Journal of Materials Chemistry

Cite this: J. Mater. Chem., 2012, 22, 19498

www.rsc.org/materials

PAPER

PEDOT:TOS with PEG: a biofunctional surface with improved electronic characteristics†

Leslie H. Jimison,[a] Adel Hama,[a] Xenofon Strakosas,[a] Vanessa Armel,[b] Dion Khodagholy,[a] Esma Ismailova,[a] George G. Malliaras,[a] Bjorn Winther-Jensen[b] and Róisín M. Owens*[a]

Received 7th April 2012, Accepted 18th May 2012
DOI: 10.1039/c2jm32188b

Devices based on conducting polymers offer great promise for interfacing with cells. Here, we use vapour phase polymerisation to create a biofunctional composite material of the conducting polymer poly(3,4-ethylenedioxythiophene):tosylate (PEDOT:TOS) and the biologically relevant poly(ethylene glycol) (PEG). On the addition of PEG, electroactivity of the PEDOT is maintained, conductivity is increased, and its performance as the active material in a transistor is unaffected. Both direct and indirect biocompatibility tests prove that PEDOT:TOS and PEDOT:TOS:PEG are biocompatible and nontoxic to mammalian cells. A functionalised PEG (PEG(COOH)) was additionally introduced into PEDOT:TOS to showcase the potential of this material for use in applications requiring biofunctionalisation.

Introduction

Conducting polymers discovered in the 1970s[1] have shown widespread promise for use as electrically active materials at the interface with biology. The significant rise in the use of conducting polymers in biomedical and bioengineering applications is due to inherent properties that make these materials ideal for interfacing with biological systems.[2] The 'soft' nature of conducting polymers assures compatibility with flexible substrates, and allows for good mechanical matching with delicate biological tissues. The unique ability of organic electronic materials to conduct ions, in addition to electrons and holes, facilitates their communication with biological systems, which rely heavily on ion fluxes. Furthermore, as van der Waals bonded solids, these materials are capable of forming ideal interfaces with electrolytes, without disruptive dangling bonds or oxides, leading to improved electronic sensitivity and reduced noise. Finally, the ability to chemically tune both molecular architecture and film microstructure allows for significant flexibility when optimising materials for a specific application.

There exist many examples of conducting polymers in contact with mammalian cells for applications in tissue engineering.[3] The bulk of the literature has focused on the relatively passive use of conducting polymers as coating materials for interface optimisation.[4–8] Recent applications have exploited the unique attributes of conducting polymers to realise new devices with novel capabilities. Smart conducting polymer surfaces can be used to control cell adhesion, proliferation and migration.[9–11] Organic electronic ion pumps (OEIPs) that can precisely control the flow of ions have been used to deliver neurotransmitters in the inner ear of a guinea pig.[12–14] The organic electrochemical transistor (OECT), first introduced in 1984,[15] has proven to be an extremely versatile device for interfacing with biological systems.[16] OECTs are being developed for a variety of biosensing applications, including ion detection,[17,18] enzymatic sensing[19–21] and whole cell sensing.[22]

The vast majority of OECTs are fabricated from poly(3,4-ethylenedioxythiophene) (PEDOT). This semiconducting polymer is degenerately p-type doped and rendered conducting with negative dopant ions, which stabilize positive holes on the conjugated backbone and balance overall charge. Common dopants include poly(styrenesulfonate) (PSS) and the tosylate anion (TOS). PEDOT doped with either PSS or TOS has shown considerable promise with respect to electrical properties, biocompatibility, and film stability and are therefore appropriate for applications in biological interfacing.

In order to further improve the material–tissue interface, there is a concerted effort to add increased biofunctionality by incorporating bioactive species into the conducting polymer. Such additives can include peptides and proteins for the purpose of encouraging cell adhesion[23–26] and directing axon growth[27] or enzymes for the purpose of bactericidal properties

[a]*Department of Bioelectronics, Ecole Nationale Supérieure des Mines, CMP-EMSE, MOC, 13541, France. E-mail: owens@emse.fr*
[b]*Materials Engineering, Monash University Clayton, 3800 Victoria, Australia. E-mail: bjorn.winther-jensen@monash.edu*

† Electronic supplementary information (ESI) available: Optical micrographs of PEDOT:TOS with and without PEG, revealing the suppression of μ-scale domains in the presence of PEG. See DOI: 10.1039/c2jm32188b

or sensing capabilities.[28] The bioactive species can be incorporated into the conducting polymer film in a number of ways. A commonly used method is to add the desired species to the electrolyte solution during electropolymerisation.[29] In this way, the bioactive species are entrapped in the conducting polymer films during growth, and often serve as a dopant or co-dopant. However, bulky biochemical molecules can have a disruptive effect on the final film. The incorporation of a neural growth factor with ligands into PEDOT films *via* electropolymerisation has been shown to result in both decreased electroactivity and poor mechanical properties,[30,31] attributed to the change in the polymerisation rate upon the addition of the biomolecules. Similarly, while the incorporation of hyaluronic acid (HA) into polypyrrole (Ppy) *via* electropolymerisation was found to encourage angiogenesis, the resulting composite film was brittle and conductivity was reduced by four orders of magnitude.[27] An alternative to molecular entrapment is to employ covalent tethering of the bioactive species to the semiconducting polymer using carboxylic acid ligand binding. For this purpose, films of Ppy with carboxylic acid functional groups, Ppy(COOH), were electropolymerised.[32] However, along with increasing functionality, Ppy(COOH) was shown to have a slightly lower doping level and drastically lower conductivity compared to Ppy. The degradation in electrical properties was attributed to the decrease in the polymerisation rate and disruption of the PPy molecular planarity caused by the additional functional group.

