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Precise Neurotransmitter-Mediated Communication with Neurons \textit{In Vitro} and \textit{In Vivo} Using Organic Electronics*

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Abstract

Attempts to interface human-made systems with neural systems are commonly based on direct electrical stimulation or exogenous drug delivery. Few techniques have attempted to mimic neurons’ own combination of electronic and chemical signaling with endogenous substances. We demonstrate below the organic electronic ion pump (OEIP), a technology which aims to accomplish just that: electronically controlled delivery of ions, neurotransmitters and other bio-substances. Based on electrophoretic migration through an organic electronic system, delivery is diffusive and non-convective, that is, without fluid flow. Various experiments involving OEIP technology are reviewed, culminating in its use, in an encapsulated form, to modulate sensory function in a living animal. As a first step towards an “artificial neuron”, this technology has significant potential for both neural system interfacing and in the treatment of various neurological disorders.

Key words: organic electronics, drug delivery, neurotransmitters, electrophoresis, \textit{in vivo}

Introduction

With recent progress in the understanding of nerve cell signaling, demand is increasing for precise human-made systems capable of interfacing with neurons. Fulfilling this demand, and thus enabling modern electronic technology to interface with biological systems, has been the focus of a significant research effort throughout the last century. Indeed, a host of technologies have been developed to address such precise “machine-to-brain” interfacing: cochlear implants, multi-electrode arrays for neural analysis, deep brain stimulation (DBS), as well as various micro- and nano-fluidic devices\textsuperscript{1,2}, to name a few. While established therapeutically, these and other technologies suffer from various drawbacks, for example, an inability to distinguish specific cell types. Until now, no technology has demonstrated behavior analogous to a nerve cell: electrically triggered release of neurotransmitters.
A major challenge in achieving such a device is bridging the gap between the signal carriers of the nervous system (ions, neurotransmitters) and those of conventional electronics (electrons). Organic conjugated polymers represent a promising solution to this challenge. Unlike the plastics encountered in everyday use, these materials have properties more akin to conventional semiconductors. However, unlike more conventional materials, their electrical properties – and indeed many of their material properties – can be influenced by the proximity of charged molecules. Conjugated polymers can thus be employed in transducing electronic signals directly into ionic/molecular signals, and vice versa. Indeed, this electrochemical facet of organic electronics has begun to merge with bio-medical research, yielding a new generation of biosensors, bio-electrodes, and drug delivery platforms.

We recently reported such a next-generation organic bioelectronic device, known as the organic electronic ion pump (OEIP) (Fig. 1), which utilizes both the electronic and ionic features of conjugated polymers. Upon application of a voltage across the device, electronic signals are converted into exact delivery of ions and bio-substances at very high spatiotemporal resolution, and in the absence of fluid flow – i.e., only ions or molecules are delivered, not solution. We have successfully applied OEIP technology in various in vitro and in vivo settings to create precision microenvironments and to precisely regulate cellular responses.

While great strides in drug delivery-based machine-to-brain technology have been achieved, there is still room for much improvement. Devices typically suffer from limitations such as low release rates and poor on/off ratios, as well as inadequate electronic control.
control of the delivered dose. Systems involving micro- and nanofluidics have had success in circumventing some of these problems and have even been used to establish precise concentration gradients – analogous to many biological systems. However, these techniques involve flowing liquids, which invariably disturb the complex chemical environment of the target biosystem. Organic electronic ion pump technology addresses these limitations, and can deliver a broad range of bio-substances – including neurotransmitters – with precise electronic control and without convective disturbances.

The Organic Electronic Ion Pump (OEIP)

The OEIP consists of a single film of the conducting polymer poly(3,4-ethylenedioxythiophene) doped with the polyanion poly(styrenesulfonate) (PEDOT:PSS) (Fig. 2). This film is photolithographically patterned into electrodes joined by an “ion channel” (Fig. 1). The channel – essentially an electrochemical salt-bridge – is formed from the same starting film of PEDOT:PSS. To deactivate electronic conduction in this region, the film is chemically over-oxidized. This process results in the irreversible disruption of the electronically conducting PEDOT phase, while leaving the ionically conducting PSS phase unaffected and maintaining a continuous film from one electrode to the other. A hydrophobic encapsulation layer, such as SU-8, covers the channel region, and provides openings over the electrodes for application of source and target electrolytes.

