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AN ORGANIC ELECTRONIC ION PUMP TO REGULATE INTRACELLULAR SIGNALING AT HIGH SPATIOTEMPORAL RESOLUTION

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ABSTRACT

Current technologies for cell stimulation suffer from a variety of drawbacks. Indeed, precise, localized, and minimally disruptive machine-to-cell interfacing is difficult to achieve. Here we present the organic electronic ion pump (OEIP), a polymer-based delivery system exhibiting high spatial, temporal, and dosage precision. Based on electrophoretic transport of positively charged species, the OEIP can deliver – with high precision – an array of biologically relevant substances *without* fluid flow, thus eliminating convective disturbance of the target system’s environment. We discuss our results to date, including oscillatory delivery profiles and stimulation of neuronal cells *in vitro*, as well as our ongoing work.

KEYWORDS

Organic electronics, drug delivery, polymer, electrochemistry, electrophoresis

INTRODUCTION

Conjugated polymer systems, doped and un-doped, can exhibit high conductivity for both ions and electrons. In their oxidized state, high concentrations of holes are easily transported along the π -conjugated backbone. Meanwhile, an open, amorphous bulk structure, in conjunction with the chemical properties of the polymer-dopant ion system, provides for fast ion conduction. We have chosen the conducting polymer system poly(3,4-ethylenedioxythiophene) doped with the polyanion poly(styrenesulfonate) (PEDOT:PSS) [1] (Figure 1) to achieve “ion pump” delivery devices that exploit this combined ionic and electronic conduction. Upon biasing these organic electronic ion pumps (OEIPs) [2], electronic addressing signals are converted into exact delivery of ions and bio-substances at very high spatiotemporal resolution,

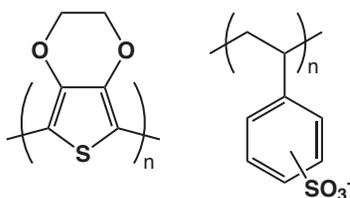


Figure 1: The π -conjugated conducting polymer poly(3,4-ethylenedioxythiophene) (left) doped with the polyanion poly(styrenesulfonate) (right) (PEDOT:PSS).

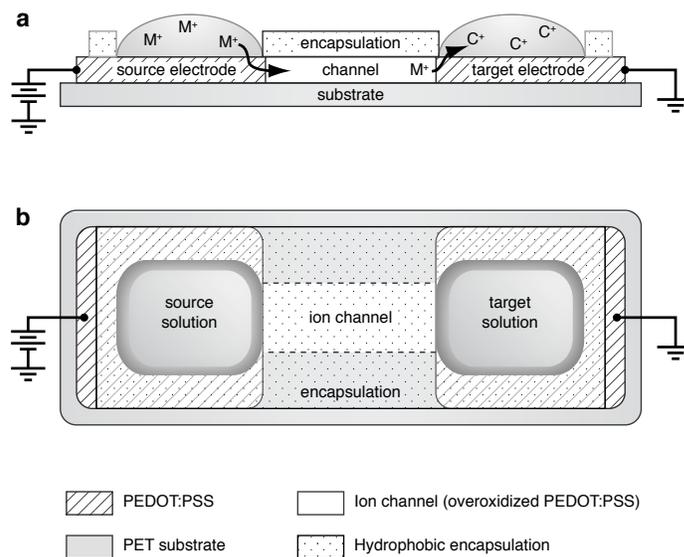


Figure 2: The organic electronic ion pump (OEIP). **a**, Side and **b**, top view of the device structure. M^+ and C^+ represent cations in source and target electrolyte, respectively, where M^+ is the species “pumped” through the channel.

and in the absence of fluid flow – *i.e.*, only molecules are delivered, not solution. We explore these devices to create precision microenvironments and to precisely regulate the cellular responses of several cell lines, such as HCN-2 neuronal cells.

In addition to their now widespread utilization in LEDs and solar cells, conjugated polymers have been explored for more unique applications based on their electrochemical characteristics. The redox-activated swelling and contraction of certain polymers has led to the development of various actuator technologies [3], whereas the electrochemical modulation of electronic conductivity has paved the way for mixed electronic-ionic transistors [4,5], and light-emitting electrochemical cells (LECs) [6]. More recently, the organic electronics field has begun to merge with bio-medical research, yielding a host of biosensors, bio-electrodes, and drug delivery platforms [7,8].

For delivery devices in particular, while many great strides in the technology have been achieved, there is still room for much improvement. These devices typically suffer from limitations such as low release rates and poor on/off ratios, as well as inadequate electronic control of the

delivered dose [9]. Micro- and nanofluidic techniques have successfully bypassed some of these problems and have been used to generate controlled concentration gradients – analogous to many biological systems [10]. However, these systems are based on fluid flow, which necessarily disrupts the fragile liquid environment comprising the target system.