Enhancement of the CP–tissue interface will allow for more stable, reliable and functional bioelectronic materials. However, this improvement should not come at the cost of electronic performance. Ideally, the electronic properties of a conducting polymer should be maintained, or even better, *improved*, on addition of the bioactive species. Vapour phase polymerisation (VPP) is an *in situ* polymerisation technique, in which an oxidant is used to coat a surface on which the EDOT monomer is then sourced, leading to the formation of a conducting polymer film.[33] This simple technique lends itself well to the incorporation of various additives to the conducting polymer film. Recent studies have demonstrated that mixing other molecules with the oxidant results in composite films, in which the additive is intimately incorporated in the conducting PEDOT matrix.[34–36] Moreover, a correctly chosen additive can serve to enhance the microstructure and electronic properties of the CP. In this study, we show that on addition of PEG to PEDOT:TOS, electroactivity is maintained and the conductivity is improved. OECTs were fabricated with the composite films, and modulation was identical compared to neat PEDOT:TOS films. PEG is a biocompatible material often used in tissue engineering applications, whose alcohol groups can be readily activated for subsequent functionalisation of bioactive species.[37] The incorporation of PEG into PEDOT:TOS films provides a means to achieve specific binding of a bioactive species, without negatively affecting the conducting polymer microstructure or electronic structure. In this approach, the protein is introduced after film fabrication, and is therefore not exposed to the harsh temperatures and solvents associated with *in situ* polymerisation techniques. We believe that the PEDOT:TOS–PEG composites in this work can act as an ideal platform for interfacing organic electronics and biological systems.

Materials and methods

Vapour phase polymerisation of PEDOT:TOS composites

PEDOT:TOS, PEDOT:TOS-PEG and PEDOT:TOS:PEG(COOH) composites were fabricated according to a previously published protocol.[35] Prior to VPP, an oxidant solution was prepared. Fe(III)tosylate (40 wt% in solution with butanol, Yacoo Chemical Company) was used as the oxidant, with pyridine (Sigma; used as received) as a weak base in all solutions. Poly(ethylene glycol) and PEG(COOH) (PEG, M_n = 20 000, and PEG(COOH), M_n = 600, Sigma Aldrich) were dissolved in a small amount of water before being added to the oxidant solution. The ratio of PEG to PEDOT was estimated on the basis that it requires 2.25 moles of Fe(III) to produce 1 mole of PEDOT.[38] The amount of PEG added to each oxidant solution was chosen in order to achieve films with 30%, 50% and 70% PEG contents as compared to PEDOT. For the purpose of lowering the final film thickness, the oxidant solutions were diluted with ethanol. To promote adhesion between the final PEDOT film and the substrate, glass slides were coated with plasma polymerised maleic anhydride[39] prior to deposition of the oxidant solution. The oxidant solution was spun onto the substrates at 1500 rpm for 30 s and placed directly in the vapour phase polymerisation chamber without a drying step. The vaporisation chamber, containing an EDOT monomer (HD Stark or Yacoo Chemical Company), was kept in an oven at 70 °C, at ambient pressure. EDOT was allowed to polymerise on the coated substrates for 30–40 minutes, at which point samples were removed from the polymerisation chamber and rinsed twice in ethanol to remove excess Fe(III)Tos and unpolymerised EDOT monomer.

Cyclic voltammetry

Cyclic voltammetry (CV) measurements were made using a multichannel potentiostat (VMP2 with EC-Lab software version 10.19) with a traditional 3-electrode setup. A standard calomel electrode was used as reference and 0.1 M NaPTS (pH 7) was used as the electrolyte, at a scan rate of 5 mV s^{-1}. The electrolyte was deaerated by bubbling through N_2.

Conductivity measurements

Conductivity measurements were performed by measuring the resistance across PEDOT:TOS and PEDOT:TOS–PEG samples of defined length and width. The thicknesses of the films were measured using profilometry and final film thickness values used in calculations were averaged from at least three measurements. Conductivity values shown were averaged over at least 5 separate samples.

OECT fabrication and characterisation

OECT fabrication consisted of first defining a conducting polymer channel on a glass substrate. Polymethyldisiloxane (PDMS) was used to define the well (active channel area: *ca.* 16 mm^2). Phosphate buffered saline (PBS) was used as the electrolyte. Ag/AgCl was used as the gate electrode. Transistor characteristics were measured using a Keithley 2612A Sourcemeter and

customised Labview software. Transient measurements were carried out in two ways. In the first method, the drain–source voltage (V_{DS}) was kept at −0.2 V, while a square voltage pulse of 0.3 V for a duration of 5 s was applied to the gate (V_{GS}), allowing 10 s recovery periods. In a second measurement, the gate voltage was stepped from 0.1 to 0.5 V in intervals of 0.1 V, while maintaining a drain voltage of −0.2 V and the same duty cycle as the previous measurement. Transconductance values were extracted from the maximum of the derivative of the steady state transfer curves, at a drain voltage of −0.6 V.

Cell culture

HeLa cells (from HPACC-health protection agency culture collections) were routinely maintained at 37 °C in a humidified atmosphere of 5% CO_2, in DMEM (Advanced DMEM Reduced Serum Medium 1X, Invitrogen) with 2 mM glutamine (Glutamax™-1, Invitrogen), 10% FBS (Fœtal Bovine Serum, Invitrogen) and Pen-strep (5000 [U mL^{-1}] penicillin–5000 [μg mL^{-1}] streptomycin, Invitrogen). Cells were detached by trypsinisation (0.05% trypsin–EDTA 1X, Invitrogen) and numbers were determined by a cell counter (Scepter™ Handheld Automated Cell Counter, Millipore).

