Graphene-Enabled Electrophoretic Ion Pump Delivery Devices

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Organic electronic ion pumps (OEIPs) have been investigated as a promising solution for precise local delivery of biological signaling compounds. OEIP miniaturization provides several advantages, ranging from better spatiotemporal control of delivery to reduced invasiveness for implanted devices. One miniaturization route is to develop OEIPs based on poly-electrolyte-filled capillary fibers. These devices can be easily brought into proximity of targeted cells and tissues and could be considered as a starting point for other “iontronic” implants. To date, OEIPs and other such iontronics exhibit a limited electrode capacity as they generally rely on poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) electrodes. While this material is well studied and viable in mixed ion-electron systems, its bulk capacitance is limited by eventual redox reactions. Graphene is an excellent alternative for high-performance electrodes and low-cost solution-processed graphene derivatives are particularly promising, exhibiting high charge mobility and ideal structural properties (lightness, flexibility). Here, the application of solution-processed reduced graphene oxide (RGO) as high-performance driving electrodes for OEIPs is presented. RGO electrodes are characterized and compared with standard PEDOT:PSS (and Ag/AgCl) electrodes. The RGO exhibits greater charge storage capacity and thus increased operational lifetime. The graphene-enabled OEIPs exhibit improved neurotransmitter transport, without imposing limitations to the applied current level.

1. Introduction
Organic bioelectronic systems and technologies are considered among the most efficient tools for applications such as biosensing, electrophysiological recording, and drug delivery.[1–3] OEIP miniaturization provides several advantages, ranging from better spatiotemporal control of delivery to reduced invasiveness for implanted devices. One miniaturization route is to develop OEIPs based on poly-electrolyte-filled capillary fibers. These devices can be easily brought into proximity of targeted cells and tissues and could be considered as a starting point for other “iontronic” implants. To date, OEIPs and other such iontronics exhibit a limited electrode capacity as they generally rely on poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) electrodes. While this material is well studied and viable in mixed ion-electron systems, its bulk capacitance is limited by eventual redox reactions. Graphene is an excellent alternative for high-performance electrodes and low-cost solution-processed graphene derivatives are particularly promising, exhibiting high charge mobility and ideal structural properties (lightness, flexibility). Here, the application of solution-processed reduced graphene oxide (RGO) as high-performance driving electrodes for OEIPs is presented. RGO electrodes are characterized and compared with standard PEDOT:PSS (and Ag/AgCl) electrodes. The RGO exhibits greater charge storage capacity and thus increased operational lifetime. The graphene-enabled OEIPs exhibit improved neurotransmitter transport, without imposing limitations to the applied current level.

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Polymer electrodes are used for biological applications such as polymer sensor electrodes, acting in amperometric\cite{14,15} or potentiometric\cite{16} mode, and stimulation electrodes;\cite{17,18} Poly(3,4-ethylenedioxythiophene) doped with polystyrene sulfonate (PEDOT:PSS) is the most studied and explored mixed ion-electron conducting polymer system and it has been used extensively in organic (bio)electronics.\cite{19–21} Moreover, PEDOT:PSS is generally the first choice as signal transducer in various bioelectronics devices operating in physiological media, such as biosensors, electrochemical transistors;\cite{22,23} and electrodes\cite{22,24} interfacing with neuronal systems, as well as the electrodes in OEIPs.\cite{12} Despite its long-term use as a material for sensing, storage, and transport of charges, in many of the abovementioned devices the operational principle relies on the capacitance of PEDOT:PSS\cite{25} and its bulk capacitance can be poor in long term use due to redox reactions.

