Active Control of Dye Release for Neuronal Tracing Using PEDOT-PSS Coated Electrodes

Stefanie Heizmann, Antje Kilias, Patrick Ruther, Ulrich Egert, and Maria Asplund

***Abstract*— Penetrating neural probes comprising arrays of microelectrodes are commonly used to monitor local field potentials and multi-unit activity in animal brain over time frames of weeks. To correlate these recorded signals to specific tissue areas, histological analysis is performed after the experimental endpoint. Even if the lesion of the penetrating probe shaft can be observed, a precise reconstruction of the exact electrode positions is still challenging. To overcome these experimental difficulties, we developed a new concept, whereupon recording electrodes are coated with a poly (3, 4-ethylenedioxythiophene/ polystyrenesulfonate) (PEDOT/PSS)-based film. The conducting polymer acts as dye reservoir over several weeks and afterwards provides controlled delivery of neurotracers. This paper presents a recording electrode based on a PEDOT/PSS bilayer optimized for dye delivery and with reduced impedance. Controlled exchange of neurotracer dye is successfully demonstrated *in vitro* using spectrofluorometry and in neuroblastoma cell cultures. A second PEDOT/PSS capping layer on top of the dye reservoir lowers the passive leakage of dye by a factor of 6.4 and prevents a direct contact of the dye filled layer with the cells. Stability tests over four weeks demonstrate the electrochemical stability of the PEDOT coating, as well as retained functionality of the dye delivery system.**

***Index Terms*— Dye delivery system, electro active PEDOT coating, neuronal electrode tracing, precise cell labelling.**

I. Introduction

TO UNDERSTAND the underlying causes of neuronal dysfunction, such as epilepsy or Parkinson's disease, it is necessary to establish technologies that allow for reliable recordings of neuronal signals in animal models and the correlation of these recordings with the morphology of the surrounding neural tissue. Penetrating silicon-based multi-electrode probes [1] are often used to track neuronal signals, i.e., local field potentials as well as multi-unit and single-unit activity from different locations in the brain [2], and thereby follow the progress of a disease. Correlating the recorded signals with the neural network structure, as well as neuron cell type and status, provides detailed additional information about the development and progression of neuronal diseases. Extracting the exact electrode positions within the tissue after sectioning the brain, however, is challenging and up to now not solved in a satisfactory way. Estimating the electrode position using the shape of the probe shaft lesion is inaccurate and not always possible, as track lesions can often not be identified at all. Another common method to locate the position of recording sites is to burn the tissue by driving high faradaic currents over the electrode site [3]. Using this method, the cells located close to the probe track which are the most relevant cells for the analysis are destroyed and important information on cell morphology and status is therefore lost.

A further strategy to identify recording positions is to stain the neurons under investigation with neurotracers which are typically lipophilic and positively charged dyes. Upon contact with the cell, neurotracers are transferred to the cellular membrane and accumulate there. The dye is then transported by diffusion within the phospholipid bilayer of the cell membrane (lipophilic dyes). In these cases the distance across which dye spreads is a function of time after application. The labelled neurons can be identified by fluorescence microscopy of the tissue.

Neurotracers, such as DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) and FastDiO (3,3'-Dilinoleyloxacarbocyanine perchlorate), belong to the family of carbocyanines. These fluorescent molecules are commercially available and used as neuron staining dyes for periods of several weeks [4]. Staining directly after electrode implantation would lead to a wide diffusion of the neurotracers into the surrounding tissue [5], possibly obscuring the site of release. In addition, glial cells are shown to take up the dye molecules and distribute them far away from the origin [6], which makes it impossible to locate exactly the recording site after electrode explantation. Furthermore, long-term exposure and a high concentration of dye can affect the normal cellular behavior, thus interfering with the experiment itself [7], [8]. Consequently, specific and noninterfering staining of the recorded cells can only be applied after long-term recording and prior to the electrode explantation.

PEDOT networks have previously been utilized as drug delivery systems for a controlled release of anti-inflammatory

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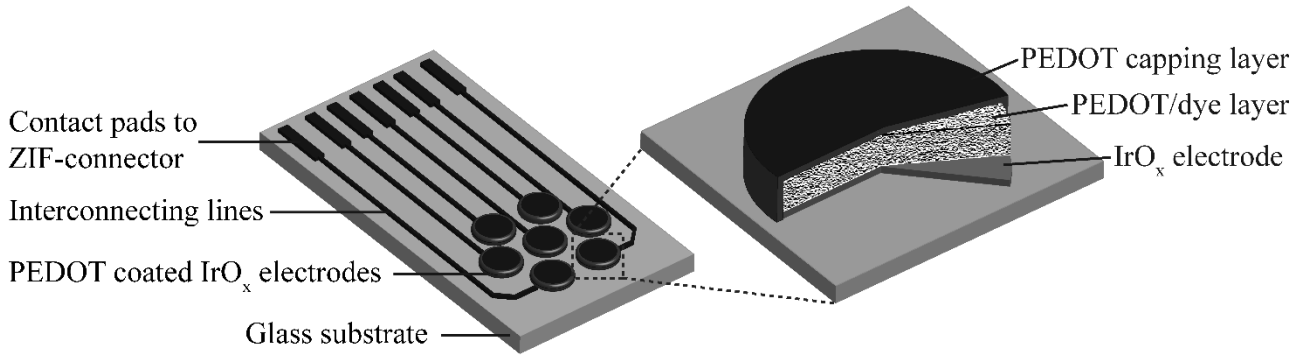

