Journal of
Materials Chemistry B

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This *Accepted Manuscript* will be replaced by the edited and formatted *Advance Article* as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about *Accepted Manuscripts* can be found in the **Information for Authors**.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard **Terms & Conditions** and the **ethical guidelines** that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these *Accepted Manuscript* manuscripts or any consequences arising from the use of any information contained in them.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A facile biofunctionalisation route for solution processable conducting polymer devices

Xenofon Strakosas[a], Michele Sessolo[a], Adel Hama[a], Jonathan Rivnay[a] Eleni Stavrinidou[a], George G. Malliaras[a], and Roisin M. Owens[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Abstract

For the majority of biosensors or biomedical devices, immobilization of the biorecognition element is a critical step for device function. To achieve longer lifetime devices and controllable functionalization, covalent immobilisation techniques are preferred over passive adhesion and electrostatic interactions. The rapidly emerging field of organic bioelectronics uses conducting polymers (or small molecules), as the active materials for transduction of the biological signal to an electronic one. While a number of techniques have been utilized to entrap or functionalize conducting polymers deposited by electro- or vapor phase polymerization, covalent functionalization of solution processed films, essential for realizing low cost or high throughput fabrication, has not been thoroughly investigated. In this study we show a versatile biofunctionalization technique for the solution processable conducting polymer (poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) PEDOT:PSS, which is a commercially available material, and has a record high conductivity. Addition of poly(vinyl alcohol) (PVA) into solution with PEDOT:PSS provides a handle for subsequent silanization with a well-characterised silane reagent, allowing for covalent linkage of biological moieties onto PEDOT:PSS films after curing of the films. We show homogenous and large-scale biofunctionalization with polypeptides and proteins, as well as maintenance of the biological functionalities of the proteins. In addition, no deleterious effects are noted on the electronic or ionic transport properties of the conducting polymer films due to incorporation of the PVA.

Introduction

Biofunctionalization is an essential step in the fabrication of a biosensor or biomedical device. For biosensor operation, the so-called 'biorecognition element' is usually immobilized on the active area of the transducer in order for the target molecule to be captured and for signal transduction to occur. For biomedical devices, active areas are increasingly being coated or functionalized with biomolecules to improve biocompatibility and facilitate integration with living systems for both *in vitro* and *in vivo* applications. A variety of methods such as passive adhesion and electrostatic interactions may be used, however for longer lifetime and better control of immobilisation, covalent interactions are preferable. For traditional electronic materials such as silicon or metal electrodes, a variety of covalent functionalization methods have been developed ranging from silanization, thiolation and the ubiquitous EDC/NHS chemistry for coupling of the commonly found biological amine and carboxyl functional groups[1, 2].

[a] *Department of Bioelectronics, Ecole Nationale Supérieure des Mines, CMP-EMSE, MOC, 13541, France. E-mail: owens@emse.fr*

† Electronic Supplementary Information (ESI) available: Incorporation of PEG into PEDOT :PSS films**. (a)** Conductivity measurements of PEDOT:PSS:PEG films. **(b)** Biofunctionalization of PEDOT:PSS:PEG films with FITC-PLL at 0, 10 and 25%. Quantitation of fluorescence intensity of FITC-PLL functionalised PEDOT:PSS:PVA films.

A rapidly emerging area of research in bioelectronics is the use of conducting polymers (CPs) as an alternative to traditional electronic materials. The field of organic bioelectronics has gained tremendous momentum in the past decade[3, 4]. In particular, the use of conducting polymers (CPs) to interface with biology has been shown to be advantageous for numerous reasons including potential low cost of the materials, mechanical flexibility, mixed electronic/ionic conductivity, improved interfaces and tunability of the materials due to their organic nature[5, 6]. Applications for organic electronic devices in biology have ranged from relatively simple biosensing [7, 8] to more complex applications for use in drug delivery[9], neurotransmitter delivery[10], toxicology[11], and neural probes[12, 13]. Devices of particular interest in biosensing are organic electrochemical transistors (OECTs) which take advantage of the mixed electronic/ionic conductivity of CPs, in order to achieve efficient ion-to-electron transduction.

The polythiophene PEDOT doped with the water dispersible polystyrene sulfonate (PSS) or with the tosylate anion (TOS), is currently the most commonly used conducting polymer in organic bioelectronic applications. This is due to a combination of factors including good thermal, and electrochemical stability, relatively high conductivity[14], and demonstrated biocompatibility[15, 16], compared with other CPs based on polypyrrole and polyaniline. A number of different methods exist for deposition of PEDOT films; solution deposition, electropolymerization, and vapor phase polymerization. Previous work on incorporation of biomolecules into either the bulk or onto the surface of PEDOT has employed the latter techniques, for example, incorporating biomolecules as dopants in the electrolyte solution during polymerization[17, 18]. We recently reported a novel method for incorporation of gelatin into

films of vapor phase polymerised PEDOT doped with tosylate (TOS), maintaining both the electrochemical properties of the conducting polymer, and also the functionality of the biomolecule[19]. A recent study on PEDOT, showed the use of a modified dopant (PSS-maleic acid) could generate films with good electrical performance but also with sufficient carboxyl groups for subsequent biofunctionalization[20]. PEDOT:PSS is often chosen because it is commercially available as a liquid dispersion suitable for solution-based deposition methods such as spin coating and inkjet printing[21, 22]. One disadvantage of the commercially available formulation of PEDOT:PSS is that it is difficult to perform chemistry on an already cured film, and also since the PSS is in excess with respect to the PEDOT[23], commonly targeted chemical modification of PEDOT would lead to inhomogeneous functionalization and disruption of conductivity. The most desirable approach for biofunctionalization is for the biomolecule to be added after film processing steps in order to avoid exposure to solvents or high temperatures that are required during fabrication, but may cause biomolecule denaturation.

Herein we demonstrate the an easy-to-use method for the covalent linkage of biomolecules to films of PEDOT:PSS deposited from commercially available dispersion. We show the successful, large area functionalization of PEDOT:PSS films with different biomolecule types: the enzyme glucose oxidase (GOx) and the extracellular matrix derived polypeptide poly-L-lysine (PLL). We demonstrate that the resulting conducting polymer devices maintain excellent electrical properties. Finally, we show not only the functionality of these immobilized biomolecules, but also applications for the functionalized films.