Cell adhesion and proliferation tests

A glass substrate (approximate area: 4.9 cm^2) was coated with the conducting polymer composite (PEDOT:TOS–PEG) at four different PEG concentrations (0%, 30%, 50% and 70%) according to the procedure described above. Each coated substrate was sterilised for 20 minutes in 70% ethanol, rinsed twice in PBS, and placed in a separate Petri dish. Cells were seeded at a concentration of 10^5 cells per Petri dish. An additional 4 mL of DMEM was added to each dish. Adhesion and proliferation were evaluated after 48 h. A calcein AM/propidium iodide assay was carried out to determine the cell viability (calcein-AM, Sigma) at 1 μg mL^{-1} and propidium iodide (propidium iodide solution, Sigma) at 2 μg mL^{-1}. To perform these tests, media in the dishes were discarded and the cells were gently rinsed two times with PBS. 3 mL of the calcein-AM–PI mixture was added to each dish and incubated for 30 min at 37 °C. Fluorescence images were taken (Axio Observer Z1, Carl Zeiss, calcein AM 485 nm/535 nm, PI 530 nm/620 nm) and cells were counted to determine viability.

MTT assay

Glass slides were placed in Petri dishes and incubated with 4 mL of DMEM at 37 °C and 5% CO_2 for 1 week. As a positive control, corresponding to 100% viability, DMEM was incubated in a dish under the same conditions. The MTT assay (MTT Cell Proliferation Assay Kit, Cayman Chemical) was carried out according to the manufacturer's instructions. Briefly, HeLa cells were seeded at a concentration of 1×10^4 cells per well in a 96 well plate and incubated until 50% confluency was reached. The medium was then discarded and replaced with 100 μL of medium previously incubated with the conducting polymer composite. Five replicates were evaluated after 24 h of culture. After the incubation, the medium was aspirated and exchanged with 100 μL of fresh medium supplemented with 10 μL of MTT reagent and the plate was incubated at 37 °C for 2 h. Finally, the medium was aspirated and each well was rinsed twice with PBS. 100 μL of a crystal dissolving reagent was added to each well and the absorbance ($A_{570\ nm}$) was measured to determine viability with a spectrophotometer (Infinite M1000, Tecan).

Biofunctionalisation

A sixteen well incubation chamber was affixed to PEDOT:TOS and PEDOT:TOS–PEG composite films on glass substrates. An alexa-fluor labeled donkey anti-rabbit IgG antibody was used for coupling experiments (A_{ex} 578 nm/A_{em} 603 nm) (Invitrogen; 2 mg mL^{-1}). A solution of EDC (Sigma; 0.4 M in DI water) and NHS (Sigma; 0.1 M in DI water) was prepared and 100 μL was added to each well (8 mm^2), with three replicates for each condition. 100 μL of DI water was used as a control in an additional three wells. Reactions were allowed to proceed at room temperature for 1 hour. EDC–NHS was then removed and samples were rinsed once with 1X PBS. Next, 100 μL of a protein solution at 0.1 mg mL^{-1} were added inside the wells. The samples were covered with aluminum foil and left to incubate for two hours at room temperature. The antibody was then removed and the samples were rinsed three times with PBS, followed by a rinse with PBS-T (0.05% Tween-20). Finally, samples were rinsed with PBS with NaCl adjusted to 0.5 M. Fluorescence measurements were performed using a spectrofluorimeter (Tecan Infinite® M1000).

X-ray photoelectron spectroscopy

XPS data were collected using an AXIS-HSi spectrometer (Kratos Analytical Inc., Manchester, UK) with a monochromated Al K_α source at a power of 144 W, a hemispherical analyser operating in the fixed analyser transmission mode and the standard aperture of 1 mm × 0.5 mm. The total pressure in the main vacuum chamber during analysis was of the order of 10^{-8} mbar. Charge neutralisation was achieved using a magnetic immersion lens, which injects low kinetic energy electrons (1–4 eV) from a heated tungsten filament into the sample. Each specimen (area of analysis = 0.35 mm^2) was analysed at an emission angle of 0° as measured from the surface normal. The information depth of this method is estimated to be smaller than 10 nm. The background was removed using either a linear or a Shirley approach. The data were analysed using Casa XPS. The survey and the C1s spectra were recorded by setting the saturated C1s peak to 285 eV.

Results and discussion

To show that the electroactivity of the PEDOT was maintained on the addition of PEG, cyclic voltammetry (CV) curves were measured for both PEDOT:TOS and PEDOT:TOS with the addition of 50% PEG (PEDOT:TOS–50PEG). The resulting curves are shown in Fig. 1. The shape and presence of peaks in the CV curves of the two films are identical, assuring that the addition of PEG does not lead to a change in the electrochemical response of the PEDOT, *i.e.* the reduction and oxidation potentials have not changed.

To further investigate electrical performance, composite films of PEDOT:TOS and PEG were prepared with various PEG to PEDOT ratios: 0% PEG, 30% PEG, 50% PEG and 70% PEG

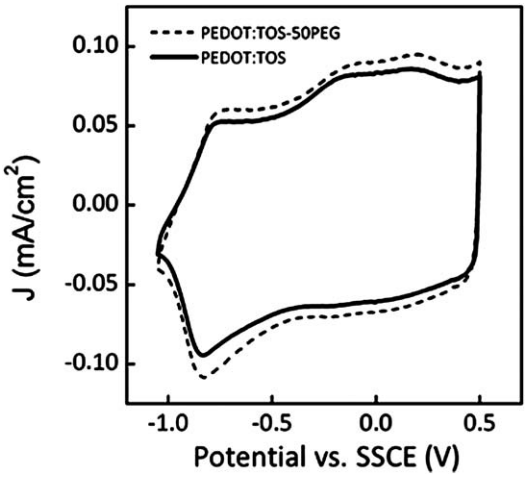

Fig. 1 Comparison of cyclic voltammetry curves for PEDOT:TOS and PEDOT:TOS–50PEG (5 mV s^{-1}, in 0.1 M NaTOS).