Fig. 1. Illustration of the electrode array applied in this study with PEDOT/PSS layers polymerized onto IrO_x electrodes.

drugs [9]–[13]. The polymer was found to be non-toxic *in vitro* [14] and suitable for recordings of neuronal signaling *in vitro* [15] and *in vivo* [16]. It can act as a reservoir for charged molecules over months [12] and support the controlled release at multiple time points over this time frame. Furthermore, PEDOT layers provide an electrode coating with low electrical impedance [15], which makes it applicable as a material for neuronal interfaces. To date there is no experimental technique available that allows for highly reliable tracing of electrode recording positions within the tissue, therefore a new staining concept is proposed in this study. This PEDOT bilayer system allows for active loading and unloading of neurotracer dyes to/from a reservoir PEDOT layer. This provides an accurate and highly controlled dye delivery system directly at the recording electrode site.

The dye reservoir is formed by depositing a thick layer of the PEDOT/PSS conducting polymer onto the base electrode site. Dye loading and release are achieved via the ion exchange method, which can be controlled by changing the redox state of the polymer network. For dye incorporation, electrochemical reduction of the polymer backbone provides a negative net charge and leads to an attraction of positively charged dye molecules from a loading medium. The ions move into the polymer layer and are retained there, electrostatically bound to the immobile counter ion PSS. For an active dye release, the subsequent oxidation of PEDOT/PSS leads to a positive net charge of the polymeric network and therefore to a release of the mobile and positively charged dye molecules.

Within this study, we successfully demonstrate the synthesis of PEDOT/PSS on iridium oxide (IrO_x) electrodes and the subsequent loading of the polymer layer with the neurotracer dye FastDiO. The IrO_x acts as an adhesion layer between the PEDOT film and a platinum-based electrode metallization [17]. We optimize the storage performance by implementing a multi-layer setup introducing an additional capping layer that covers the dye reservoir. The additional capping prevents the direct contact of the tissue with the reservoir. This layer was shown to enhance the long-term stability of the system *in vitro* and thus allows for a convenient, precise and controllable labelling of cells in the microenvironment of the electrode surface. The coated electrodes have similar impedances as the uncoated IrO_x electrodes which is important for the subsequent recordings. We present here the proof of concept for the active ion exchange of the positively charged and lipophilic neurotracer FastDiO into electrodeposited PEDOT/PSS coatings, providing them with active dye delivery functionality, and suggest the system as a method for the precise reconstruction of the positions of recording electrodes after implantation.

II. Methods

IrO_x electrodes with a diameter of 1600 μm are realized on a glass substrate as follows. A metallization is patterned onto glass wafers using a lift-off process. The metallization consists of titanium (Ti), gold (Au), platinum (Pt), and Ti with thicknesses of 30 nm, 200 nm, 100 nm, and 30 nm, respectively. While Ti layers serve as adhesion promoters to the underlying glass substrate and a subsequently deposited passivation layer, Au is used to lower the line resistance of interconnecting lines between the electrodes and contact pads located at the chip edge (Fig. 1). The metallization is further insulated by a 1.5 μm thick stress compensated layer stack of silicon oxide (SiO_x) and silicon nitride (Si_xN_y) deposited by plasma enhanced chemical vapor deposition (PECVD). Electrodes and contact pads are defined by reactive ion etching (RIE) and the selective removal of the Ti layer using 1% hydrofluoric acid providing access to the Pt surface [1]. The IrO_x electrodes, with a total thickness of 300 nm, are finally deposited by reactively sputtering iridium in an oxygen plasma and patterned using a lift-off process. The electrode arrays are used to evaluate the dye delivery capabilities and electrochemical stability of dye filled PEDOT/PSS coatings.

A. PEDOT/PSS Polymerization

PEDOT/PSS layers [Fig. 2 (a)] are polymerized onto individual IrO_x electrodes of the electrode array (Fig. 1) from a solution of 3,4-ethylenedioxythiophene (EDOT) (0.01 M, Sigma Aldrich) in deionized water, supplemented with NaPSS (71 μM, Sigma Aldrich). A potentiostat (PGSTAT 102N, Metrohm Autolab) is used in a three-electrode setup to control the electro-deposition parameters. The micro-fabricated IrO_x electrodes are used as the working electrode, while a stainless steel and a silver/silver chloride electrode are used as counter and reference electrodes, respectively. PEDOT/PSS is polymerized by applying a constant current density of 500 μA/cm^2