Materials and methods

Preparation of conducting polymer (CP) films

PEDOT:PSS (Heraeus, Clevios PH 1000) was used as the conducting polymer active layer. Dodecylbenzenesulfonic acid (DBSA) (0.5 µL/mL) and Ethylene glycol (Sigma Aldrich) (50 µL/mL) were added to the PEDOT:PSS dispersion to improve film formation and to increase conductivity. The formulation was sonicated and filtered to avoid aggregates. A stock solution of PVA average M_w 130,000 5 wt% was prepared; heating for ~30s in a microwave oven assisted dissolution until a clear solution was obtained. The amount of PVA added to the solution was calculated according to the mass of PEDOT:PSS. Four different PEDOT:PSS:PVA composite solutions (10, 20, 25, 50 wt%) were made and mixed thoroughly and filtered before deposition. Following PEDOT:PSS:PVA spin - coating, samples were baked for 30 minutes at 140 °C under atmospheric conditions.

Conductivity measurements of conducting polymer films/devices

PEDOT:PSS and PEDOT:PSS:PVA composites were coated onto glass slides which were cleaned by sonication in acetone, isopropyl alcohol, and DI water, followed by a drying step with nitrogen and brief exposure to oxygen plasma. Sheet resistance was measured using Jandel four point probe device. The thicknesses of the films were measured using a Veeco Dektak 150 Profilomer. The conductivity was calculated from the measured sheet resistance and thickness.

Electrochemical transistor fabrication and operation

The fabrication process, similar to that reported previously[24] included the deposition and patterning of gold, parylene, and PEDOT:PSS:PVA. Source/drain contacts were patterned by a lift-off process, using S1813 photoresist, exposed to UV light through a SUSS MBJ4 contact aligner, and developed using MF-26 developer. 5 nm of chromium and 100 nm of gold were subsequently deposited using a metal evaporator, and metal lift-off was carried out in acetone. Metal interconnects and pads were insulated by depositing 2 µm of parylene C using an SCS Labcoater 2, with a silane adhesion promoter. A dilute solution of industrial cleaner (Micro-90) was subsequently spin coated to act as an anti-adhesive for a second, sacrificial 2 µm parylene C film. Samples were subsequently patterned with a 5 µm thick layer of AZ9260 photoresist and AZ developer (AZ Electronic Materials). The patterned areas were opened by reactive ion etching with an oxygen plasma using an Oxford 80 Plasmalab plus. PEDOT:PSS:PVA 25 wt% and PEDOT:PSS + 1 wt% GOPS in solution, usual formulation for *in vivo* applications, were spin coated at 3000 rpm, and baked for 90 sec in 100 ^{o}C. The second layer of parylene was peeled off with a subsequent rinsing in DI water and baking at 140 ^{o}C for 30 min. All characterization was done using a 100 mM NaCl solution in DI water as the electrolyte and a Ag/AgCl wire (Warner Instruments) as the gate electrode. The measurements were performed using a National Instruments PXIe-1062Q system. The channel of the OECT was biased using one channel of a source-measurement unit NI PXIe-4145. The gate voltage was applied using a NI PXI-6289 modular instrument. In the case of the IV-characteristics and transconductance characterization, the drain current was measured using the SMU channel used for the bias. Concerning the time response characterization of the OECT, one NI-PXI-4071 digital multimeter measured drain current, and a channel of the NI PXI-6289 equipment measured gate voltage. All the measurements were triggered through the built-in PXI architecture.

Measurement of ion mobility in PEDOT:PSS:PVA films

Ionic mobility was measured as previously described[25]. Briefly, a PEDOT:PSS:PVA film, deposited on a parylene-coated glass substrate, was coated with a layer of SU-8 serving as an ion barrier. Using photolithography, a well was created in the SU8/PEDOT:PSS stack and filled with an electrolyte, forming a planar junction with an area A of 16 mm. An Ag/AgCl counter electrode was immersed into the electrolyte, and an Au pad provided contact with the conducting polymer composite film, positioned at a distance L = 32 mm from the electrolyte interface. The experiment was carried out by applying a positive bias at the Ag/AgCl electrode while simultaneously measuring the current flow in the device and recording the electrochromic changes associated with the propagation of the dedoping front using an inverted Carl Zeiss Axio Observer Z1, in the bright field mode with 1X objective. We used 10 mM KCl electrolyte in order to measure the mobility of potassium.

Silanization and fluorescence imaging of patterned films

To investigate the distribution of the fluorescence we prepared patterns with PEDOT:PSS and PEDOT:PSS:PVA composites using photolithograpy and the parylene C peel-off technique. Prior to the peel-off, 3-glycidoxypropyltrimethoxysilane (GOPS) was deposited by vapor deposition at 90 ^{o}C for 45 minutes under vacuum. FITC labelled poly-L lysine (PLL), with concentration 50

μg/mL in phosphate buffered saline (PBS) with the addition of 50 mM NaOH final pH 8-9, was drop-cast on to the sample allowed to react for two hours at room temperature. The resulting films were thoroughly cleaned with 0.1 M PBS, 0.1 M NaCl, and finally DI water. Finally, samples were examined with a fluorescent microscope (Carl Zeiss Axio Observer Z1).

Quartz crystal microbalance (QCM)

Glucose oxidase (GOx) immobilization monitoring was undertaken using a Q – sense E4 system. PEDOT:PSS:PVA 25 wt% was spin coated on top of quartz crystal sensors, with additional GOPS deposition as mentioned above. The samples were transferred to a QCM flow module part of the Q-sense E4 device and equilibrated in Di water for 60 min. GOx 50 μg/mL in PBS with 50 mM of NaOH was introduced to the axial flow sample chamber and a constant flow rate of 20 μL/min for 180 min and then rinsed with DI water at a constant rate of 60 μL/min.

Glucose sensing assay using GOx functionalised OECTs

All PEDOT:PSS:PVA 25 wt% transistors were patterned using a parylene peel-off technique, and immobilization of glucose oxidase (1 mg/mL PBS plus NaOH), both of which are described above. 20 mM glucose was prepared in PBS. Due to its hydrophobicity, parylene C served as a virtual well for the drop of the electrolyte. Finally, a platinum wire was immersed in the electrolyte from the top serving as a gate of the transistor. The measurements were recorded by a Keithley 2612A and customized Labview software.