Graphene poses an interesting alternative to PEDOT:PSS electrodes. Owing to its 2D structure and the resulting outstanding electronic properties, graphene has attracted significant interest for use in electronic and optoelectronic applications.\cite{26} Graphene provides excellent electrochemical properties, high flexibility and mechanical strength, and is therefore an ideal electrode candidate for bioelectronics.\cite{27} To date, the most promising method for preparing graphene electrode films over large and flexible areas is the solution processing of graphene oxide (GO), mainly due to the simplicity and high throughput of the fabrication technique. GO can be easily produced upon exfoliation of graphite powder in solution to form sheets comprising single or multiple layers of carbon atoms decorated with oxygenated species. Large-area GO-based layers can then be fabricated through solution-casting of GO onto a specific substrate. However, the relatively high sheet resistance of such layers is a drawback for high-performance electrode applications. A way to overcome this obstacle is by deoxygenation of GO, yielding reduced GO (RGO), to reach a material as close in structure and properties to graphene as possible via chemical reaction with oxygen reducing agents or high-temperature annealing.\cite{28–30} Unfortunately, most of the efficient GO reduction methodologies are not only incompatible with temperature or chemically sensitive substrates but are time-consuming and complicated. One of the most efficient reduction methods that addresses such limitations is laser-induced reduction, which is a simple, single-step, chemical-free, and room temperature technique that can also be applied for in situ reduction of GO thin films deposited on plastic substrates as well as for post-reduction of complete GO-based devices.\cite{31,32}

Here we investigate the electrode capacity limitation of PEDOT:PSS versus RGO driving electrodes in ion pump applications, where the ideal electrode would not exhibit any such limitation. Glass capillary-based ion pumps\cite{4,33} comprising a polyanionic CEM “ion channel” were used for delivery of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and comparison of the various electrode configurations (Figure 1a–c). Fiber capillary ion pumps pose a particular challenge for electrode capacity limitations, since the larger three-dimensional CEM (compared to older planar OEIPs) comprises a higher amount of total fixed charge. This fixed charge corresponds to the amount of charge that must be built-up/exchanged in the electrodes to “flush out” the ion channel and effectively delivery from source to target. In this way, demonstrating the highest possible capacity electrodes with the longest possible sustained operation is of great importance for future applications.

2. Results and Discussion

GO films of ≈130 nm thickness were deposited using a spray-gun technique on plain polyethylene terephthalate (PET) as...
well as on PET substrates pre-coated with PEDOT:PSS.\textsuperscript{[34]} Scanning electron microscopy (SEM) and atomic force microscopy (AFM) showed that the morphology of the as-deposited GO layers is practically identical for both substrates. Therefore, only GO films on PET were considered in the following work. A typical SEM image of the surface of such films is presented in Figure 2a (cross section shown in Figure S1a, Supporting Information). RGO films were subsequently produced by UV laser irradiation of the pristine GO layers.\textsuperscript{[35,36]} Typical SEM and AFM images of the RGO layers, presented in Figure 2b and Figure S1a,b (Supporting Information), respectively, showed that the film roughness is not practically affected by the laser treatment process (see also Figure S1c, Supporting Information). At a specific laser power, the yellowish color of the initial GO film gradually turned to black (Figure S1d, Supporting Information) with the elevation of the irradiation time. At the same time, the conductivity of the respective films progressively increased with exposure time (Figure 2e) and laser fluence (Figure S2, Supporting Information), indicating that the reduction degree of GO could be well controlled. It is evident that a more than two order of magnitude improvement in conductivity can be realized via proper selection of laser fluence and irradiation time. The corresponding UV–vis spectra of GO and the four RGO layers prepared upon UV laser irradiation of GO layers at a fluence of 20 mJ cm\textsuperscript{−2} for 5, 10, 20, and 100 s, respectively. e) Electrical conductivity for the different layers studied in this work.

![Figure 2.](image)

Figure 2. a) Typical SEM image of an as-prepared GO layer. b) Typical SEM and c) AFM images of RGO layers. All scale bars: 5 µm. d) UV–vis spectra of GO and the four RGO layers prepared upon UV laser irradiation of GO layers at a fluence of 20 mJ cm\textsuperscript{−2} for 5, 10, 20, and 100 s, respectively. e) Electrical conductivity for the different layers studied in this work.

