Journal of
Materials Chemistry B

REVIEW

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Biofunctionalization of polydioxythiophene derivatives for biomedical applications

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Cite this: *J. Mater. Chem. B*, 2016, 4, 4952

Received 6th April 2016,
Accepted 16th June 2016

DOI: 10.1039/c6tb00852f

www.rsc.org/MaterialsB

The polydioxythiophenes PEDOT and more recently ProDOT have emerged as champion materials in the field of organic bioelectronics, both in the domain of biosensing and also for integration with living cells (both *in vitro* and *in vivo*). Although polydioxythiophenes in their pristine forms have shown great promise for bioelectronics, in order to broaden the spectrum of applications, a biofunctionalization step is essential. In this review we summarise the methods that have been used thus far to biofunctionalize polydioxythiophenes in an effort to improve the biotic/abiotic interface. We provide an introduction to this class of materials, focusing particularly on the different methods of synthesis (chemical oxidative polymerization, vapor phase polymerization or direct electrochemical polymerization) and discuss the implications of synthesis on biofunctionalization. Rather than provide an exhaustive review, we chose to highlight key examples of biofunctionalization techniques for polydioxythiophenes for specific biomedical applications. Finally, we conclude with a brief discussion of the importance of biofunctionalization methods in future bioelectronics applications, and some ideas for future directions in this field.

Introduction

Rationale for biofunctionalization

It is becoming increasingly clear that the development of effective biomedical devices relies on engineering of the biotic/abiotic interface.[1,2] Most biosensors or biomedical devices consist of a physical, mechanical, or electrical transducer coupled to a biorecognition element. Improved performance for these sensors and devices can be achieved through biofunctionalization,

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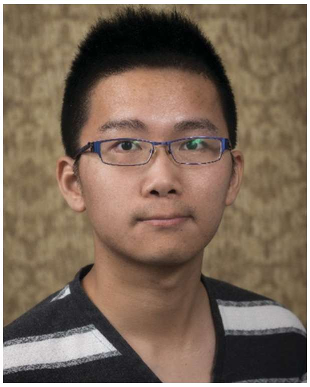

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where a biorecognition element is physically or chemically coupled to the transducer. Improved performance may be attributed to numerous factors including long-term stability, increased signal transduction, and of course specificity. The nature of the biofunctionalization can vary from relatively weak van der Waals type reversible bonds, to strong (and usually irreversible) covalent bonds. The method of biofunctionalization used depends on the surface exposed by the transducer, the nature of the biomolecule to be bound, and the particular needs of each application. For long term stability, covalent binding of the biomolecule is preferred, whereas for more acute applications, electrostatic or even secondary interactions may suffice. In this review we focus on the biofunctionalization of a particular class of materials called conducting polymers (CPs) which are coming to the forefront as novel alternatives to traditional electronics in certain niche applications. More specifically, we focus on polydioxythiophene based conducting polymers, including poly(3,4-ethylene dioxythiophene) (PEDOT) and poly(3,4-propylene dioxythiophene) (PProDOT) (Fig. 1).

CPs came to the forefront in 1976 with research done by Alan MacDiarmid, Hideki Shirakawa and Alan Heeger, and have since been shown to display many desirable characteristics for a variety of applications.[3,4] An important example is their mixed conductivity; in CPs, electrons move primarily along the conjugated backbone of the polymer, but charge hopping between chains also occurs. CPs typically have an open microstructure that allows for facile ionic transport. This mixed (electronic and ionic) conductivity has been shown to be very useful for certain biomedical applications.[5] The most frequently used CPs for biomedical applications include polypyrrole, polyaniline and polythiophenes.[6] Although polypyrrole lead the charge as the candidate material for use in biomedical applications, of late, functionalized polythiophenes derivatives such as PEDOT and more recently PProDOT have emerged as the dominant materials as we will describe below. Early uses of CPs in biomedical engineering included as coatings on metal electrodes, resulting in significantly lower impedance and higher charge storage capacity electrodes, thereby improving the quality of recordings and stimulation.[7,8] Later, CPs were used as platforms for interfacing with cells due to their soft nature and ease of chemical functionalization for tissue engineering.[6,9,10] In the past, tuning of the chemical structure was focused on optimizing charge transport and photon absorption or generation, for applications such as OLEDs or OPVs.[11] For bioelectronics, tuning of the interface for interaction with biological molecules or tissues/organisms is of particular interest. The latter is done for two main reasons: (i) to improve the biotic/abiotic interface for interfacing with live cells and (ii) to introduce biological molecules such as nucleotides or proteins for functionality, *e.g.* for sensing.