Cell culture

PC12 cells from Health Protection Agency culture collections (Salisbury, UK) were cultured at 37°C, 5% CO_2 in humidified atmosphere. RPMI 1640 media supplemented with 10% horse serum (HS, Invitrogen), 5 % feotal bovine serum (FBS, Invitrogen), 2 mM glutamine (Glutamax™, Invitrogen), 25 U/mL penicillin-streptomycin (PenStrep, Invitrogen), 50 ng/mL gentamicin (Gentamicin, Invitrogen). During maintenance, the cells were cultivated in suspension and numbers were determined by a cell counter (Scepter handheld automated cell counter, Millipore).

Cell patterning using collagen and PLL functionalised conducting polymer films

Patterned substrates (approximate area: 4.9 cm²) were coated with the conducting polymer PEDOT:PSS:PVA 25 wt% concentration according to the procedure described above. Each coated substrate was sterilized for 20 minutes in 70% ethanol, rinsed twice in PBS, and placed in a separate 6-well plate. Cells were seeded at a concentration of 10^5 cells/well. The final volume of each well was 3 mL. Adhesion was evaluated after 24 h. A Calcein AM/Propidium Iodide assay 2 μg/mL was carried out to determine the cell viability (Calcein-AM, Sigma) and Propidium Iodide (Propidium Iodide Solution, Sigma). To perform these tests, media in the well was discarded and the cells gently rinsed two times with PBS. 3 mL of the Calcein-AM/PI mixture was added to each well and incubated for 30 min at 37°C. Fluoresence images were then recorded (Axio Observer Z1, Carl Zeiss, Calcein AM 485 nm/535 nm, PI 530 nm/620 nm). After 24h of culture, the media was changed to discard the cells in suspension using differentiation media: RPMI 1640 supplemented with 1 % (v/v) HS, 2 mM glutamine, 50 ng/mL nerve growth factor (NGF, Sigma), 25 U/mL penicillin-streptomycin, and 50 ng/mL gentamicin. The media was changed every two days until the end experiment.

Results and discussion

As mentioned in the introduction, silanization is a covalent functionalization technique, widely used for glass or metal oxide surfaces[2]. A silane reagent can form a covalent linkage with free hydroxyl groups on the surface via a condensation reaction. The most useful silanization reagents are heterobifunctional, containing an alkoxysilane as well as an organic functional group that can selectively bind a second organic molecule. Different examples of silanization reagents include aminosilanes (such as APTS (3-aminopropyltriethoxysilane)) and glycidoxysilanes such as GOPS, which yield amine and epoxy functional groups respectively, for subsequent linkage to a secondary organic molecule[2]. The lack of hydroxyl groups on polythiophenes such as PEDOT, would appear to rule out this method of biofunctionalization for films or devices made from these polymers. Therefore, we sought to add a hydroxyl containing polymer to solutions of commercially available PEDOT:PSS, that could later be used for biofunctionalizing the films via silanization, but would not cause deleterious effects to the film forming poorly soluble in aqueous solutions, and the polyanion PSS is used as a means to generate an aqueous dispersion that has good film forming properties and high conductivity[26]. One disadvantage of the PEDOT:PSS solution however, is that it is generally considered a finely balanced solution which can be disturbed by addition of extraneous additives

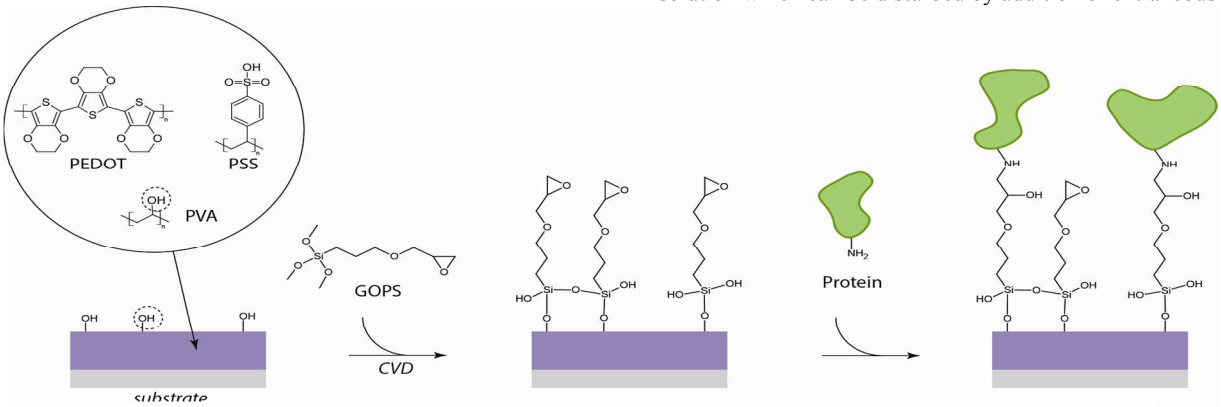

Figure 1. Reaction scheme for biofunctionalization of PEDOT:PSS by incorporation of PVA.