Upon application of a voltage between the electrodes, an electrochemical circuit is established, resulting in the oxidation of PEDOT at the anode (Eq. (1)) and reduction of PEDOT at the cathode (Eq. (2)) according to the half reactions:

\[
\text{PEDOT}^0 + M^+ + \text{PSS}^- \rightarrow \text{PEDOT}^+:\text{PSS}^- + M^+ + e^- (1)
\]

\[
\text{PEDOT}^+:\text{PSS}^- + M^+ + e^- \rightarrow \text{PEDOT}^0 + M^+:\text{PSS}^- (2)
\]

where M is the source cationic species. Since the channel region is electronically insulating and the polyanionic PSS essentially forms a cation exchange membrane, the only mechanism whereby electronic current can be sustained is for cations from the anode/source side of the device be transported to the cathode/target side, thus the presence of the same M in Eqs. (1) and (2). Owing to the thin film nature of the PEDOT:PSS electrodes (250 nm thick) and the difference in ion concentrations between the electrodes and their accompanying electrolytes, ions can be rapidly exchanged between the electrodes and their electrolytes. The source electrolyte thus provides a reservoir for the positively charged species intended for delivery, and species emerging from the target side of the ion channel are rapidly delivered into the target electrolyte.

The electrochemical relationships of Eqs. (1) and (2) ensure that the delivery rate of positively charged species into the target system is directly proportional to the current measured in the electronic branch of the circuit. As seen in the equations, for each M that is transported from the anodic to the cathodic side of the circuit, exactly one electron must have been transported through the control electronics. The electrophoretic nature of the transport through the channel further ensures that it is only charged species (M\(^+\)), and not...
solution, that are transported through the channel. Delivery is thus non-convective and the OEIP therefore represents an ideal platform for precise, non-disruptive studies of biological systems.

Results and Discussion

Delivery Efficiency
To verify basic functionality of the OEIP, monovalent metal ion delivery was characterized. Using a source electrolyte of 0.1 M KCl(aq) and a target electrolyte of 0.1 M NaCl(aq), biasing the device (between 2–10 V) causes K+ ions to be transported through the channel and delivered into the target electrolyte. Meanwhile, Cl– ions in the target electrolyte are prevented from flowing in the opposite direction by the cation selectivity of the PSS– in the channel. By comparing [K+] in the target electrolyte -- by means of atomic absorption spectroscopy -- to the integrated current measured in the driving circuitry, a precise linear relationship can be established between the delivered concentration and the driving current (Fig. 3). Indeed, the correspondence of K+ to e– is one-to-one, i.e., for each electron measured in the electronic portion of the circuit, exactly one K+ ion is transported through the channel (the ionic portion of the circuit). In addition to K+, such a precise electron-to-molecule ratio can be defined for all species successfully pumped to date, including mono- and divalent ions as well as various neurotransmitters8,12-14.

Using the OEIP to Induce pH Gradients and Oscillations
In cell signaling, ion fluxes are essential in conveying information, and specificity for diverse cellular mechanisms is achieved by spatial and temporal regulation of these fluxes. Creation of both stable gradients

![Figure 3](image-url)  
Figure 3. Ion-to-electron equivalence as demonstrated by the linear relationship between target ion concentration (that is, number of intended ions transported through the channel) to number of electrons transported through the control circuitry.

![Figure 4](image-url)  
Figure 4. pH gradients and oscillations. a, pH gradient established in target electrolyte as a function of lateral distance from ion delivery point and time. b, pH oscillations elicited by pulsing the bias voltage.
and oscillations with a wide dynamic range are of utmost importance. The significance of controlling such a range of oscillations is exemplified by neurotransmitter exocytosis, triggered within microseconds and, at the other end of the timescale, cell proliferation with periodicities of several hours\(^{15}\). Because of the diffusive nature of the delivery from the OEIP, gradients can be established in the target electrolyte by proper choice of delivery rate – that is, applied voltage. Such gradients were demonstrated by delivery of $\text{H}^+$ to establish a pH gradient\(^{12}\) (Fig. 4 (a)). Likewise, dynamic concentration oscillations can be achieved owing to the switchable nature of delivery; since delivery is determined by applied voltage, cycling the voltage on/off effects similar on/off behavior in the electrophoretic transport. This functionality was demonstrated by oscillatory delivery of $\text{H}^+$ (Fig. 4 (b)). When voltage was applied (5 V, 10 s) $\text{H}^+$ were delivered into the target system and the pH decreased rapidly at the channel outlet. When the voltage was turned off the pH increased as the high concentration of $\text{H}^+$ near the outlet diffused into the bulk of the electrolyte. The diffusion process was slower than the delivery through pumping, and thereby limits the frequency at which pulses can be applied to generate a regular pH oscillation. In this example, the periodicity of generated oscillations was in the range of minutes. Oscillations operating over minutes are physiologically relevant time frames in, for example, $[\text{Ca}^{2+}]$ waves known to regulate cellular processes such as gene transcription\(^{15}\).