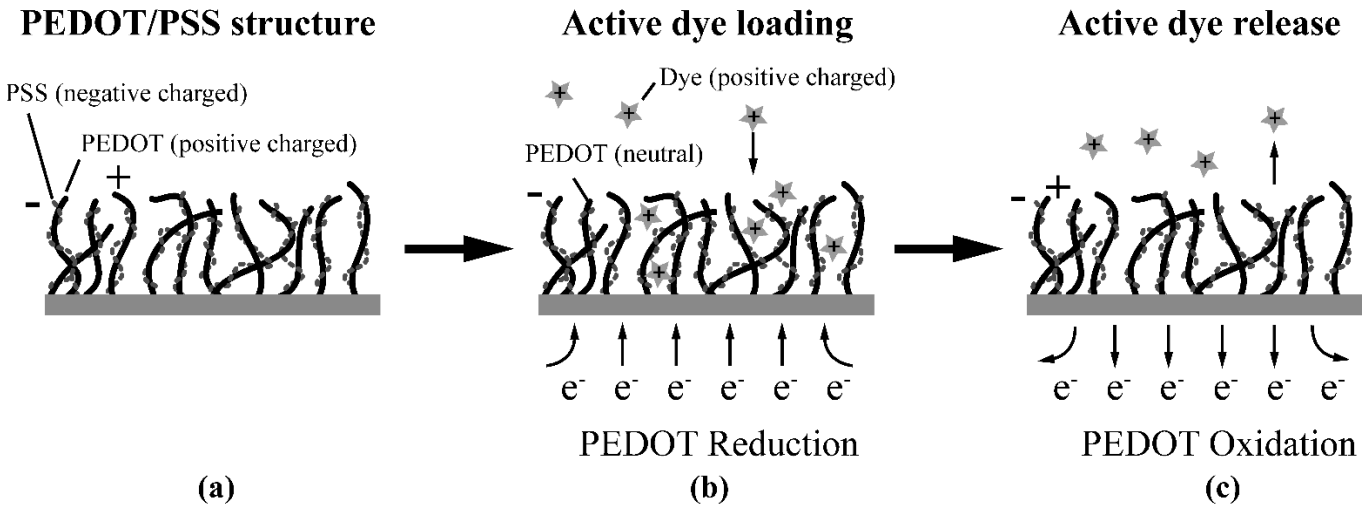

Fig. 2. Schematic of a PEDOT layer with the counter ion PSS **(a)**. Reducing the PEDOT backbone **(b)** leads to an incorporation of positively charged dye molecules, while PEDOT oxidation leads to an active dye release **(c)**.

until a total charge of 300 mC/cm^2 is transferred. Charge capacity before and after PEDOT deposition is evaluated by cyclic voltammetry (CV) in 0.01 M PBS from −0.5 to 0.8 V. In order to evaluate impedance effects of adding the PEDOT/PSS layer with and without dye molecules, the electrode impedance was compared to uncoated IrO_x recording electrodes by impedance spectroscopy (10 mV_{pp}, 100 kHz to 0.1 Hz). Profilometry is used to evaluate the typical thickness of the PEDOT coatings.

B. Ion Exchange

An ionic liquid solution of FastDiO (Molecular Probes) is dissolved in dimethylsulfoxide (DMSO) to a concentration of 1 mg/ml. Potassium perchlorate ($KClO_4$) is added as supporting electrolyte at 0.1 M and FastDiO is actively incorporated into the PEDOT/PSS layer by sweeping the potential in five cycles from −0.45 to 0.2 V vs. Ag/AgCl at a scan rate of 0.1 V/s, while immersing the electrodes in the dye solution. The stop potential was set to −0.3 V. Fig. 2 (b) shows a schematic of the dye loading process. After dye loading, the electrodes are rinsed in ethanol, to remove dye adhering to the chip and electrode surfaces.

C. Capping Layer

A second PEDOT/PSS layer is grown on top of the PEDOT/PSS/FastDiO composite from an EDOT/PSS solution by applying a constant current density of 500 μA/cm^2 until a total charge of 30 mC/cm^2 is transferred. Finally, the chips are washed first in ethanol for 3 × 10 min and in DMSO for 3 × 10 min, to remove residually attached dye molecules from the insulated chip surface. Fluorescence microscopy is used to exclude the presence of contaminating residuals on the chips after the final washing step.

D. Dye Release

In order to allow spectrophotometric quantification of the lipophilic molecules, the electrode system is immersed in a chamber filled with 1 ml of a 0.1 M $KClO_4$ dissolved in DMSO. By oxidation of the composite film, the dye molecules are expected to move out of the film to the surrounding measurement solution [Fig. 2 (c)]. To repeatedly oxidize the PEDOT film, CV in the voltage range from −0.2 to 0.7 V vs. Ag/AgCl is applied at a scan rate of 0.1 V/s for up to 30 min. The dye amount accumulating in the surrounding solution is measured by excitation of the solution at 480 nm and measuring the peak emission at 526 nm using a luminescence spectrometer (LS55, Perkin Elmer).