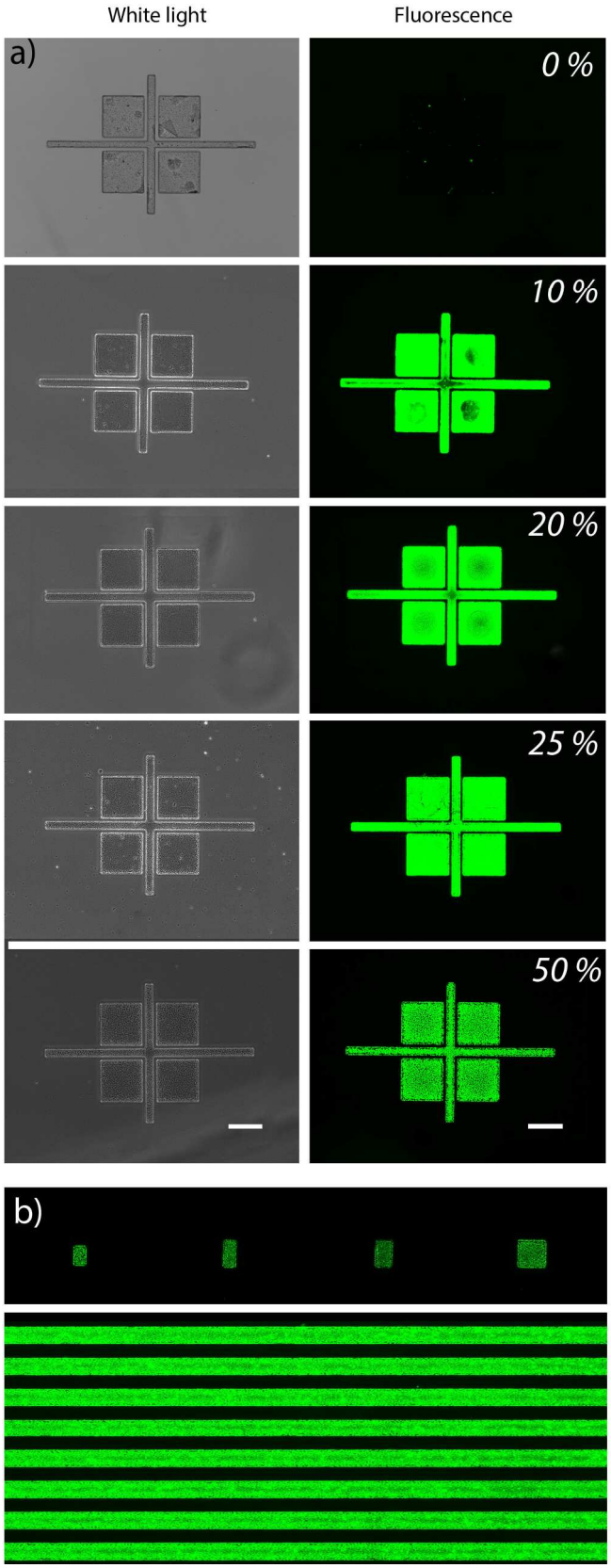

Figure 2 Biofunctionalization of Poly-L-Lysine on PEDOT:PSS:PVA films. **(a)** Different concentrations of PVA added to PEDOT:PSS; 0, 10, 20, 25 and 50 wt% PVA. **(b)** Patterning of FITC-PLL on PEDOT:PSS:PVA films.

or solvents. Nevertheless, several reports have been published on addition of compounds to PEDOT:PSS to enhance properties such as conductivity for application in a variety of device types including solar cells and displays. Additives to PEDOT:PSS include diols, such as ethylene glycol, diethylene glycol, or poly(ethylene glycol) (PEG), where addition to PEDOT:PSS emulsions before spin-coating increases the conductivity of the resultant thin films[27 28 29]. Addition of the sugar alcohol sorbitol is also known to enhance the conductivity of PEDOT:PSS thin films by three orders of magnitude[30]. Given that alcohol containing polymers have often been used as additives for improving conductivity, we decided to incorporate PVA as a handle for subsequent silanization of PEDOT:PSS. The proposed reaction scheme is outlined in Figure 1. The polyalcohol is mixed with the PEDOT:PSS in solution, after which the film is cast and cured. Following curing, GOPS is added by chemical vapour deposition to the film whereupon a condensation reaction occurs and the silane reagent is bonded to the polymer. Subsequent addition of amine containing polypeptides/proteins under basic conditions allows formation of a secondary amine bond with the biomolecule.

In order to estimate the degree of biofunctionalization of PEDOT:PSS films using the above method, we immobilized a FITC-labelled Poly-L-Lysine on a photolithographically defined pattern. Figure 2a shows fluorescence images of the biofunctionalized pattern. Different amounts of PVA were added to the PEDOT:PSS dispersion (in concentrations ranging from 0 to 50 wt%). We observed the most homogeneous and brightest fluorescence (and thus biofunctionalization) for PVA concentrations around 20-25 wt%. High PVA loading (wt50%) resulted in more aggregated fluorescence morphology. It should be noted that the films were stringently washed first with PBS, and then with a high concentration salt solution (0.1 M NaCl) and finally with deionised water to remove any non-specific or electrostatically bound polypeptide, thus making sure that the remaining polypeptide is covalently linked to the conducting polymer. In addition, the absence of fluorescent label on the 0 wt% PVA sample indicates that the PVA is necessary for the binding of the polypeptide to the surface, and that GOPS does not react with the PEDOT:PSS film or the Parylene C. The same procedure was attempted with PEG, however the patterning obtained with PEDOT:PSS:PEG was less homogenous and showed some evidence of aggregation or phase separation (Supplementary Figure 1b). The optimal concentration of PVA (25 wt%) was used to functionalise additional patterns as shown in Figure 2b.

We measured ion mobility in the PEDOT:PSS:PVA 25 wt% using planar junctions between the polymer and the KCl electrolyte. When a positive voltage is applied in the electrolyte with respect to the film, potassium ions are injected from the electrolyte into the film and at the same time holes are extracted from the film through a Au electrode in order to preserve electroneutrality in the film. The film becomes dedoped and due to an electrochromic effect the color changes, allowing monitoring of the optical transmission of the film. From the change of the optical transmitted intensity with respect to the initial state we define the drift length of ions at each time. The one dimensional geometry, where ions and holes move in the same direction, allows for the determination of ion mobility using the relationship $l = 2 \cdot \mu \cdot V \cdot t$, where l is the drift length of ions, μ is the ion mobility through the film and V is the applied voltage. PEDOT:PSS:PVA has a slightly improved K^+ mobility,

$5.3*10^{-4}$ cm^2/Vs compared to PEDOT:PSS+GOPS(1 wt%) which is $1.9*10^{-4}$ cm^2/Vs[25].

An important consideration when adding compounds to conducting polymers which will later be used in devices, is that the electrical characteristics of the films remain unchanged. The conductivity of a range of different concentrations of PVA into PEDOT:PSS was measured (Figure 3a). The conductivity of the 25 wt% PVA sample remained high, within 10% of the PEDOT:PSS sample. Unlike addition of polymers such as PEG to PEDOT:TOS via vapor phase polymerization[31, 32], addition of PVA did not enhance conductivity. A similar result was shown with addition of PEG to PEDOT:PSS (Supplementary Figure 1a). Previous studies have reported the combination of PVA with PEDOT:PSS for a variety of purposes: for example taking advantage of increased mechanical flexibility in free standing films of PEDOT:PSS:PVA films[33] and for preparation of conducting nanofibers[34, 35]. Reynolds and co-workers showed increases in conductivity upon addition of PVA to PEDOT:PSS but with maximum reported conductivity of 1 S/cm. Addition of polymers such as PEG and PVA to PEDOT:TOS most likely induce significant morphological rearrangements resulting in concurrent improvements in conductivity[31, 36], however addition of such polymers to already highly conducting PEDOT:PSS solutions would appear to have merely a dilution effect after a certain point. It should be noted that we routinely add ethyleneglycol (EG) to improve conductivity of PEDOT:PSS films.