**Bio-Signaling by Metal Ion Delivery**

The PEDOT:PSS surfaces of the OEIP have been demonstrated to be biocompatible\(^{8}\). Cells can thus be cultured – often without the need of cell adhesion promoters such as poly-L-lysine or fibronectin – directly on the electrodes, enabling cell-signaling studies in vitro. An ideal ion to demonstrate such functionality is the metal ion $\text{K}^+$ which is implicated in numerous cell signaling pathways. High extracellular $[\text{K}^+]$ will depolarize excitable cells resulting in the opening of membrane-bound ion channels and influx of $\text{Ca}^{2+}$. Using $\text{KCl}$ as the source electrolyte as in the efficiency characterization described above, $\text{K}^+$ can be precisely delivered to cells cultured on the target electrode. The subsequent changes in $[\text{Ca}^{2+}]$ can be monitored optically by pre-loading the cells with the $\text{Ca}^{2+}$-sensitive ratiometric probe FURA-2 AM. This study was realized by microscopy-based real-time single-cell $\text{Ca}^{2+}$ imaging of human cortical neurons\(^{8}\) (Fig. 5). The results demonstrate that the OEIP can successfully modulate nerve cell signaling by diffusive (non-convective) controlled delivery of signal substances – in this case monovalent cations.

**Bio-Signaling by Neurotransmitter Delivery**

The delivery repertoire of the OEIP was recently expanded from protons and metal ions to also include positively charged neurotransmitters such as acetylcholine (ACh), glutamate (Glu), aspartate, and $\gamma$-aminobutyric acid (GABA)\(^{13,14}\). Neurotransmitters are endogenous...
chemicals, which upon binding to their respective receptors modulate signaling between nerve cells. Along with this new catalog of materials, the ion channel dimensions were miniaturized, from a width of millimeters down to a few microns, that is, equal to or smaller than the size of individual nerve cells. Furthermore, the temporal resolution of the device was enhanced, reducing the time required to initially fill the channel with the intended delivery species before delivery into the target system begins (this can be observed in Fig. 5 as the 5 min lag before the cell at the outlet begins to respond). This temporal enhancement was achieved by incorporating a secondary channel and target for preloading the primary channel before initial delivery into the primary target. This modification reduced the delivery lag time from minutes to seconds and delivery of undesired residual ions was minimized\textsuperscript{14}. Such a device with miniaturized channel was recently utilized in neurotransmitter-mediated single-cell studies using ACh\textsuperscript{14}, one of the most important signaling molecules of the nervous system. Microscopy-based $\text{Ca}^{2+}$ imaging was again utilized to visualize, in real-time, the effects of delivery in human neuroblastoma SH-SY5Y cells cultured on the target electrode. Pulsed delivery of ACh induced oscillatory $\text{Ca}^{2+}$ fluxes with a temporal pattern mimicking naturally occurring $\text{Ca}^{2+}$ oscillations (Fig. 6). Importantly, as seen in the figure, the duration of the voltage pulse (that is, the dosage) determines the magnitude of the response.

**Neurotransmitter Delivery in vivo**

The results described above, based on the planar geometry shown in Fig. 1 (a), demonstrate the feasibility of using the OEIP as the basis of an “artificial neuron”. In the OEIP, electrical signals are converted into the controlled diffusive release of neurotransmitters, which selectively activate cells expressing the appropriate receptor. This is analogous to biological neurons’ internal, electrical action potential communication and subsequent neurotransmitter-based chemical signaling at the synaptic interface to succeeding neurons. To begin to realize such “artificial neuron” functionality, the OEIP was redesigned from its original open planar geometry to a fully encapsulated, syringe-like form, allowing its use in vivo\textsuperscript{13}. As depicted in Fig. 1 (b), since the ion channel is essentially a salt bridge between the electrodes, an additional electrolyte can be incorporated within the channel without loss of ionic conductivity. If this central electrolyte is envisaged as the new target system, the two halves of the device can be “folded” together, allowing the two electrode-electrolyte systems to enter the central target electrolyte from the same side, rather that opposite sides as shown in Fig. 1 (b). Adding an encapsulating tube around each electrolyte and modifying the geometry of the electrodes results in the device of Fig. 1 (a) and (b).