Raman spectra recorded from pristine GO and RGO layers (Figure S3, Supporting Information) showed the characteristic broad peaks at 1354, 1580, and 2695 cm\textsuperscript{−1}, corresponding to the D, G, and 2D bands, respectively. To account for the crystalline quality of the RGO sheets, the D/G intensity ratio (I\textsubscript{D}/I\textsubscript{G}), which is a measure of the lattice disorder, was monitored upon increasing the number of irradiation pulses at constant incident fluence (20 mJ cm\textsuperscript{−2}). As shown in Table S1 (Supporting Information), I\textsubscript{D}/I\textsubscript{G} progressively increased with the number of pulses. Therefore, thermal effects become more and more pronounced\textsuperscript{[31]} giving rise to photothermal breakage of carbon bonds, which increases the number of defects in the lattice and in turn leads to the formation of smaller and smaller crystalline graphene sp\textsuperscript{2} domains of improved lattice order.\textsuperscript{[31]} This also complies with the observed reduction in the width of the G band upon increasing the irradiation time (Table S1, Supporting Information). We conclude that removal of oxygen atoms from GO sheets takes place, without causing damage to the lattice, by precise tuning of the laser power and pulse number, in compliance with recent theoretical calculations.\textsuperscript{[33]}

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We proceeded to investigate using the RGO films as alternative high-capacity electrodes for driving ion pumps. For this purpose, fiber capillary ion pumps (25 µm inner diameter, 15 mm channel length, Figure 1) filled with a 2-acrylamido-2-methylpropane sulfonic acid (AMPSA) polyanion membrane (i.e., CEM) were used with three different electrode configurations: PEDOT:PSS, Ag/AgCl paste (another common electrode used in iontronics), and RGO (all 70 × 4 mm). In all three cases, the same type of electrode was used for both the anode (inside the ion pump reservoir) and cathode (in the target solution, Figure 1). The ion pumps were characterized by loading the channel with an aqueous electrolyte containing the inhibitory neurotransmitter GABA (100 × 10⁻³ M) with the pH adjusted by the addition of HCl to 3. The added H⁺ in the source exhibit higher ionic mobility than GABA, but previous results have shown that pH 3 is optimal for cationic GABA transport due to a combination of GABA:H⁺ ratio and amenable GABA configuration for passing through the CEM.[38] The devices were mounted in a target solution of 100 × 10⁻³ M KCl(aq) and a constant potential difference (1 V) was applied between the two electrodes for 12 h with continuous recording of the electronic current.

Compared to PEDOT:PSS and Ag/AgCl paste electrodes, RGO presented a higher steady-state current and more stable behavior (Figure 1a). After 8 h, the PEDOT:PSS electrodes transitioned to the de-doped “reduced” state and lost their conductivity.[39] This is characteristic behavior for all our published PEDOT:PSS-based ion pumps but is rarely shown in the publications.[40] The device resistance dramatically increases as the electron-to-ion exchange reactions shift from the redox-active PEDOT:PSS to the more distant metal/carbon contacts (illustrated as the black contacts at the top of the RGO electrodes in Figure 1a). The steady-state currents, ranging from 30–60 nA yield current densities (for the 25 µm diameter channel cross-section) of 6–12 mA cm⁻², in good agreement with previous planar devices (13 mA cm⁻²).[38] From the steady-state currents, the ionic conductivity and total delivered charge were calculated for the different electrodes at 4 h (Figure 3b). The conductivity and total delivered charges were determined to be lower for PEDOT:PSS and Ag/AgCl compared to the RGO electrodes. The next step was to apply the same constant voltage for 4 h using the four different RGO film electrodes prepared. The corresponding devices presented a different steady-state current which complies with the conductivity of the RGO films (Figure 3c). As shown in Figure 3c,d, the lower the optical density, and thus the reduction degree of RGO films, the higher the observed steady-state current. The morphology of the RGO electrode is also important, as the presence of visible cracks in highly-reduced layers (3rd and 4th RGO electrode) results in lower steady-state currents (see photographs in Figure 3c,e).