In order to test both electronic and electrochemical properties of PEDOT:PSS:PVA blends, they were used as active materials in OECTs The organic electrochemical transistor (OECT) is a device which has been used by a number of groups in a variety of different biosensing applications. The geometry of the OECT device varies considerably depending on the particular application. In certain cases, e.g. for applications for neural probes *in vivo* very fast transistors are required. We demonstrate the successful employment of PVA modified PEDOT:PSS films as the channel in an OECT (channel dimensions: 10 µm width and 5 µm length) (Figure 3). Since the devices compared have the same channel dimensions, and are spun under the same conditions, direct comparison of transistor parameters allows for elucidation of the added effect of PVA in an OECT. Here we measure average transconductance values of 1.86 and 1.59 mS for devices of PEDOT:PSS:PVA 25 wt% and PEDOT:PSS+GOPS 1 wt% in solution respectively. The latter is a typical formulation for stable high performance OECTs used *in vivo,* and achieves a tranconductance similar to that previously reported[24]. The response times to a constant gate voltage pulse, τ, (measured with an exponential fit) also compare favourably. PEDOT:PSS:PVA results in a τ of 20.4 µs compared to 27.5 µs for PEDOT:PSS+GOPS 1 wt% when a gate pulse of V_G = 0.6 V is applied. The slight enhancement in response time with the addition of PVA is likely related to the trend in ion mobility reported above.

A second relevant test of the effectiveness of this biofunctionalization method was the immobilization of the enzyme Glucose oxidase (GOx) on PEDOT:PSS:PVA films for development of a glucose sensor. To verify the immobilization of GOx onto PEDOT:PSS:PVA 25 wt% we monitored the binding and the stability of the enzyme with QCM. A thin film was deposited onto a quartz crystal sensor, where frequency is inversely proportional to the mass of the film. As we introduce enzyme to the

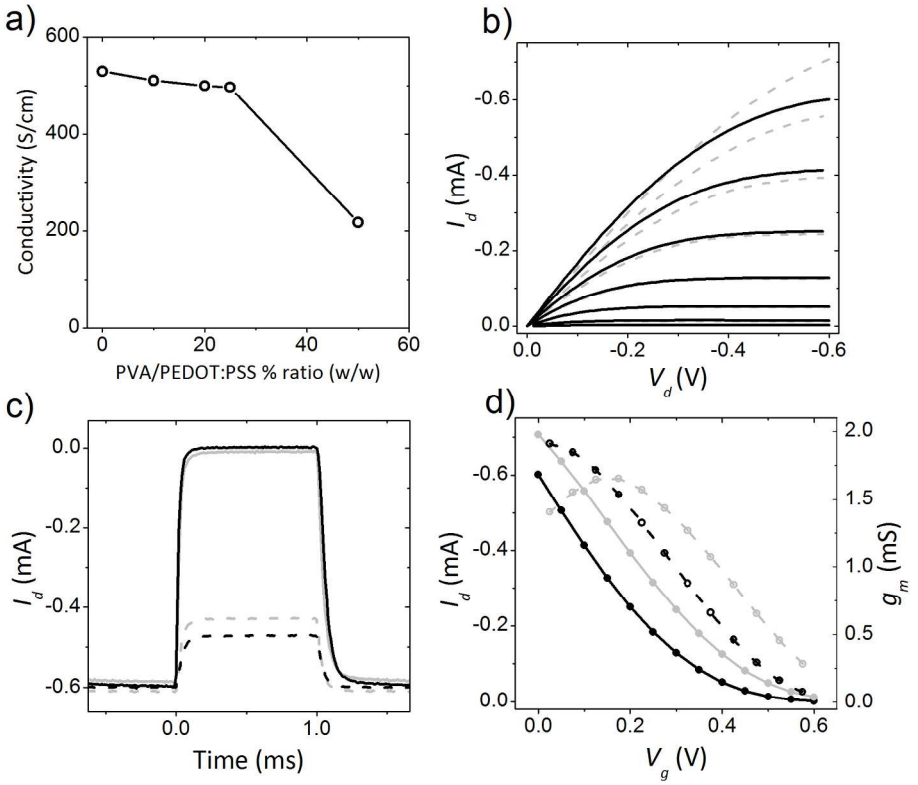

Figure 3. (a) Effect of different concentrations of PVA on PEDOT:PSS film conductivity.**(b), (c)** and **(d)** Electrical characterization of OECTs prepared with either PEDOT:PSS+PVA(25%) (black), or PEDOT:PSS+GOPS (grey). **(b)** Output IV curves for Vg=0-0.6V. **(c)** Time response of device in response to a Vg=0.1V (dashed), and Vg=0.6V (solid) square pulse. **(d)** Corresponding transfer curves (solid line/symbols), and transconductance, gm, dashed line/open symbols.