E. Cell Culture Tests

The neuroblastoma cell line SH-SY5Y (Sigma Aldrich 94030304) was used to test the concept of dye delivery and cell labelling. The cells are cultured with Ham's F12: EMEM (EBSS) (1:1) with supplements (2 mM Glutamine (Sigma Aldrich D8062), 15% Fetal Bovine Serum (Sigma Aldrich F0804), 1% Penicillin-Streptomycin (Sigma Aldrich P4333) and 1% of MEM Non-essential Amino Acid solution (Sigma Aldrich M7145)) until 80 to 100% cell confluency is reached. Cells are passaged with Trypsin/EDTA solution (Sigma Aldrich T3924) and transferred 1:5 in medium to the multi-electrode chip. One day after passage, the chip is connected to a PCB via a zero-insertion force (ZIF) connector and used for active release by the cycling method described above. After dye release, the cells are cultured for a further 24 h in cell culture medium, to provide a sufficient time for the uptake of the released dye. Cells are kept in the dark, to prevent phototoxicity effects after dye uptake. Subsequently, the chip is washed in PBS (1 × 10 min), fixed with Histofix (Roth, 2213.4) (1 × 20 min), washed with PBS (1 × 10 min) and stained with DAPI (Sigma Aldrich D9564), to mark all cell nuclei. Chips with cells are embedded with DAKO mounting medium (DAKO S3023) and dried over night at 4 °C. Fluorescence microscopy (ZEISS observer.Z1 inverted microscope with 81 HE filter set, Carl Zeiss Microscopy GmbH) is used to evaluate the FastDiO release and its uptake by the cells.

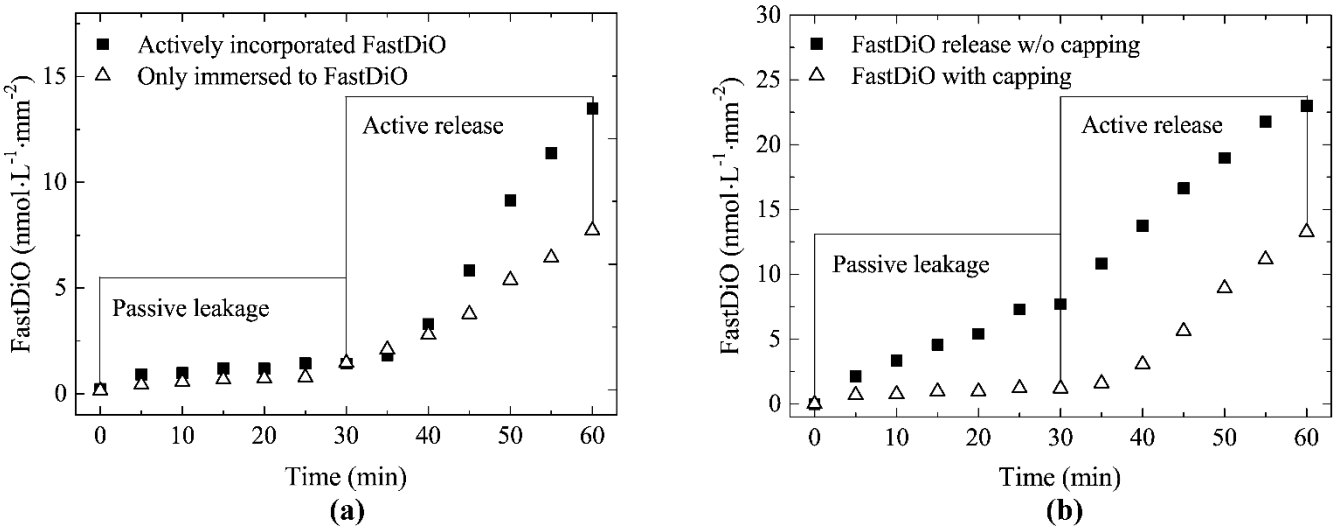

Fig. 3. **(a)** Representative data on the exchanged amount of dye, measured using spectrofluorometry, for the release of FastDiO from n = 3 measurements (mean values) with a PEDOT/PSS bilayer assembly. The squares show passive leakage and subsequent active FastDiO release out of a PEDOT/PSS/FastDiO system, where FastDiO was actively incorporated by ion exchange. In comparison, the triangles show the amount of passively and actively released FastDiO, when PEDOT/PSS-electrodes were only immersed in the FastDiO solution. **(b)** Effect of capping the PEDOT/PSS/FastDiO layer with a second PEDOT/PSS layer on the passive leakage and active release behavior of FastDiO (n = 3). FastDiO was actively incorporated in both experimental sets. The squares represent the polymer coating without a second PEDOT/PSS layer compared to the capped one (triangle).

Image analysis and fluorescence quantification is done using the ZEN Imaging Software (Carl Zeiss Microscopy GmbH).

III. Results

The typical thickness of the PEDOT/PSS/dye/capping layer stack was measured to 900 ± 50 nm using profilometry.

The active release of FastDiO was evaluated by measuring the fluorescence intensity of an ionic solvent solution before and after cycling the PEDOT/PSS/FastDiO layer within this solution. Prior to the active release, passive leakage of dye from the film into the ionic solvent solution was analyzed for comparison.

A. Active Release Versus Passive Leakage of FastDiO

We compared the active and passive dye release within the same electrode actively loaded with FastDiO (Fig. 3 (a), squares). First, we allowed for passive leakage over 30 min, followed by an active release driven by cycling the electrodes between −0.2 and 0.7 V vs. Ag/AgCl. Cycling led to a significantly higher amount of dye being released from the electrode compared to the passive dye leakage (approximately 10:1).