(referred to as PEDOT:TOS, PEDOT:TOS–30PEG, PEDOT:TOS–50PEG and PEDOT:TOS–70PEG, respectively). For the range of PEG contents measured in this study, the film conductivity was found to increase with increasing PEG concentration (Fig. 2a). These data confirm previous observations of increasing PEDOT conductivity on the addition of PEG,[35,36] and similar non-conducting materials.[40–43] This effect has been attributed to a number of factors relating to film microstructure and energetics. As observed previously, we saw that the addition of PEG suppresses the formation of μ-scale domains (ESI†), presumably providing a smoother energetic landscape for charge transport. Perhaps the most important factor for the increase in conductivity is the decrease of the solid–solid phase transition temperature by as much as 70 °C observed for PEDOT–PEG blends compared to PEDOT alone.[35] This strongly implies that the order of PEDOT in the blended materials is maintained, but the energy required to disrupt this order is significantly reduced. Thus, the composite materials are more suitable for undergoing the necessary conformational changes along the conjugated backbone required for efficient charge transfer.

Having established an increase in the conductivity, we wanted to validate the potential of PEDOT:TOS–PEG as the active material in an OECT. As mentioned in the introduction, OECTs are important devices in the field of bioelectronics, used for biosensing and ionic signal transduction. OECTs are comprised of a conducting polymer film acting as the transistor channel, with metallic source and drain electrodes. The CP, in this case PEDOT:TOS or PEDOT:TOS–PEG, is in direct contact with an electrolyte, and a gate electrode is submerged in the electrolyte. The application of a positive gate–source bias encourages migration of ions: tosylate anions leave the polymer film as cations from the electrolyte enter the polymer film. Both of these actions serve to de-dope the CP. As the number of mobile holes is reduced, the drain current decreases. In this way, the OECT translates changes in ion flux to a change in electrical current, making it an ideal platform for the integration of electronics and biological systems.

We fabricated OECTs with PEDOT:TOS and PEDOT:TOS–PEG. The transient response of the OECT drain current to a square gate voltage pulse for films with different PEG to PEDOT ratios is shown in Fig. 2b. As discussed above, the addition of PEG results in an increase in film conductivity. This is manifested as a shift in the steady state drain current of the device on the addition of PEG. On application of the positive gate voltage, all devices show efficient de-doping, as evidenced by the rapid decrease in drain current. Upon removal of this voltage, the drain current recovers to the original steady state value. The surface plot in Fig. 2c illustrates the effect of both applied gate voltage and film composition on the transistor response (defined as the change in drain current on application of a positive gate voltage, normalised by the baseline current). In all films, an increase of the gate voltage results in an increase of the normalised modulation, while the PEG content has a negligible effect.

It has been demonstrated previously that OECT behaviour can be modelled as a combination of an electronic circuit, which accounts for hole transport in the polymer channel, and an ionic circuit, which accounts for the ionic transport from the electrolyte to the polymer channel.[44] As a simple approximation, the behaviour of the ionic circuit resembles that of an RC circuit, with R determined by the resistance of the electrolyte and C being an effective capacitance of the channel–electrolyte interface (in the case where a Ag/AgCl electrode is used as the gate). The time response of the device to a square voltage pulse on the gate is

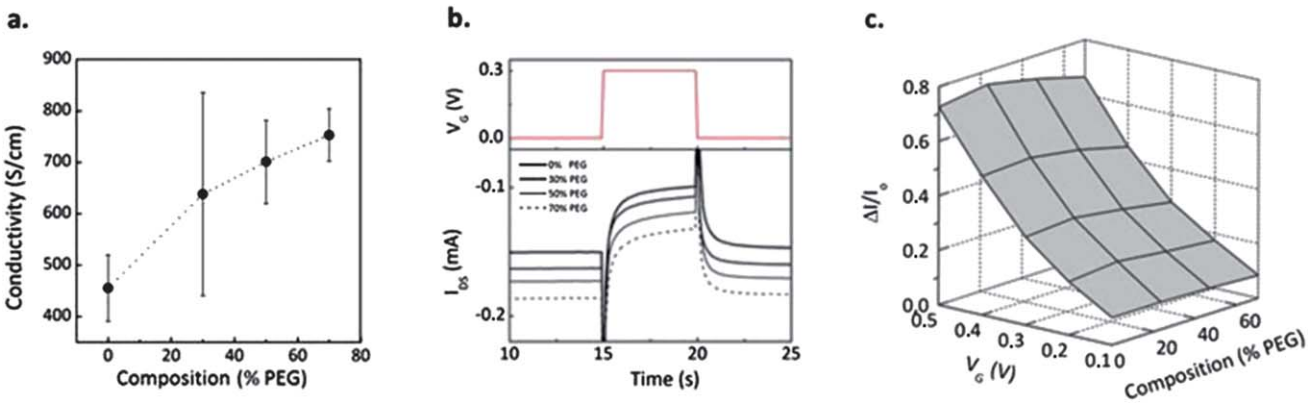

Fig. 2 (a) Conductivity as a function of PEG content. Error bars represent standard deviation from the mean. (b) Transient OECT response for films with varying PEG content, and (c) normalised OECT response as a function of gate voltage and film composition (PEG content).

related to charging of that "effective" capacitor, and it is related to the ability of the polymer channel to store charge. Data presented in Fig. 2b and c show that the addition of PEG does not alter the RC time. From a physical perspective, this implies that the PEG content presents no additional barrier for ion migration both into and out of the film during the de-doping/doping process. The efficiency of the doping and de-doping process is further evidenced by the very high transconductance (average of 768 μS, or 274 μS mm^{-1} when normalised for channel width) measured in the OECTs, which is an order of magnitude greater than that of typical organic field effect transistors.[45]

The data presented here assure that the addition of PEG is not harmful to device performance, and in fact, improves conductivity. Since our efforts are directed towards organic electronic devices for biological applications, it is also necessary to determine the biocompatibility of these composite films. It has been observed previously that unpolymerised EDOT monomers can be toxic to cells,[46] but once polymerised, PEDOT is generally accepted to be cytocompatible. A number of studies interfacing PEDOT with a variety of cell types have been reported with both PSS[47] and TOS as dopants.[10,48,49] Likewise, PEG has been used extensively in tissue engineering, generally as an inactive ingredient or scaffold.[50–52] Nevertheless, we wanted to confirm that there was no toxic effect of the PEDOT:TOS–PEG composites on cell activity or growth.