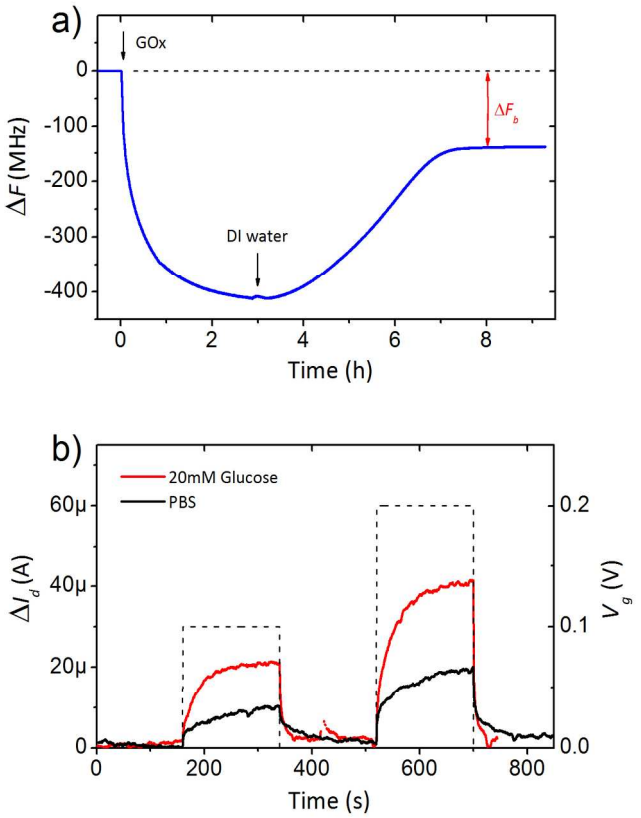

Figure 4. GOx functionalized PEDOT:PSS:PVA. **(a)** QCM monitoring of GOx deposition on PEDOT:PSS:PVA films. **(b)** Sensing of glucose with OECT, V_d=-0.4V

cell (Figure 4a), we see a fast drop of the vibration frequency with a subsequent slow stabilization, which indicates interactions between the polymer and the enzyme, resulting in an increase in the mass of the polymer. However, after introducing deionised water to the chamber as a rinsing step, the film loses mass until it stabilizes at a higher frequency value, which implies the removal of weakly bonded enzyme. In the frequency scan, the difference between the starting value and the value after washing Δf_b can be attributed to the presence of enzyme immobilised on the surface. The use of the OECT as a glucose sensor has previously been demonstrated by a number of groups, however with the GOx immobilised on a Pt gate electrode [37]. Here, we demonstrate the operation of GOx functionalized PEDOT:PSS:PVA OECTs (Figure 4b). It should be noted that in contrast to the OECTs characterized in the preceding section, the OECTs developed for glucose sensing require significantly different geometry, with the conducting polymer channel area many-fold greater than that of the gate electrode. This has been described in detail elsewhere[38, 39], along with the mechanism of the OECT for glucose sensing[40]. In this case a Pt gate electrode was used to avoid the necessity of adding a redox mediator[7]. The dimensions of the device used here were 1 cm wide and 1 mm long. Additionally, the gate area was selected such that the ratio of the gate area to the channel area was < 1/10. Glucose was added, and a substantial increase in the modulation of the source-drain current was observed. Despite the limited amount of GOx immobilized on the surface (compared to experiments where the enzyme is present in solution) a clear difference is observed upon addition of glucose, compared to PBS for different values of gate voltage (Figure 4b). The successful operation of the OECT suggests that the enzyme is properly

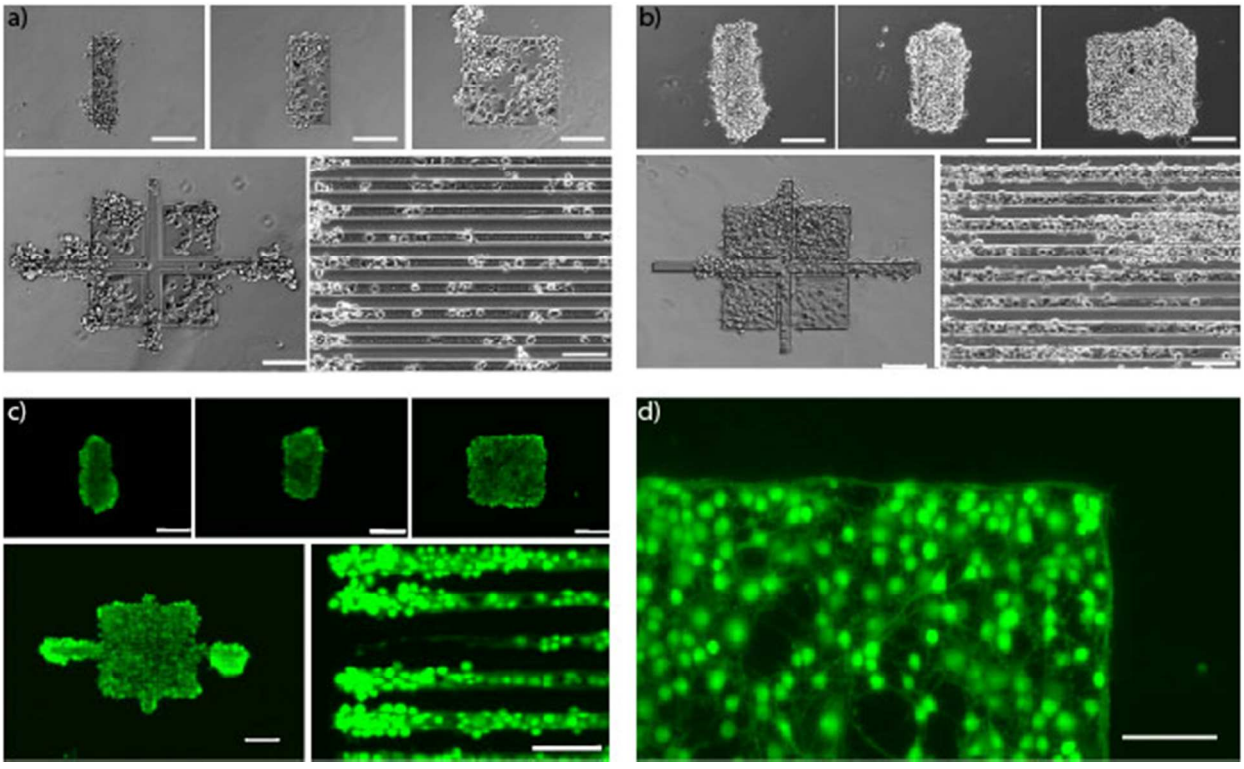

Figure 5. Growth and differentiation of PC12 cells on PLL functionalized PEDOT:PSS:PVA patterns.**(a)** Optical micrograph of PC12 cells 1 day after seeding.**(b)** Optical micrograph of PC12 cells 5 days after seeding. **(c)** Fluorescence imaging of live (green) /dead (red) stained of PC12 cells 5 days after seeding on PEDOT:PSS:PVA patterns. **(d)** Differentiation of PC12 cells on PEDOT:PSS:PVA pattern. Scale bars are 100 μm for all the panels.

immobilized, and that biological activity of the enzyme is maintained.