In order to investigate whether ion exchange is the main driving force incorporating the dye, or whether it is the adsorption of dye to the PEDOT surface, one set of electrodes was only immersed into the FastDiO solution and compared to a set of electrodes actively reduced in order to incorporate the dye. The total time (2 min) each sample was immersed in the solution was equal. Loosely bound FastDiO molecules on the surface were removed by washing the electrodes in ethanol and DMSO prior to immersion into the measurement solution. Both sets of electrodes were cycled, to actively drive the FastDiO release.

Fig. 3 (a) shows the measured dye concentration, released per electrode area, resulting from the release of actively incorporated FastDiO (squares) in comparison to an electrode, which was solely immersed in the dye solution (triangles). The cycling driven release ratio between actively incorporated samples and samples only subjected to passive adsorption was 1.7:1. This confirms that the positively charged FastDiO is attracted to the polymer by applying a negative potential.

It also becomes clear that both processes, adsorption and electrostatic interaction, contribute in parallel to dye incorporation into the PEDOT layer. Fig. 5 (a) shows a cyclic voltammogram during active dye release from a PEDOT/PSS/FastDiO coated electrode. The potential was swept for 200 cycles. It could be observed, that the measured current decreased with increasing cycle number (see Fig. 5, dashed box).

B. PEDOT/PSS Bilayer Setup

In order to reduce the passive leakage of FastDiO from the coating, we implemented a PEDOT/PSS bilayer system. Capping the PEDOT/PSS/FastDiO coating with a thin PEDOT/PSS layer (30 mC/cm^2) reduced the amount of passive leakage by factor 6.4, compared to a non-capped coating, shown in Fig. 3 (b). It was confirmed that the capping still enables an active release of FastDiO from the polymer, where the ratio for active FastDiO release from uncapped vs. capped electrodes is 1.3:1.

C. Impedance Spectroscopy

Bode Plots of FastDiO filled PEDOT/PSS electrodes were compared to those of bare IrO_x electrodes and purely PEDOT/PSS coated electrodes [Fig. 4 (a)].

The measurement shows a cut-off frequency shift from approx. 5 Hz to 1 Hz, when both types of PEDOT layers are applied. This is primarily a capacitive effect, as can be seen from the phase plot given in the bottom graph of Fig. 4 (a). This is the expected behavior of PEDOT known to be a highly