Two different tests were carried out: a direct test where cells were grown on the PEDOT:TOS–PEG films and assessed for growth, and an indirect test, where films were incubated in cell culture media, which was later used in cell culture. Fig. 3 clearly shows that after 48 hours, PEG did not have a negative effect on cell growth or proliferation. Cells were seeded on the composite materials and allowed to grow for 48 hours, at which point images were recorded and viability was determined using a live/dead assay. Calcein AM was used for live cells (shown in green), and propidium iodide for dead cells (shown in red). Very few dead cells were observed. It should be noted that the cell rounding observed in the micrographs in Fig. 3 is due to a rinsing step in PBS and is not an effect of the PEDOT:TOS–PEG surface. Prior to the PBS rinsing steps, HeLa cells exhibited the typical, elongated morphology.

While every attempt is made to ensure that toxic compounds from the polymer processing steps are removed before the addition of cells (multiple wash steps with ethanol and PBS), it is possible that over time compounds can be released into the cell medium.[53] The indirect test was designed to investigate changes in cell metabolism caused by substances that may leach out of the PEDOT:TOS–PEG films (such as ethylene glycol oligomers, tosylate ions and unpolymerised EDOT monomers). Substrates coated with PEDOT:TOS–PEG were submerged in media for one week. This exposed medium was then used to culture HeLa cells, previously seeded and cultured in unexposed media. An MTT assay was performed to assess cellular metabolism (viability), and results shown in Fig. 4 indicate that over the course of one week, viability of cells cultured using the exposed media was decreased only slightly compared to that of control cells cultured with unexposed media. The PEG content had a negligible effect on cell viability. The indirect assay shows that cytotoxic compounds released after a week's incubation were

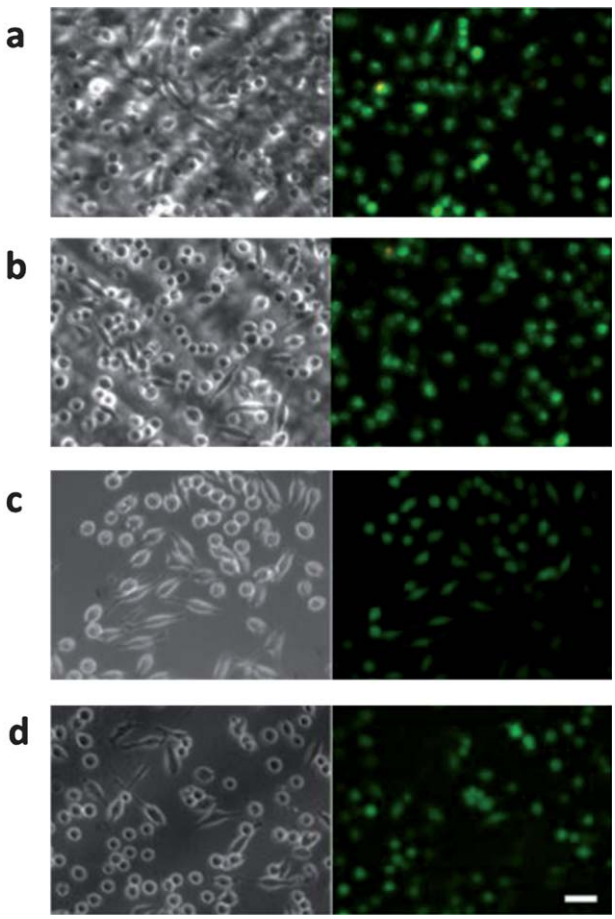

Fig. 3 Biocompatibility of HeLa cells on PEDOT:TOS–PEG composites for (a) PEDOT:TOS, (b) PEDOT:TOS–30PEG, (c) PEDOT:TOS–50PEG, and (d) PEDOT:TOS–70PEG. The left-hand panels are representative brightfield images showing cell coverage. The right-hand panels are representative fluorescent images of cells stained with calcein AM and propidium iodide. Live cells are shown in green, dead cells are shown in red. The scale bar corresponds to 50 μm and is applicable to all panels.

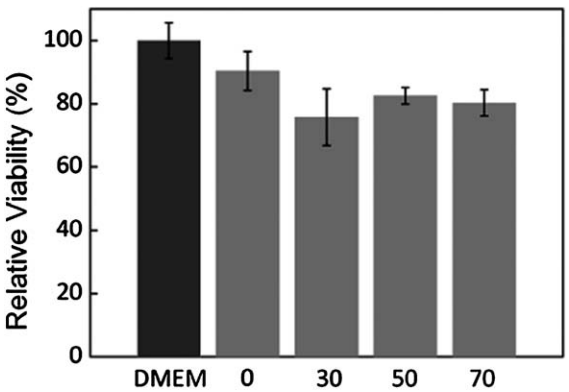

Fig. 4 Assessment of the effect of PEG on the viability of HeLa cells. Results shown are an average of five replicates. Error bars represent standard deviation from the mean. DMEM condition represents the positive control without polymer and is used to determine the 100% viability. Conditions where the DMEM was left in direct contact with PEDOT:TOS–PEG are labelled according to the PEG content of the film.

minimal. Furthermore, the release of such compounds was not facilitated by the incorporation of PEG.