Conducting polymer devices have shown great potential for use in devices integrated with living cells. The ability to pattern cells or cell-networks is advantageous for a number of applications, for example controlling the growth and connectivity of neurons for studies of artificial neuronal networks. PEDOT:PSS and PEDOT:TOS have been used as simple electrode coatings, but also as active materials for controlling cell adhesion[41-43], proliferation[44, 45] or migration[46, 47]. Improving the interface by coating with proteins such as extracellular matrix components can have advantages for biocompatibility and may be essential in *in vivo* applications for avoiding rejection of implants by tissue[48, 49]. Examples include multielectrode arrays [50] and biosensors for toxicology[11, 51]. Although a variety of methods exist for coating active surfaces, as mentioned above, covalent linkage of biomolecules offers gains in terms of stability and control of binding. Figure 5 shows micron-scale patterning of PEDOT:PSS:PVA 25 wt% on glass slides which were subsequently seeded with PC12 cells, a cell line which is often used as a model for neuronal differentiation. Figure 5a shows optical micrographs of PLL patterned surfaces with PC12 cells seeded at Day 1, illustrating an excellent confinement of the cells to the patterned PEDOT:PSS:PVA. The same pattern is shown with cells 5 days after differentiation, demonstrating maintenance of the pattern (Figure 5b). A live/dead cell staining assay was performed on PC12 cells grown on the patterns for 5 days, and confirms that the cells are alive and that the PEDOT:PSS:PVA material is biocompatible for this application and duration (Figure 5c). A close-up of one of the patterned areas from the same samples reveals connections formed between individual cells on the pattern, demonstrating that this functionality of the cells is also maintained (Figure 5d).

Conclusions

We have demonstrated an easy-to-use, novel biofunctionalization method that is compatible with solution based processing of PEDOT:PSS films and devices. The method involves modification of the existing method for preparation of PEDOT:PSS films in three simple steps: 1. addition of a polyalcohol into the CP solution before film casting, 2. chemical vapor deposition of a silane reagent onto the cast film, and 3. addition of biomolecule. This method can therefore be easily incorporated into existing device preparation schemes, with the major advantage that the biomolecule is added after film preparation, meaning that it will not undergo denaturation due to harsh processing conditions such as high temperature and presence of solvents. We conclusively demonstrate a high-degree of biofunctionalization and demonstrate in two different applications that the biofunctionality of these molecules is maintained. Further we demonstrate that the conductivity is only negligibly changed, and that devices made from the mix of PVA with PEDOT:PSS have comparable performance to PEDOT:PSS with 1% GOPS devices used for *in vivo* applications. Future work will include more in-depth studies of different polyalcohols and silane reagents, as well as studies on functionalizing within the bulk of the conducting polymer to facilitate direct interactions between biomolecules and CPs.

Acknowledgements

R.O. acknowledges funding from the European Research Council (ERC grant agreement no. 258966), and a Cifre grant from ANR-T to X.S. M.S. and J.R. acknowledge the support of Marie Curie post-doctoral research fellowships. The authors would like to thank Pierre Leleux for fruitful advice and discussions and Paul Molino from University of Wollongong for advice pertaining to the QCM experiments.

References

1. N. Nakajima and Y. Ikada, *Bioconjugate Chem*, 1995, **6**, 123-130.
2. G. Hermanson, *Bioconjugate techniques*, Academic Press, San Diego, 1996.
3. M. Berggren and A. Richter-Dahlfors, *Adv. Mater.*, 2007, **19**, 3201-3213.
4. R. M. Owens and G. G. Malliaras, *Mrs Bull*, 2010, **35**, 449-456.
5. L. H. Jimison, J. Rivnay and R. M. Owens, in *Org Electron*, Wiley-VCH Verlag GmbH & Co. KGaA, 2013, pp. 27-67.
6. J. Rivnay, R. M. Owens and G. G. Malliaras, *Chem Mater*, 2013.
7. N. Y. Shim, D. A. Bernards, D. J. Macaya, J. A. DeFranco, M. Nikolou, R. M. Owens and G. G. Malliaras, *Sensors-Basel*, 2009, **9**, 9896-9902.
8. F. Yan, S. M. Mok, J. J. Yu, H. L. W. Chan and M. Yang, *Biosens. Bioelectron.*, 2009, **24**, 1241-1245.
9. M. R. Abidian, D. H. Kim and D. C. Martin, *Adv. Mater.*, 2006, **18**, 405-+.
10. D. T. Simon, S. Kurup, K. C. Larsson, R. Hori, K. Tybrandt, M. Goiny, E. H. Jager, M. Berggren, B. Canlon and A. Richter-Dahlfors, *Nature Materials*, 2009, **8**, 742-746.
11. L. H. Jimison, S. A. Tria, D. Khodagholy, M. Gurfinkel, E. Lanzarini, A. Hama, G. G. Malliaras and R. M. Owens, *Adv. Mater.*, 2012, **24**, 5919-5923.
12. D. Khodagholy, T. Doublet, P. Quilichini, M. Gurfinkel, P. Leleux, A. Ghestem, E. Ismailova, T. Herve, S. Sanaur, C. Bernard and G. G. Malliaras, *Nat Commun*, 2013, **4**, 1575.
13. D. H. Kim, J. A. Wiler, D. J. Anderson, D. R. Kipke and D. C. Martin, *Acta Biomaterialia*, 2010, **6**, 57-62.
14. N. K. Guimard, N. Gomez and C. E. Schmidt, *Progress in Polymer Science*, 2007, **32**, 876-921.
15. E. Ismailova, T. Doublet, D. Khodagholy, P. Quilichini, A. Ghestem, S. Y. Yang, C. Bernard and G. G. Malliaras, *Int J Nanotechnol*, 2012, **9**, 517-528.
16. S. M. Richardson-Burns, J. L. Hendricks and D. C. Martin, *Journal of Neural Engineering*, 2007, **4**, L6-L13.
17. M. L. M. Asplund, E. M. Thaning, T. A. Nyberg, O. W. Inganas and H. von Hoist, *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, 2010, **93B**, 407-415.
18. M. Asplund, H. von Holst and O. Inganas, *Biointerphases*, 2008, **3**, 83-93.
19. M. Bongo, O. Winther-Jensen, S. Himmelberger, X. Strakosas, M. Ramuz, A. Hama, E. Stavrinidou, G. G. Malliaras, A. Salleo, B. Winther-Jensen and R. M. Owens, *J Mater Chem B*, 2013, **1**, 3860-3867.
20. J. E. Collazos-Castro, G. R. Hernández-Labrado, J. L. Polo and C. García-Rama, *Biomaterials*, 2013, **34**, 3603-3617.