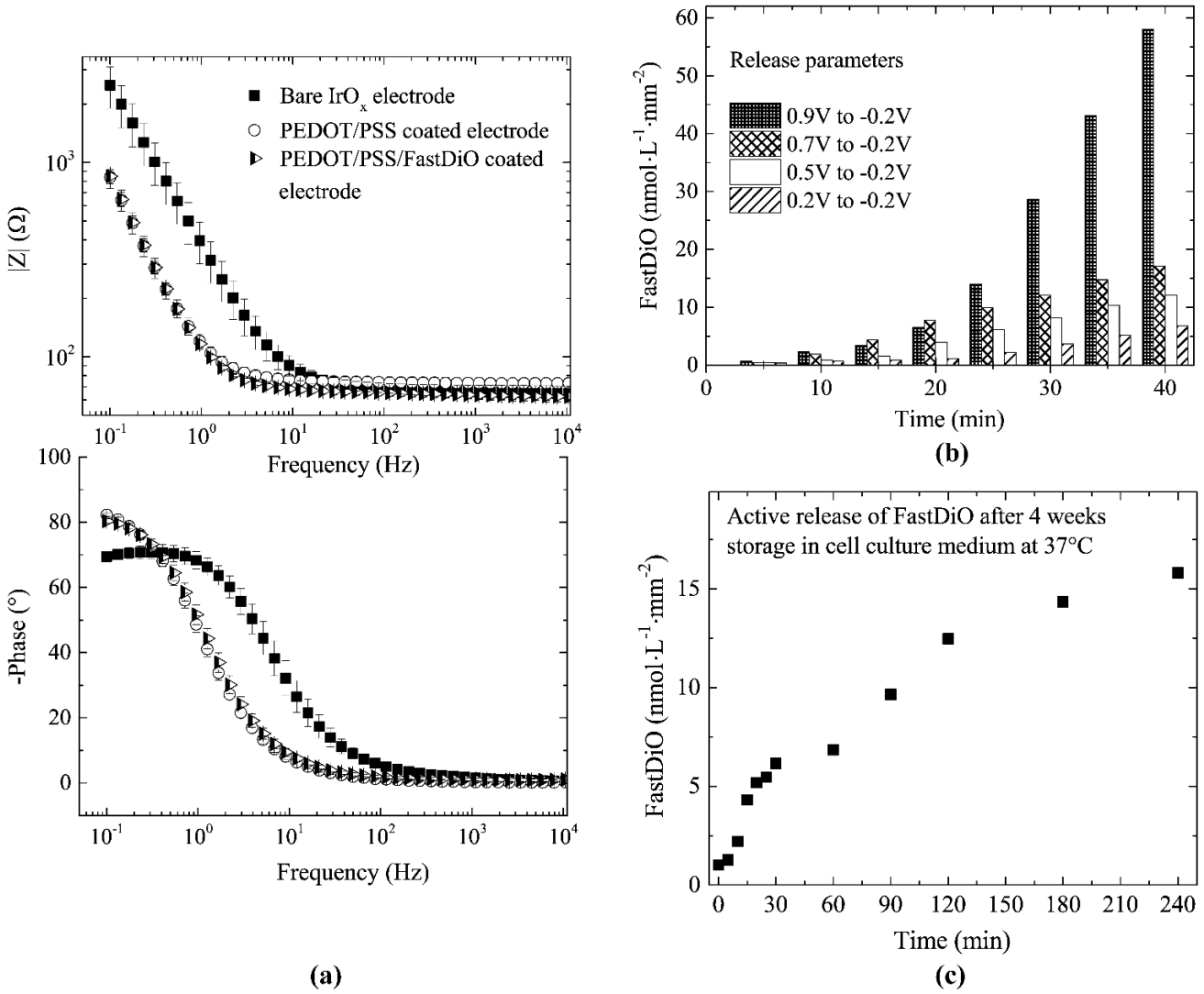

Fig. 4. **(a)** Impedance characteristic of bare IrO_x and polymer coated electrodes in a Bode plot (n = 5). The filled squares show the impedance of the bare IrO_x electrodes compared to PEDOT/PSS coated electrodes (open circles) and the coated one, filled with FastDiO (triangles). **(b)** Different cycling parameters (from V_{pos} to −0.2 V where V_{pos} is varied from 0.9 to 0.2 V) were applied to release FastDiO, showing the dependence of different positive potential rates to the active release of FastDiO in PEDOT/PSS (mean values from n = 2). **(c)** FastDiO filled PEDOT/PSS coated electrodes stored in cell culture medium for 4 weeks at 37 °C. Subsequently, FastDiO was released from the stored electrodes using cycling in ranges of −0.2 to 0.7 V vs. Ag/AgCl.

capacitive electrode material. The additional FastDiO load led to a small impedance reduction in the higher frequency range (10 Hz to 100 kHz), compared to the purely PEDOT/PSS coated electrode. This observation can be explained by the additional ions (dye), inserted into the PEDOT/PSS layer, leading to a higher conductivity of the FastDiO filled PEDOT/PSS system.

D. Evaluation of Different Cycling Parameters for Active Dye Release

In order to investigate the impact of the driving signal on release efficiency, a range of different release cycles was applied, where the positive vertex potential was varied from 0.2 V up to 0.9 V, while the negative vertex remained at −0.2 V. Fig. 4 (b) shows the dependency of actively released dye concentration to the applied range of release potentials. With increasingly positive potential ranges more dye molecules were driven out of the PEDOT/PSS layer to the surrounding liquid. The widest range (−0.2 to 0.9 V) led to the highest amount of released dye molecules, and the dye amount decreased in the logical order for narrower sweep ranges. The highest positive potentials led to a partial delamination of the PEDOT/PSS coating in response to mechanical stress inflicted on the layer. It was observed that the low release range from −0.2 to 0.2 V is still potent enough to deliver dye molecules above the passive leakage rate and with increasing dye amounts over time.

E. Storage Stability of PEDOT/PSS/FastDiO Coatings in Cell Culture Medium at 37 °C

To investigate the long-term stability of the PEDOT/PSS/FastDiO systems, test chips were stored in cell culture medium for four weeks. After storage, the amount of dye that could be actively released from the reservoir into the ionic liquid was quantified. Fig. 4 (c) shows the released dye amount per electrode area as a function of time.