To address the need for conducting polymer surfaces with specifically covalently incorporated proteins we carried out preliminary work to biofunctionalise the surface of our composite materials by taking advantage of the readily activated alcohol groups on the PEG. That is, composites comprised of PEDOT:TOS and a carboxylic acid functionalised PEG, referred to as PEG(COOH), were made. XPS data confirm that incorporation of 70% PEG into PEDOT:TOS films (both PEG–COOH and PEG) results in a surface layer of approximately 18–20% PEG. In the PEDOT:TOS–PEG(COOH) composite, approximately 2% of the total carbon can be attributed to the COOH groups. EDC–NHS (EDC: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride–NHS: *N*-hydroxysuccinimide) chemistry was used to incorporate fluorescent proteins onto these surfaces *via* the carboxyl groups present in the functionalised PEG. EDC reacts with carboxylic acid groups in the presence of NHS to form a stable amide bond between the carboxyl group and ubiquitous primary amines found in all proteins. Subsequent stringent wash steps were included (3× PBS washes, one wash with PBS containing detergent, followed by a wash step with 0.5 M NaCl) in an attempt to exclude the possibility of electrostatic binding of proteins to the surfaces tested. A standard curve obtained using the fluorescent antibody serially diluted and quantitated for fluorescence in solution, in an identical well, on a PEDOT:TOS coated film – data not shown – was used to approximate the protein bound, with the maximum amount found to correspond to approximately 1 μg of protein. The amount of protein coupled is low; however this is something that may be optimised for future experiments.

The data presented in Fig. 5 show the fluorescence intensity of four different surfaces: PEDOT:TOS–PEG with COOH (PTP-C), PEDOT:TOS–PEG without COOH (PTP), PEDOT:TOS alone (PT), and PEDOT:TOS coated with plasma polymerised maleic anhydride (PT-MA). It can be seen that the PEDOT:TOS–PEG(COOH) surface has a low level of specific binding of protein, which does not occur in the absence of the EDC–NHS treatment. In contrast, the PEDOT:TOS–PEG surface does not show any difference between the control or the EDC–NHS treated surface. Interestingly, a PEDOT:TOS surface, tested as a control, showed specific binding of protein in the presence of EDC–NHS treatment, but not without this treatment. Although this surface does not have an available carboxylic acid group, nonetheless there is a specific reaction occurring which we speculate is *via* direct attack of the EDC on PEDOT. As an additional control, we coated a PEDOT:TOS–PEG surface with plasma polymerised maleic anhydride, which has been shown to result in high concentrations of carboxyl groups on the surface.[39] Indeed, this sample exhibited the highest fluorescent intensity, indicating that –COOH groups are covalently bound with the protein. We believe that both PEDOT and –COOH groups on the PEG are participating in the EDC–NHS chemistry, forming a specific linkage with the protein. While further elucidation of this reaction mechanism is necessary, we are currently presented with at least directions to proceed. First, we could substitute the functional group on the PEG and use orthogonal chemistry that does not interfere with the PEDOT. Second, we could specifically block the PEDOT functional groups before proceeding with the PEG functionalization. Finally, we could take advantage of the unexpected specific binding allowed with EDC–NHS chemistry, and functionalize the PEDOT itself.

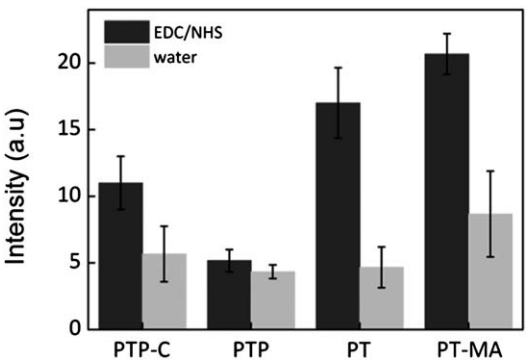

Fig. 5 Biofunctionalisation of conducting polymer surfaces. Data shown are the mean fluorescence values of 8 mm^2 wells on a variety of CP surfaces, each carried out in triplicate. Error bars represent standard deviation from the mean. Samples from left to right: PEDOT:TOS–70PEG(COOH) (PTP-C), PEDOT:TOS:70PEG (PTP), PEDOT:TOS (PT), and PEDOT:TOS treated with a plasma coating of maleic anhydride (PT-MA).

Conclusions

In this work we have demonstrated that composite materials comprised of the conducting polymer PEDOT:TOS and the nonconducting polymer PEG show electrical properties equal or superior to PEDOT:TOS alone. We showed that these composite materials are biocompatible through rigorous direct and indirect tests and that the degree of biocompatibility is not dependent on the concentration of PEG. Furthermore, we have demonstrated the potential of the incorporated PEG to provide an avenue for the specific incorporation of biomolecules onto PEDOT:TOS surfaces in a manner that is compatible with the sensitive nature of most of these moieties. We believe that the development of PEDOT:TOS–PEG biofunctional materials can lead to the improvement of biomaterials and bioelectronic devices.

Acknowledgements

The authors acknowledge funding from the European Research Council (ERC grant agreement no. 258966), and MASK, a Marie Curie International Research Staff Exchange Scheme. We thank Matthias Hilder for assisting with XPS and Dr Moshe Gurfinkel and Dr Michele Sessolo for fruitful discussions.

Notes and references

1 H. Shirakawa, E. J. Louis, A. G. Macdiarmid, C. K. Chiang and A. J. Heeger, Synthesis of electrically conducting organic polymers – halogen derivatives of polyacetylene, (Ch)X, *J. Chem. Soc., Chem. Commun.*, 1977, 578–580.
2 R. M. Owens and G. G. Malliaras, Organic electronics at the interface with biology, *MRS Bull.*, 2010, **35**, 449–456.
3 N. K. Guimard, N. Gomez and C. E. Schmidt, Conducting polymers in biomedical engineering, *Prog. Polym. Sci.*, 2007, **32**, 876–921.
4 D. H. Kim, M. R. Abidian, S. Richardson-Burns, L. Povlich, S. Spanninga, J. L. Hendricks and D. C. Martin, Soft, Fuzzy, and Bioactive Conducting Polymers for Improving the Chronic Performance of Neural Prosthetic Devices, in *Indwelling Neural*