21. D. Nilsson, T. Kugler, P. O. Svensson and M. Berggren, *Sensors and Actuators B-Chemical*, 2002, **86**, 193-197.
22. P. Andersson, D. Nilsson, P. O. Svensson, M. X. Chen, A. Malmstrom, T. Remonen, T. Kugler and M. Berggren, *Adv. Mater.*, 2002, **14**, 1460-+.
23. E. Stavrinidou, P. Leleux, H. Rajaona, M. Fiocchi, S. Sanaur and G. G. Malliaras, *J Appl Phys*, 2013, **113**.
24. D. Khodagholy, J. Rivnay, M. Sessolo, M. Gurfinkel, P. Leleux, L. H. Jimison, E. Stavrinidou, T. Herve, S. Sanaur, R. M. Owens and G. G. Malliaras, *Nat Commun*, 2013, **4**, 2133.
25. E. Stavrinidou, P. Leleux, H. Rajaona, D. Khodagholy, J. Rivnay, M. Lindau, S. Sanaur and G. G. Malliaras, *Adv. Mater.*, 2013, **25**, 4488-4493.
26. M. Fabretto, C. Hall, T. Vaithianathan, P. C. Innis, J. Mazurkiewicz, G. G. Wallace and P. Murphy, *Thin Solid Films*, 2008, **516**, 7828-7835.
27. S. Xiong, L. Zhang and X. Lu, *Polymer Bulletin*, 2013, **70**, 237-247.
28. B. Y. Ouyang, C. W. Chi, F. C. Chen, Q. F. Xi and Y. Yang, *Adv. Funct. Mater.*, 2005, **15**, 203-208.
29. D. A. Mengistie, P.-C. Wang and C.-W. Chu, *Journal of Materials Chemistry A*, 2013, **1**, 9907-9915.
30. A. M. Nardes, M. Kemerink, M. M. de Kok, E. Vinken, K. Maturova and R. A. J. Janssen, *Org Electron*, 2008, **9**, 727-734.
31. L. H. Jimison, A. Hama, X. Strakosas, V. Armel, D. Khodagholy, E. Ismailova, G. G. Malliaras, B. Winther-Jensen and R. M. Owens, *Journal of Materials Chemistry*, 2012, **22**, 19498-19505.
32. M. Fabretto, C. Jariego-Moncunill, J.-P. Autere, A. Michelmore, R. D. Short and P. Murphy, *Polymer*, 2011, **52**, 1725-1730.
33. C.-h. Chen, A. Torrents, L. Kulinsky, R. D. Nelson, M. J. Madou, L. Valdevit and J. C. LaRue, *Synthetic Met*, 2011, **161**, 2259-2267.
34. N. Liu, G. Fang, J. Wan, H. Zhou, H. Long and X. Zhao, *Journal of Materials Chemistry*, 2011, **21**, 18962-18966.
35. D. Cho, N. Hoepker and M. W. Frey, *Materials Letters*, 2012, **68**, 293-295.
36. K. Zuber, M. Fabretto, C. Hall and P. Murphy, *Macromol Rapid Comm*, 2008, **29**, 1503-1508.
37. H. Tang, F. Yan, P. Lin, J. Xu and H. L. W. Chan, *Adv. Funct. Mater.*, 2011, **21**, 2264-2272.
38. G. Tarabella, C. Santato, S. Y. Yang, S. Iannotta, G. G. Malliaras and F. Cicoira, *Appl Phys Lett*, 2010, **97**, 123304.
39. F. Cicoira, M. Sessolo, O. Yaghmazadeh, J. A. DeFranco, S. Y. Yang and G. G. Malliaras, *Adv. Mater.*, 2010, **22**, 1012-+.
40. S. Y. Yang, J. A. DeFranco, Y. A. Sylvester, T. J. Gobert, D. J. Macaya, R. M. Owens and G. G. Malliaras, *Lab on a Chip*, 2009, **9**, 704-708.
41. A. M. D. Wan, D. J. Brooks, A. Gumus, C. Fischbach and G. G. Malliaras, *Chemical Communications*, 2009, 5278-5280.
42. K. Svennersten, M. H. Bolin, E. W. Jager, M. Berggren and A. Richter-Dahlfors, *Biomaterials*, 2009, **30**, 6257-6264.
43. K. Svennersten, M. Berggren, A. Richter-Dahlfors and E. W. H. Jager, *Lab on a Chip*, 2011, **11**, 3287-3293.
44. J. Y. Lee, J. W. Lee and C. E. Schmidt, *Journal of the Royal Society Interface*, 2009, **6**, 801-810.
45. J. W. Lee, F. Serna, J. Nickels and C. E. Schmidt, *Biomacromolecules*, 2006, **7**, 1692-1695.
46. B. C. Thompson, R. T. Richardson, S. E. Moulton, A. J. Evans, S. O'Leary, G. M. Clark and G. G. Wallace, *J Control Release*, 2010, **141**, 161-167.
47. A. Gumus, J. P. Califano, A. M. D. Wan, J. Huynh, C. A. Reinhart-King and G. G. Malliaras, *Soft Matter*, 2010, **6**, 5138-5142.
48. L. K. Povlich, J. C. Cho, M. K. Leach, J. M. Corey, J. Kim and D. C. Martin, *Biochimica et biophysica acta*, 2013, **1830**, 4288-4293.
49. J. A. Chikar, J. L. Hendricks, S. M. Richardson-Burns, Y. Raphael, B. E. Pfingst and D. C. Martin, *Biomaterials*, 2012, **33**, 1982-1990.
50. M. Sessolo, D. Khodagholy, J. Rivnay, F. Maddalena, M. Gleyzes, E. Steidl, B. Buisson and G. G. Malliaras, *Adv Mater*, 2013, **25**, 2135-2139.
51. C. Yao, C. Xie, P. Lin, F. Yan, P. Huang and I. M. Hsing, *Adv Mater*, 2013, n/a-n/a.