To demonstrate the OEIPs efficacy in a living animal, the hearing organ of guinea pigs was used as a model system. In the cochlea, sound waves travel through fluid, the perilymph. These sound waves are transduced into neural signals predominantly by the inner hair cells, a type of neuron specific to the cochlea that is activated by mechanical

![Figure 6. Ca$^{2+}$ imaging of SH-SY5Y cells on delivery of ACh, the numbers to the right of the voltage pulses are the duration in seconds. Inset shows the chemical structure of ACh. Figure reproduced with permission from Ref. 18, courtesy GIT VERLAG.](image-url)
agitation of stereocilia ("hairs") on its surface. The total volume of the guinea pig cochlear perilymph is less than 10 µl\(^1\) and in the human, approximately ten times that\(^2\). Hence, substance delivery involving fluid flow – for example, using osmotic pumps – tends to increase the pressure inside the cochlea and would affect the extremely mechanosensitive hair cells. The OEIP, with its non-convective delivery, is thus a promising technology for delivery into the inner ear.

The inner hair cells utilize Glu as the primary neurotransmitter, and accordingly, express the Glu receptor. On the contrary, the outer hair cells, the secondary transducers of sound waves into neural signals, do not use Glu and therefore do not express the Glu receptor. The inner ear thus represents a model system wherein both the efficacy and specificity of neurotransmitter delivery can be ascertained.

The encapsulated OEIP (Fig. 1 (d)) was mounted on the round window membrane (Fig. 7 (a) and (b)), an established point of diffusive access to the cochlea. This procedure is non-invasive to the cochlea and can be accomplished with routine surgical procedures. The effect of 1 h of Glu delivery (~1.5 µA device current) was assessed by real-time monitoring of the auditory brainstem response (ABR). As shown in Fig. 7 (c), delivery of Glu resulted in significant change in hearing sensitivity as compared to delivery of H\(^+\) (control), thus answering the question of efficacy in an \textit{in vivo} setting. Furthermore, histological analysis indicates that by delivering a particular neurotransmitter, the OEIP was able to target the inner hair cells (Fig. 7 (d)), leaving the outer hair cells unaffected, thus
answering the question of specificity. These results represent the first successful realization of an organic electronic device capable of modulating mammalian sensory function by precise delivery of neurotransmitters and thus establish the OEIP as a viable stepping stone on the way to artificial neurons.

**Conclusion**

Organic conjugated polymers represent a unique class of materials that sit at the interface of traditional electronics and biomimetic ionic and molecular systems. We have leveraged these materials and their distinctive properties in the development of the organic electronic ion pump. The device is integrable both with conventional electronic hardware and directly into biological systems. The polymer composition of the devices provides a flexible, mechanically biocompatible structure, while the manufacturing processes common to organic electronics are easily scaled up or incorporated with other organic or inorganic solid-state systems. From establishing electronically controlled pH gradients to the demonstration of electronic control of mammalian sensory function in a living animal, the OEIP has demonstrated itself as a viable platform for interface with nerve cells – *in vitro* as well as *in vivo* – where complex high-resolution signaling patterns can be generated to control cell physiology. In its ability to translate electronic signals into the controlled release of chemical messengers, the OEIP mimics biological neurons (Fig. 8). Indeed, the OEIP represents the first step towards a true “artificial neuron”.

Many prominent neurological disorders, such as epilepsy and Parkinson’s disease, occur as a result of malfunctioning signal transduction pathways. The OEIP offers a unique possibility to establish a system where endogenous signaling substances – ions and neurotransmitters – can be delivered to modulate, or even restore, the activity of these signaling pathways. This technology promises to increase our understanding of the pathophysiology of neurological diseases and is certain to be pivotal in the development of new treatments for a variety of disorders.

Figure 8. The OEIP compared to a biological nerve cell. Figure reproduced with permission from Ref. 18, courtesy GIT VERLAG.
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