Implants: Strategies for Contending with the in vivo *Environment*, CRC Press (2008), ed. W. M. Reichert, CRC Press, Durham, North Carolina, 2007.
5 D. H. Kim, J. A. Wiler, D. J. Anderson, D. R. Kipke and D. C. Martin, Conducting polymers on hydrogel-coated neural electrode provide sensitive neural recordings in auditory cortex, *Acta Biomater.*, 2010, **6**, 57–62.
6 M. R. Abidian, J. M. Corey, D. R. Kipke and D. C. Martin, Conducting-polymer nanotubes improve electrical properties, mechanical adhesion, neural attachment, and neurite outgrowth of neural electrodes, *Small*, 2010, **6**, 421–429.
7 D. H. Kim, S. M. Richardson-Burns, J. L. Hendricks, C. Sequera and D. C. Martin, Effect of immobilized nerve growth factor on conductive polymers: electrical properties and cellular response, *Adv. Funct. Mater.*, 2007, **17**, 79–86.
8 L. Poole-Warren, N. Lovell, S. Baek and R. Green, Development of bioactive conducting polymers for neural interfaces, *Expert Rev. Med. Devices*, 2010, **7**, 35–49.
9 J. Y. Wong, R. Langer and D. E. Ingber, Electrically conducting polymers can noninvasively control the shape and growth of mammalian cells, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 3201–3204.
10 A. M. D. Wan, D. J. Brooks, A. Gumus, C. Fischbach and G. G. Malliaras, Electrical control of cell density gradients on a conducting polymer surface, *Chem. Commun.*, 2009, 5278–5280.
11 C. Salto, E. Saindon, M. Bolin, A. Kanciurzewska, M. Fahlman, E. W. H. Jager, P. Tengvall, E. Arenas and M. Berggren, Control of neural stem cell adhesion and density by an electronic polymer surface switch, *Langmuir*, 2008, **24**, 14133–14138.
12 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, Organic electronics for precise delivery of neurotransmitters to modulate mammalian sensory function, *Nat. Mater.*, 2009, **8**, 742–746.
13 J. Isaksson, P. Kjall, D. Nilsson, N. D. Robinson, M. Berggren and A. Richter-Dahlfors, Electronic control of Ca^{2+} signalling in neuronal cells using an organic electronic ion pump, *Nat. Mater.*, 2007, **6**, 673–679.
14 K. Tybrandt, K. C. Larsson, A. Richter-Dahlfors and M. Berggren, Ion bipolar junction transistors, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 9929–9932.
15 G. P. Kittlesen, H. S. White and M. S. Wrighton, Chemical derivatization of microelectrode arrays by oxidation of pyrrole and *n*-methylpyrrole – fabrication of molecule-based electronic devices, *J. Am. Chem. Soc.*, 1984, **106**, 7389–7396.
16 H. S. White, G. P. Kittlesen and M. S. Wrighton, Chemical derivatization of an array of 3 gold microelectrodes with polypyrrole – fabrication of a molecule-based transistor, *J. Am. Chem. Soc.*, 1984, **106**, 5375–5377.
17 J. T. Mabeck and G. G. Malliaras, Chemical and biological sensors based on organic thin-film transistors, *Anal. Bioanal. Chem.*, 2006, **384**, 343–353.
18 D. A. Bernards, G. G. Malliaras, G. E. S. Toombes and S. M. Gruner, Gating of an organic transistor through a bilayer lipid membrane with ion channels, *Appl. Phys. Lett.*, 2006, **89**, 053505.
19 S. Y. Yang, F. Cicoira, R. Byrne, F. Benito-Lopez, D. Diamond, R. M. Owens and G. G. Malliaras, Electrochemical transistors with ionic liquids for enzymatic sensing, *Chem. Commun.*, 2010, **46**, 7972–7974.
20 S. Y. Yang, F. Cicoira, R. Byrne, F. Benito-Lopez, D. Diamond, R. M. Owens and G. Malliaras, *Chem. Commun.*, 2010, **46**(42), 7972–7974.
21 J. M. Green, R. C. Pritchett, D. C. Tucker, T. R. Crews and J. R. McLester, Sweat lactate response during cycling at 30 degrees C and 18 degrees C WBGT, *J. Sports Sci.*, 2004, **22**, 321–327.
22 P. Lin, F. Yan, J. J. Yu, H. L. W. Chan and M. Yang, The application of organic electrochemical transistors in cell-based biosensors, *Adv. Mater.*, 2010, **22**, 3655.
23 L. Zhang, W. R. Stauffer, E. P. Jane, P. J. Sammak and X. Y. T. Cui, Enhanced differentiation of embryonic and neural stem cells to neuronal fates on laminin peptides doped polypyrrole, *Macromol. Biosci.*, 2010, **10**, 1456–1464.
24 B. Teixeira-Dias, L. J. del Valle, F. Estrany, J. F. Mano, R. L. Reis and C. Alemán, Dextrin- and conducting-polymer-containing biocomposites: properties and behavior as cellular matrix, *Macromol. Mater. Eng.*, 2011, 359–368.
25 Y. Xiao, C. M. Li, S. Wang, J. Shi and C. P. Ooi, Incorporation of collagen in poly(3,4-ethylenedioxythiophene) for a bifunctional film with high bio- and electrochemical activity, *J. Biomed. Mater. Res., Part A*, 2010, **92**, 766–772.
26 W. R. Stauffer and X. T. Cui, Polypyrrole doped with 2 peptide sequences from laminin, *Biomaterials*, 2006, **27**, 2405–2413.
27 J. H. Collier, J. P. Camp, T. W. Hudson and C. E. Schmidt, Synthesis and characterization of polypyrrole-hyaluronic acid composite biomaterials for tissue engineering applications, *J. Biomed. Mater. Res.*, 2000, **50**, 574–584.
28 B. Teixeira-Dias, L. J. del Valle, D. Aradilla, F. Estrany and C. Alemán, A conducting polymer/protein composite with bactericidal and electroactive properties, *Macromol Mater Eng*, 2011, 427–436.
29 S. Cosnier, Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films. A review, *Biosens. Bioelectron.*, 1999, **14**, 443–456.
30 M. L. M. Asplund, E. M. Thaning, T. A. Nyberg, O. W. Inganas and H. von Hoist, Stability of poly(3,4-ethylene dioxythiophene) materials intended for implants, *J. Biomed. Mater. Res., Part B*, 2010, **93**, 407–415.
31 R. A. Green, N. H. Lovell and L. A. Poole-Warren, Impact of co-incorporating laminin peptide dopants and neurotrophic growth factors on conducting polymer properties, *Acta Biomater.*, 2010, **6**, 63–71.
32 J. W. Lee, F. Serna, J. Nickels and C. E. Schmidt, Carboxylic acid-functionalized conductive polypyrrole as a bioactive platform for cell adhesion, *Biomacromolecules*, 2006, **7**, 1692–1695.
33 B. Winther-Jensen, J. Chen, K. West and G. Wallace, Vapor phase polymerization of pyrrole and thiophene using iron(III) sulfonates as oxidizing agents, *Macromolecules*, 2004, **37**, 5930–5935.
34 M. Fabretto, C. Jariego-Moncunill, J.-P. Autere, A. Michelmore, R. D. Short and P. Murphy, High conductivity PEDOT resulting from glycol/oxidant complex and glycol/polymer intercalation during vacuum vapour phase polymerisation, *Polymer*, 2011, **52**, 1725–1730.
35 B. Winther-Jensen, K. Fraser, C. Ong, M. Forsyth and D. R. MacFarlane, Conducting polymer composite materials for hydrogen generation, *Adv. Mater.*, 2010, **22**, 1727–1730.
36 B. Winther-Jensen, T. Knecht, C. Ong, J. Vongsvivut and N. Clark, Inhomogeneity effects in vapor phase polymerized PEDOT: a tool to influence conductivity, *Macromol. Mater. Eng.*, 2011, **296**, 185–189.
37 T. Cha, A. Guo, Y. Jun, D. Pei and X.-Y. Zhu, Immobilization of oriented protein molecules on poly(ethylene glycol)-coated Si(111), *Proteomics*, 2004, **4**, 1965–1976.
38 X. Y. Wu, Q. A. Xie, Z. G. He and D. X. Wang, Free and forced Rossby waves in the western South China Sea inferred from Jason-1 satellite altimetry data, *Sensors*, 2008, **8**, 3633–3642.
39 Z. Ademovic, J. Wei, B. Winther-Jensen, X. Hou and P. Kingshott, Surface modification of PET films using pulsed AC plasma polymerisation aimed at preventing protein adsorption, *Plasma Processes Polym.*, 2005, **2**, 53–63.
40 M. Fabretto, M. Mueller, K. Zuber and P. Murphy, Influence of PEG-ran-PPG surfactant on vapour phase polymerised PEDOT thin films, *Macromol. Rapid Commun.*, 2009, **30**, 1846–1851.
41 M. Fabretto, J.-P. Autere, D. Hoglinger, S. Field and P. Murphy, Vacuum vapour phase polymerised poly(3,4-ethyelendioxythiophene) thin films for use in large-scale electrochromic devices, *Thin Solid Films*, 2011, **519**, 2544–2549.
42 K. Zuber, M. Fabretto, C. Hall and P. Murphy, Improved PEDOT conductivity *via* suppression of crystallite formation in Fe(III) tosylate during vapor phase polymerization, *Macromol. Rapid Commun.*, 2008, **29**, 1503–1508.
43 B. Winther-Jensen, D. W. Breiby and K. West, Base inhibited oxidative polymerization of 3,4-ethylenedioxythiophene with iron(III)tosylate, *Synth. Met.*, 2005, **152**, 1–4.
44 D. A. Bernards and G. G. Malliaras, Steady-state and transient behavior of organic electrochemical transistors, *Adv. Funct. Mater.*, 2007, **17**, 3538–3544.
45 Y. S. Wang, H. Weinacker and B. Koch, A Lidar point cloud based procedure for vertical canopy structure analysis and 3D single tree modelling in forest, *Sensors*, 2008, **8**, 3938–3951.
46 L. J. del Valle, D. Aradilla, R. Oliver, F. Sepulcre, A. Gamez, E. Armelin, C. Aleman and F. Estrany, Cellular adhesion and