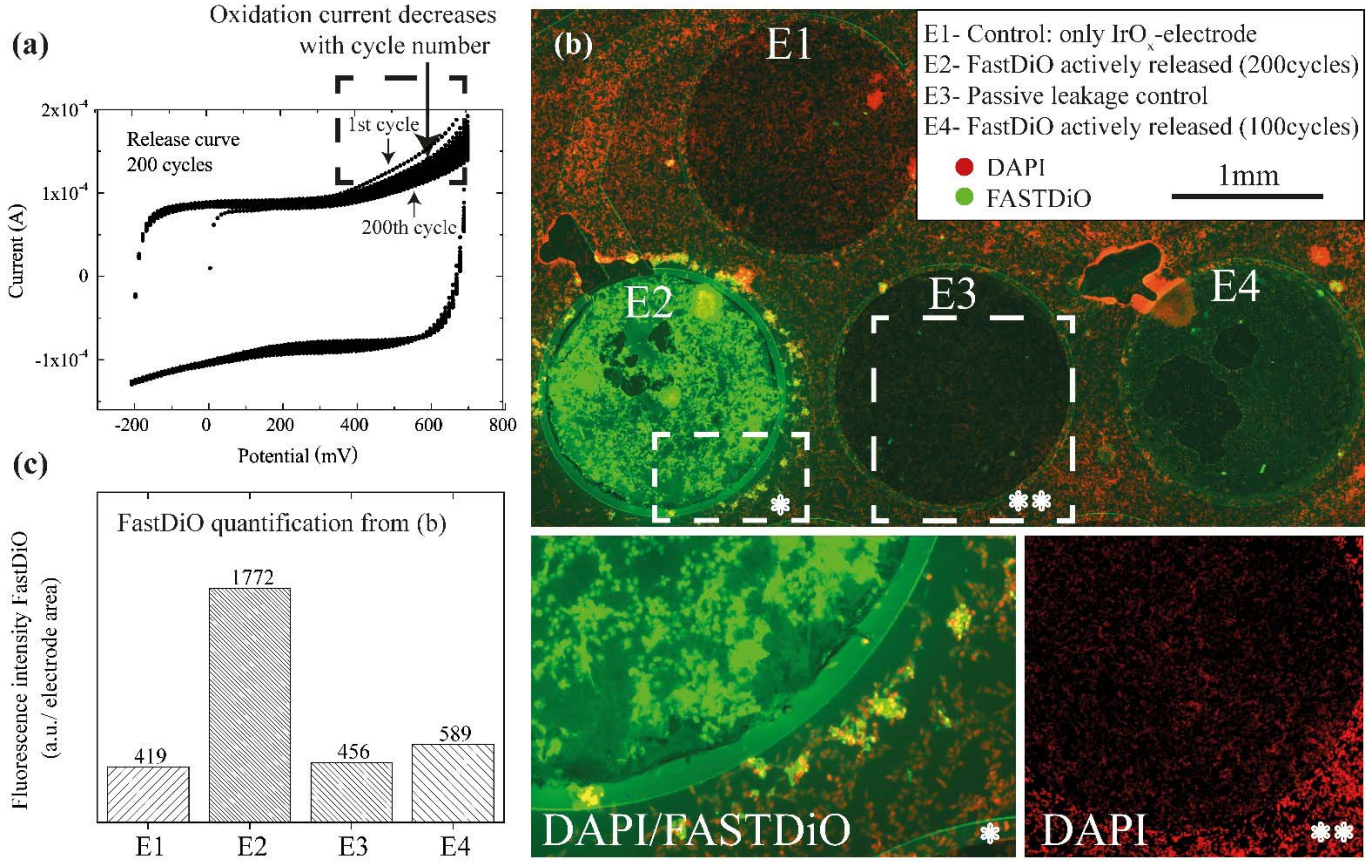

Fig. 5. **(a)** A typical release cyclovoltammogram of a PEDOT/PSS/FastDiO-coated electrodes. Electrodes were cycled with 200 cycles, cycling range from −0.2 V to 0.7 V vs. Ag/AgCl, scan rate 0.1V/s. **(b)** Neuroblastoma cells cultured on a chip with different stages of FastDiO dye release and subsequent DAPI staining. Electrodes were cycled with 200 (E2) and 100 cycles (E4) with a cycling range from −0.2 to 0.7 V vs. Ag/AgCl, scan rate 0.1 V/s. One coated electrode (E3) was not cycled and taken as passive control, whereas (E1) displays an uncoated IrOx electrode. **(c)** Quantification of fluorescence intensity on the electrode areas E1 to E4 from the FastDiO channel.

As indicated by Fig. 4 (c), FastDiO is still integrated in the PEDOT/PSS coating after four weeks of immersion, as confirmed by the active release of dye that can be triggered at this point. The actively driven release after four weeks was determined with 16.5± 2.55 nmol L^{-1} mm^{-2} (n =4).

F. Proof of Concept With Cell Culture Measurements

A cell culture model was used to demonstrate that released dye is accessible for uptake by surrounding cells Fig. 5 (b) shows a SH-SY5Y neuroblastoma cell line, seeded directly on a test chip with PEDOT coated IrO_x electrodes. Individual electrodes were used to investigate the influence of the release time on the extent of FastDiO staining. Cells were visualized by a subsequent DAPI staining.

Neuroblastoma cells grew on the PEDOT/PSS/dye coated electrodes for two days, suggesting that these surfaces are not toxic for at least this short period of time. Fig. 5 (b) shows the cell nuclei staining with DAPI after cell cultivation, indicating a comparable cell density on the control electrodes (PEDOT/PSS/dye and IrO_x) as well as on the chip substrate (Si_xN_y). Comprehensive studies would be necessary to confirm non-toxicity *in vivo*, which is beyond the scope of this proof-of-principle study. As indicated by Fig. 5 (b), released dye molecules are taken up by the cells and the active release led to a distinct labelling of cellular structures. It can also be noticed, that only cells growing directly on top or in the immediate vicinity of the electrodes were labelled with FastDiO. It was observed, that the active cycling of the electrodes leads to a significant release of FastDiO molecules, compared to the passive controls, where dye was actively incorporated into the films but not released by active cycling. Fig. 5 (b) demonstrates the dependency of the extent of released dye molecules on the number of release cycles. The software assisted quantification of the released dye fluorescence intensity on the electrodes is shown in Fig. 5 (c). It is observed that 200 release cycles (E2) led to a significantly higher fluorescence than 100 cycles (E4). The passive release control (E3) shows nearly no fluorescence signal, comparable to the uncoated IrO_x electrode (E1).