To determine the efficiency of GABA delivery through the various devices, chemical quantification was performed.
20 mJ cm\(^{-2}\) with a specific number of pulses, using a constant fluence of 25 \(\mu\)m \(\times\) 125 \(\mu\)m. Capillaries were treated in sulfuric acid (concentrated) to remove the polyamide coating (>100 °C under slow stirring for 20 min), followed by a DI water rinse. Capillaries were then bonded onto a Luer lock syringe adapter using glue and heat gun and connected vertically, via a 5 mL disposable polypropylene syringe, to a nitrogen line fitting for flushing. The syringe reservoir was connected to the nitrogen supply line to give the desired flow rate of the different solvents. The first process step was to flush the capillary with \(N_2\) at 5 bar for 5 min. The capillaries were then flushed with 2 m KOH for 2 h to allow for etching of the inner surface. This etching step increases the hydrophilicity of the capillary (KOH solution increases the surface silanol concentration as hydroxide ions react with the silanol groups of the silica surface to produce silicate ions\(^{[43]}\)). After KOH etching, the capillaries were flushed with DI water for 10 min and dried by \(N_2\) flushing for 5 min. The next process step was the silanization. Capillaries were flushed with the vinylic silane 3-(trimethoxysilyl) propyl methacrylate (10 wt% in toluene) for 1 h followed by drying with \(N_2\) flushing for 5 min and ethanol flushing for 10 min. The process introduces vinylic groups on the inner surface of the fused silica capillary and ensures that the polymer is attached covalently to the capillary wall. The silanizing agent furthermore reacts with silanol groups on the glass and methacrylic groups are expressed on the surface providing hydrophobic characteristics\(^{[44]}\). The last process step was acrylate monomer (2-acrylamido-2-methylpropane sulfonic acid (AMPSA), Sigma-Aldrich, \(M_w\) 2000, 50 wt%) flushing/filling. AMPSA was mixed with polyethylene glycol diacrylate (PEG-DA, Sigma-Aldrich, \(M_w\) 575, 2 wt%) and a photoinitiator (2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropophenone, Sigma Aldrich, 0.5 wt%) that promotes cross-linking in DI water and flushed through the capillaries from a dark syringe tube for 20 min (dark to prevent unwanted photopolymerization).

Finally, using a UV light (UVS-28 EL Series 8-W UV lamp, 254 nm) monomer in the capillary was polymerized after 10 min. After the polymerization, the capillary was cut into 20 desired device lengths (15 mm) by a fiber cleaver and assembled directly onto heat-shrink tubes/reservoirs (Figure 1a). Assembled capillary devices were immersed and stored in 1 \(\times\) 10\(^{-3}\) M KCl(aq) before use to hydrate the polyanion membrane\(^{[4,13,33]}\). All electrodes (RGO, PEDOT:PSS, or Ag/AgCl) were painted with carbon paint (DuPont 7082) to provide a robust electrical contact point (Figure 1a).

3.3. Capillary Ion Pump Fabrication

Glass fiber capillaries coated with polyimide were used to provide abrasion resistance and to maintain mechanical strength for ion pump fabrication (Polymicro Technologies, CM Scientific). The inner diameter was 25 \(\mu\)m and the outer was 125 \(\mu\)m. Capillaries were treated in sulfuric acid (concentrated) to remove the polyamide coating (>100 °C under slow stirring for 20 min), followed by a DI water rinse. Capillaries were then bonded onto a Luer lock syringe adapter using glue and heat gun and connected vertically, via a 5 mL disposable polypropylene syringe, to a nitrogen line fitting for flushing. The syringe reservoir was connected to the nitrogen supply line to give the desired flow rate of the different solvents. The first process step was to flush the capillary with \(N_2\) at 5 bar for 5 min. The capillaries were then flushed with 2 m KOH for 2 h to allow for etching of the inner surface. This etching step increases the hydrophilicity of the capillary (KOH solution increases the surface silanol concentration as hydroxide ions react with the silanol groups of the silica surface to produce silicate ions\(^{[43]}\)). After KOH etching, the capillaries were flushed with DI water for 10 min and dried by \(N_2\) flushing for 5 min. The next process step was the silanization. Capillaries were flushed with the vinylic silane 3-(trimethoxysilyl) propyl methacrylate (10 wt% in toluene) for 1 h followed by drying with \(N_2\) flushing for 5 min and ethanol flushing for 10 min. The process introduces vinylic groups on the inner surface of the fused silica capillary and ensures that the polymer is attached covalently to the capillary wall. The silanizing agent furthermore reacts with silanol groups on the glass and methacrylic groups are expressed on the surface providing hydrophobic characteristics\(^{[44]}\). The last process step was acrylate monomer (2-acrylamido-2-methylpropene sulfonic acid (AMPSA), Sigma-Aldrich, \(M_w\) 2000, 50 wt%) flushing/filling. AMPSA was mixed with polyethylene glycol diacrylate (PEG-DA, Sigma-Aldrich, \(M_w\) 575, 2 wt%) and a photoinitiator (2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropophenone, Sigma Aldrich, 0.5 wt%) that promotes cross-linking in DI water and flushed through the capillaries from a dark syringe tube for 20 min (dark to prevent unwanted photopolymerization).