proliferation on poly(3,4-ethylenedioxythiophene): benefits in the electroactivity of the conducting polymer, *Eur. Polym. J.*, 2007, **43**, 2342–2349.
47 J. E. Collazos-Castro, J. L. Polo, G. R. Hernandez-Labrado, V. Padial-Canete and C. Garcia-Rama, Bioelectrochemical control of neural cell development on conducting polymers, *Biomaterials*, 2010, **31**, 9244–9255.
48 M. H. Bolin, K. Svennersten, D. Nilsson, A. Sawatdee, E. W. H. Jager, A. Richter-Dahlfors and M. Berggren, Active control of epithelial cell-density gradients grown along the channel of an organic electrochemical transistor, *Adv. Mater.*, 2009, **21**, 4379.
49 K. Svennersten, M. H. Bolin, E. W. Jager, M. Berggren and A. Richter-Dahlfors, Electrochemical modulation of epithelia formation using conducting polymers, *Biomaterials*, 2009, **30**, 6257–6264.
50 J. M. Green, R. C. Pritchett, T. R. Crews, J. R. McLester, Jr and D. C. Tucker, Sweat lactate response between males with high and low aerobic fitness, *Eur. J. Appl. Physiol.*, 2004, **91**, 1–6.
51 X. J. Liu and W. H. Tan, Development of an optical fiber lactate sensor, *Microchim. Acta*, 1999, **131**, 129–135.
52 P. Gopalakrishna, S. K. Chaubey, P. S. Manogaran and G. Pande, Modulation of alpha 5 beta 1 integrin functions by the phospholipid and cholesterol contents of cell membranes, *J. Cell. Biochem.*, 2000, **77**, 517–528.
53 D. K. Cullen, A. R. Patel, J. F. Doorish, D. H. Smith and B. J. Pfister, Developing a tissue-engineered neural-electrical relay using encapsulated neuronal constructs on conducting polymer fibers, *J. Neural Eng.*, 2008, **5**, 374–384.