IV. Discussion

Functional coatings on microelectrodes, like PEDOT/PSS/FastDiO, offer the possibility to improve neuronal recording quality and simultaneously act as a convenient and precise cell tracing system for implanted electrodes. We demonstrated here that it is possible to actively incorporate the positively charged neurotracer FastDiO by cycling the electrodes over a negative potential

scan range. The active dye release showed that in the case of actively loaded electrodes more dye can be released compared to electrodes that were only immersed into a dye solution [Fig. 3 (a)]. This result correlates well with the theory that the reduction of the positively charged PEDOT backbone drives electrostatic binding of positively charged ions within the polymer film [18]. In accordance with this, we observed that the oxidation of the polymer led to an active release of dye molecules further supporting the electrostatic mechanism. There is a tenfold increase of the concentration of released dye when the electrode is actively driven in comparison to a passive dye leakage [Fig. 3 (a)]. The observation that oxidizing the conducting polymer backbone releases mobile, positively charged molecules was also made by other researchers [19], [20], where dopamine and chlorpromazine were released by using positive electrode potentials. The cyclic voltammogram, used for an active dye release, showed a decrease of the oxidation current by increasing the cycle number (Fig. 5 (a), dashed box). This can be explained with an increased oxidation of the film and the release of dye, but can also be an indicator for partial degradation of the polymer film. Nevertheless, our measurements demonstrate the possibility to electrochemically drive a release process, which is highly beneficial for accomplishing the precise temporal control of released quantities of charged molecules, such as neurotracers.

By introducing a bilayer system with a second PEDOT/PSS layer on top of a dye filled electrode coating, it was possible to decrease passive leakage [Fig. 3 (b)] without preventing an active release of the dye. Similar results were reported previously with the neurotracer DiI [21]. The lipophilic character of the neurotracer is expected to limit its possibility to passively diffuse into the aqueous environment *in vivo*, but it is assumed that the direct contact with a lipophilic membrane is necessary to allow dye to leave the electrode surface. The direct contact of cells with the dye filled PEDOT layer before a defined release in time can thus be prevented using a dye free, but electrically conducting capping layer made for instance of PEDOT/PSS.

Parametric testing showed an increased dye release by applying higher positive potentials [Fig. 4 (b)], confirming the correlation between oxidation rate of the electrode and release of positively charged dye. This is important, considering that it would be advantageous, to use lower potentials and drive lower currents *in vivo*, to avoid electrochemical reactions and damages to the surrounding tissue. Voltage ranges between −0.2 and 0.2 V already released significant amounts of dye, which might be sufficient to label cells *in vivo*. Longer release times also allowed more dye to be released, suggesting that a narrower sweep range can be compensated for by increasing the release time.

Stability tests showed that dye can be released from loaded electrodes even after four weeks storage in cell culture medium at 37 °C [Fig. 4 (c)]. The conducting polymer can still be electrochemically oxidized and fluorescent dye can be released in similar concentrations as from freshly prepared samples. Mobility and functionality of the dye seem to withstand long-term storage in an aqueous environment and excessive leakage over this time period is not expected, as the concentration of actively released molecules is not reduced. We thus assume that the system maintains its functionality during long-term implantations *in vivo*.

Fig. 4 (a) demonstrated that the electrode impedance is lowered significantly by coating IrO_x electrodes with PEDOT/PSS at a minimal shift of the cut-off frequency from 5 Hz to 1 Hz. Even the incorporation of lipophilic tracer molecules did not negatively influence this property, which plays an important role for signal-to-noise ratio in electrophysiological recordings *in vivo*.

A neuroblastoma cell line was successfully cultured on the test chip with PEDOT coated IrO_x electrodes. Neuroblastoma cells perfectly adhered to the coated electrodes. It was furthermore confirmed that FastDiO was actively and controllably released from the electrodes. We did not observe any negative effect of the system, on the cultured cells, as also reported by Nyberg [15] for pure PEDOT/PSS systems. The identification of recording sites *in vivo* would also benefit from an improved adherence of cells to the electrode material. Since lipophilic dyes do not readily diffuse across an aqueous gap between an electrode surface and the phospholipid bilayer of the cell membrane, dye uptake is improved by direct contact between the cell membrane and a release surface.

The *in vitro* experiment with the neuroblastoma cells showed that cells on top of and in close proximity to the electrodes incorporated and accumulated the released dye and could thereby be traced using fluorescence microscopy. The passive leakage control shown in Fig. 5 (b) indicates barely detectable dye leakage, further supporting the concept that passive leakage can be effectively blocked by using a capping layer. The amount of released dye can actively be tuned by varying the number of voltage cycles, and the used potential range applied to the electrodes, demonstrating the precise control opportunity offered by this new concept.

It is obvious that individual layers can be further optimized in terms of releasable dye amount and type of dye to be loaded to the electrode coating. Future work comprises long-term studies in mouse hippocampus over four weeks *in vivo*, with simultaneous recording of local field potentials and multi-unit activity, to evaluate the systems suitability as recording electrode.

V. Conclusion

We demonstrated here the successful implementation of a dye delivery system on recording electrodes, based on the storage in and delivery from the recording electrode itself. Fluorescent dye molecules were selectively incorporated into a conducting PEDOT/PSS coating deposited on top of IrO_x electrodes. It was demonstrated, that the system can act as a dye storage layer and support controllable dye delivery over time frames of weeks. The electrical impedance of recording electrodes was reduced, making the system highly potent for neuronal recording applications employing microelectrodes. To prove the biological applicability of this release concept, experiments were performed in a neuroblastoma cell culture, where neuronal cells were precisely and controllably labelled with the neurotracer FastDiO.

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