The OEIP reported here addresses these limitations, and can deliver a broad range of bio-substances – including neurotransmitters – with precise electronic control and without convective disturbances.

THE OEIP DEVICE

The OEIP consists of a single film of PEDOT:PSS photolithographically patterned into electrodes joined by an “ion channel” (Figure 2). The channel region, based on the same original film of PEDOT:PSS, is over-oxidized, thus deactivating electronic conduction while preserving ionic conduction [11]. 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 and reduction of PEDOT at the cathode. 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 to be transported to the cathode/target side. Owing to the rapid ion exchange between the thin film PEDOT:PSS electrodes and their accompanying electrolytes, the source electrolyte serves as a reservoir for the positively charged species to be delivered, and the target electrolyte serves as the system into which species emerging out of the ion channel are immediately delivered (Figure 2).

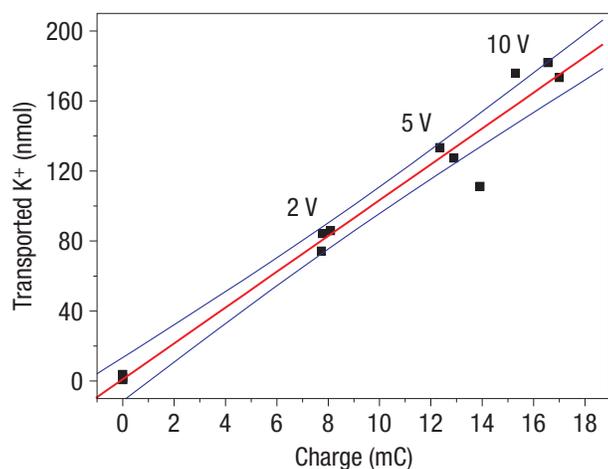


Figure 3: K^+ delivered to the target system versus charge measured in the electronic circuit (i.e., integrated current) at various driving voltages.

This electrophoretic delivery mechanism ensures that the delivery rate of positively charged species through the channel and into the target system is directly (and exactly) proportional to the current measured in the electronic branch of the circuit. Furthermore, since charged species, and not solution, are transported through the channel, material is delivered into the target system *without convective flow*. The OEIP therefore represents an ideal platform for precise, non-disruptive study of biological systems.

RESULTS AND DISCUSSION

Delivery Characteristics and Efficiency

Using KCl as the source electrolyte, upon biasing the device, K^+ ions are transported through the channel and delivered into the target electrolyte. By comparing the amount of K^+ delivered into the target electrolyte (e.g., by 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 (Figure 3). Indeed, these results indicate an approximately 100% efficiency, i.e., for every electron measured in the electronic circuit, exactly one K^+ ion is transported through the channel. In addition to K^+ , this precise electron-to-molecule ratio has been observed for all species successfully pumped to date. In addition, one or several miniaturized electrodes or ion channels can be incorporated to further refine the spatial resolution of the device.

Using the OEIP to Induce pH Oscillations

Many cell signaling events are defined by concentration oscillations. Here, we show that the OEIP device can be used to induce pH oscillations [12] in the target reservoir (Figure 4). When the voltage was applied (5 V, 10 s) protons were pumped out and the pH decreased rapidly close to the channel outlet. When the voltage was turned off the pH increased due to diffusion in the electrolyte. The diffusion process was slower than the delivery through pumping, and thereby limiting the frequency of which pulses can be applied to generate a regular pH oscillation.

Pumping K^+ to Regulate the Intracellular Ca^{2+} Level in Neuronal Cells

K^+ is an activator of excitable cells such as HCN-2 neuronal cells. High extracellular $[K^+]$ depolarizes the plasma membrane, thus activating voltage-operated Ca^{2+} channels (VOCCs) in the membrane. The open channels allow a rapid influx of Ca^{2+} ions from the extracellular environment into the cytoplasm. We investigated whether K^+ pumping could activate the Ca^{2+} homeostasis in HCN-2 cells. The cells were loaded with Ca^{2+} sensitive ratiometric probe FURA-2 AM and the intracellular Ca^{2+} level was recorded during the pumping experiment.