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3.4. Microscopic and Spectroscopic Characterization

UV-vis absorption spectra were recorded using a Perkin Elmer LAMBDA 950 UV/VIS/NIR spectrophotometer. The morphology of the GO surface electrode was examined by tapping mode atomic force microscopy (Park XE-7 AFM). All measurements took place in air immediately after laser irradiation.
3.5. Electrical Characterization

Ion transport was evaluated via electrical measurements. Capillary ion pumps were characterized by loading the channel with an aqueous electrolyte containing the substance of interest (GABA, loaded from a 100 × 10⁻³ M source electrolyte). Electrical characterization was performed using a Keithley 2602 SourceMeter with a custom-designed LabVIEW control program. A constant potential difference was applied to between the source (anode) and target (cathode) electrodes for 12 h, and then 4 h, to measure simultaneously the resulting current for the different electrode varieties. The measurements of the electrical conductivity of the different layers were carried out using the four-probe technique via a Van der Pauw Ecopia HMS-3000 Hall Measurement System.

3.6. Quantification of GABA in the Target Electrolyte

Several target electrolyte samples were collected after GABA delivery from the different devices, and thereafter chemical quantification of GABA was performed via ELISA assay (LDN/BA E-2500) measured on a BioTek Synergy H1m plate reader, following the assay supplier’s protocol. The efficiency of the device was estimated by calculating the amount of delivered GABA divided by the number of electrons passed through the circuit, and averaging the values obtained from the various experiments.

4. Conclusions

We have successfully demonstrated a new methodology for the development of miniaturized ion pump devices within a capillary fiber form factor, using laser-fabricated graphene-based electrodes. These results mark the first successful marriage of our well-developed iontronics toolkit with the equally promising and developing world of graphene-based electronics. Specifically, our findings demonstrate that graphene-based electrodes can provide the high capacitance and longevity required for iontronics applications and indeed that graphene-based ion pumps can effectively delivery one of the more challenging substances we have delivered to date: the neurotransmitter GABA. This successful delivery of GABA indicates (proves) that such graphene-based ion pumps—and other such iontronics components—can effectively deliver “simpler” compounds such as Na⁺, K⁺, OH⁻, H⁺, and the neurotransmitter acetylcholine. In addition, the versatile laser techniques used in this study were applied over large areas, but in future work can be used for fine-scale patterning of ion pump electrodes as well as wiring and driving circuits. We thus believe that this demonstration will pave the way for many future applications and enhancements of organic bioelectronics/iontronics coupled to graphene-based electronics.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

M.B. and D.T.S. are shareholders in the small, researcher-controlled intellectual property company OBOE IPR AB (oboeipr.com), which own patents related to the ion pumps used in this research. The authors declare no additional competing financial interests.

Author Contributions

M.Se., M.Sy., and K.S. carried out the fabrication and characterization of the graphene-based electrodes. M.Se. fabricated and characterized the devices and wrote the manuscript. M.B., E.S., and D.T.S. supervised the project. All authors discussed the results and contributed to the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

bioelectronics, drug delivery, electrophoresis, graphene oxide, ion exchange membranes, ion pumps, laser ablation, neurotransmitters, organic electronics, reduced graphene oxide

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