26 times greater than the approximately 0.5 pA across the unmodified membrane).

B. A Non-Lipophilic Conductive Polymer Does Not Increase Membrane Conductance

In order to verify that the conductance changes were directly related to EHPT's lipid solubility, we compared it to the non-lipophilic polythiophene HPT. As seen in Figure 4, injection of 300 μL of HPT did not produce significant changes in conductance, whereas 300 μL of EHPT (split between front and back chamber to mitigate membrane disruption) did. The HPT in DMF was visually verified to mix with the aqueous phase just as the EHPT solution did. However, we did find that HPT was slightly less soluble in DMF than EHPT, as judged by a lighter color of the saturated solution.

C. The Conductance Changes Caused By Free EHPT Diminish Over Time

Figure 5 illustrates a decrease in the effect of EHPT with time. As shown in Curves **A** through **C**, one BLM showed a decay back to its base characteristics within 22 minutes from the initial exposure. This decay did not occur at a constant rate, as an I-V recording at 12 minutes showed no significant change. Moreover, further application of another 105 μL to the opposite half chamber raised the conductance above its prior peak, as seen in **D**. When the membrane was again allowed to rest, the current again declined to roughly half its maximum, this time within only eight minutes. Stirring of the solution did not further increase the conductance at any point during these recordings (data not shown). These findings represent only one membrane and have not yet been replicated.

IV. Discussion

These initial data establish that the conductive polymer EHPT is able to significantly increase the current across a lipid bilayer. As noted in the Introduction, we have previously achieved a tethering of this compound to metal electrodes in a biocompatible self-assembled monolayer. Given that other investigators have measured the separation between a neuron and a protein-containing monolayer as 50 to 100 Angstroms,

we believe that tethered polymer chains should be able to reach and enter a biological membrane [16]. However, we have not yet verified that the polymer is spanning the bilayer to produce this increase in conductance. Bridging of the membrane remains the simplest explanation: the DMF is not causing a steady-state leak current (although it does appear to cause temporary membrane disruptions that may be aiding polymer entry), and the lack of effect with HPT implies that at least part of the polymer must dissolve in the lipid phase in order to produce the observed conductance. One alternate explanation for the data is that the EHPT molecules are assembling into pore-like structures or otherwise disrupting the membrane to allow passage of ions. This seems unlikely given that there is no hydrophilic part of the molecule to form a stable ion-conducting channel, but cannot be ruled out. We also assume that the presence of EHPT in the aqueous phase has no effect on conduction of current to the membrane, given that the polymer is less conductive than the saline.

The observed decay in conductance over time is potentially concerning. While we have not confirmed the mechanism for this decay, we believe that it may be due to a diffusive process occuring within the BLM. Artificial membranes such as these consist of an area of bilayer surrounded by a thicker annulus of bulk phospholipid. If the EHPT chains are fully entering the lipid phase of the bilayer (which seems likely), they may be migrating to the annulus, where they would no longer be able to affect the conductivity. If this is true, then this effect would not be a concern in an actual device; a tethered polymer cannot migrate, and a cell membrane has no annulus.

It may be noted that in this particular study, the largest currents we were able to pass through the membrane were 13 pA at 200 mV. Injected currents should be at least ten times larger in order to effectively depolarize a neuron. However, this is also unlikely to present a barrier to the device concept diagrammed in Figure 1. In other work currently in progress, we have been able to electrochemically oxidize monolayers of EHPT to produce a drop in electrical impedance. The EHPT used in this set of experiments was in its native "undoped" state and therefore does not represent the true performance achievable.

Based on these results, we believe that EHPT and associated polythiophenes show promise as an electrode coating for neuroprosthetic devices. The self-assembly process can form a monolayer on any exposed noble metal surface, and thus should be immediately integrable with most of the electrode systems currently in use. Moreover, if it can be verified that these polymers do in fact integrate into lipid membranes, it may be possible to adapt them into new forms of biosensors. Polythiophenes may be functionalized with a wide diversity of side chains and end groups, potentially including probes for environmental molecules of interest. We are currently experimenting with alternate methods of delivering polythiophenes to the bilayer and methods for more closely examining their behavior at and within the membrane in order to further explore these possibilities.

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