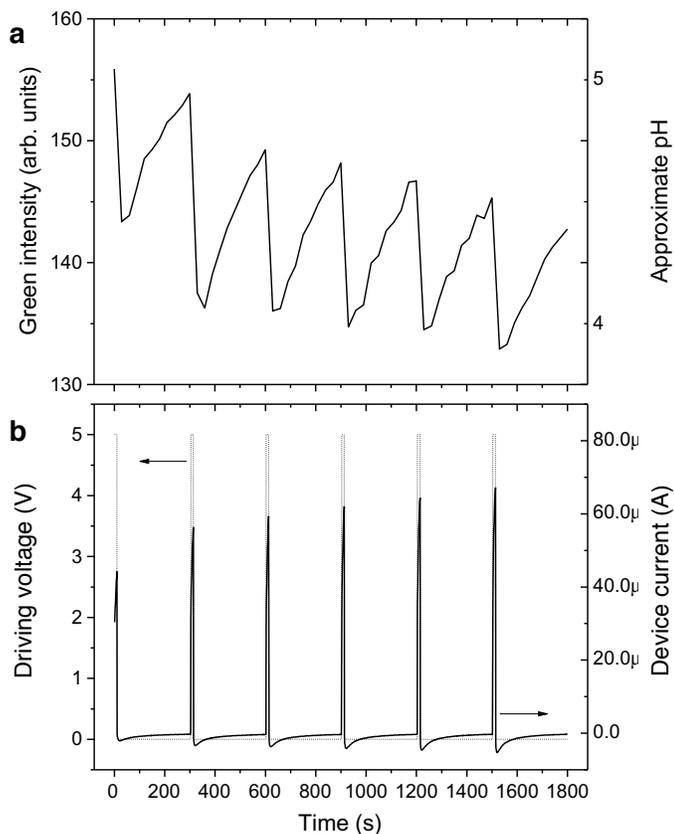


Figure 4: pH modulation using the OEIP. **a**, The increase in pH closest to the outlet of the ion channel into the target electrolyte. The pH was measured using a red pH indicator and analyzing the green intensity of subsequent microscope images. **b**, The associated device current (solid) and voltage (dotted).

Figure 5 shows how the intracellular $[Ca^{2+}]$ was modulated in HCN-2 cells by pumping of K^+ . The dashed line shows how the intracellular $[Ca^{2+}]$ increased after a few minutes of pumping. When the voltage-operated Ca^{2+} channels were blocked by Gd^{3+} (solid line) or nifedipine (dotted line) the intracellular $[Ca^{2+}]$ remained unaffected thus proving the physiological relevance of the Ca^{2+} influx.

In cell signaling research, spatiotemporal control of ion fluxes is of great importance. To achieve local stimulation of cells the channel width was shrunk down to 50 μm. When K^+ was pumped with this device geometry, only cells close to the channel outlet responded while cells 500 μm away were unresponsive.

ONGOING WORK AND CONCLUSION

Building on the results reported above, we are currently investigating further development of the OEIP platform. One focus is on the decrease of the device dimensions for even better spatial and temporal delivery resolution [13]. This will allow for true single-cell stimulation *in vitro*. In parallel, we are developing the device in an encapsulated scheme to enable its use *in vivo* as well as in expanded *in vitro* applications [14]. The

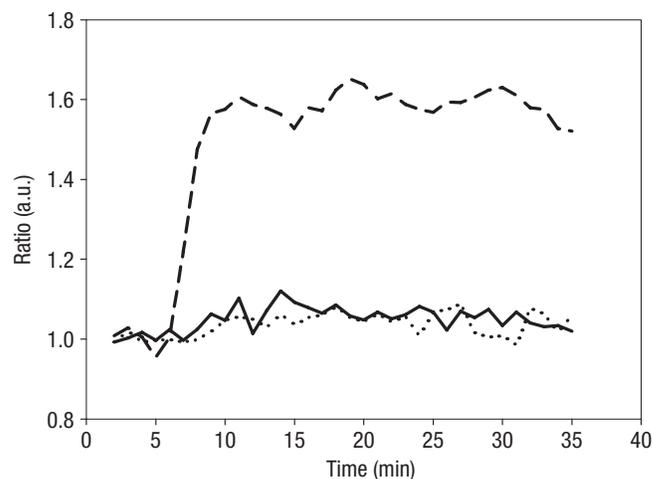


Figure 5: Fluorescence microscopy of intracellular Ca^{2+} levels in HCN-2 neuronal cells versus time. At 4 min, the pump was turned on, delivering K^+ which resulted in (dashed line) increased response due to K^+ -opened Ca^{2+} channels, and (solid and dotted lines) no response when Ca^{2+} channels were blocked by secondary substances.

encapsulated form of the OEIP could lead toward fully implantable drug delivery devices. Finally, we are exploring new delivery profiles which could expand the stimulation capabilities of the OEIP, thus enabling better machine-to-biosystem interfacing.

The findings above, and our ongoing research efforts, promise for a versatile technology platform to precisely regulate physiology and signaling *in vitro* as well as *in vivo*.

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