CPs have repeatedly been shown to be compatible with a wide variety of live cells, and cell components.[12–14] This is likely due to the fact that these polymeric materials contain no dangling surface bonds, resulting in a closer interaction with cells hosted on their surface, and thus facilitating adhesion and promoting ionic interactions. This concept has been extensively discussed by Rivnay *et al.*[15] This principle, along with the fact that CPs are known to swell in aqueous milieu, thus taking on something of a 'hydrogel' like nature,[16] are key to the understanding that CPs work well in complex electrolytes such as biological buffers or media. Unlike traditional semiconductors, they are not isolated from the sensing events taking place at the interface.[14] It should be noted that thus far, in biomedical applications, CPs have typically been used to either coat relatively

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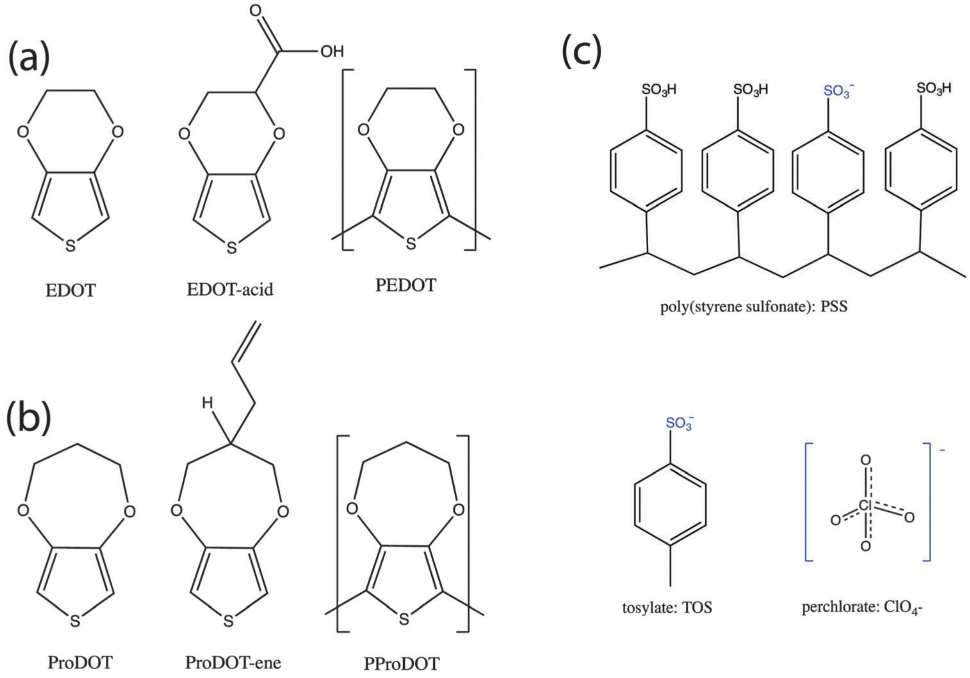

Fig. 1 Structures of polydioxythiophene monomers and dopants. (a) Structure of EDOT monomer and modified EDOT with different pendant groups (carboxyl shown). (b) Structure of ProDOT monomer and modified ProDOT with different pendant groups (ProDOT-ene shown). (c) Usual dopants such as polystyrene sulfonate (PSS) polymer, tosylate (TOS) monomer, and perchlorate monomer.

passive devices such as recording or stimulating electrodes, or used as the channel material in organic thin film transistors. For the latter, the most frequent embodiment is in the organic electrochemical transistor (OECT), where the electrolyte is in direct contact with the conducting polymer channel as well as the gate. The OECT belongs to a category of organic transistors called electrolyte gated transistors (EGTs). Electrolyte gated organic field effect transistors (EGOFETs) are another example of EGTs. The main difference between OECTs and EGOFETs is that in EGOFETs the ions don't penetrate the channel (a typical material is P3HT), and so only a thin area of the channel next to the electrolyte interface charges the doping state, in contrast to the OECT where the whole volume of the channel may be doped. Several biosensing devices using the OECT have focused on biofunctionalization of the gate electrode using non-polydioxythiophene electrodes, however we will allude only briefly to these examples as they do not fall under the scope of this review.

Biofunctionalization of CPs is a step that can enhance their ultimate properties such as biocompatibility, stability, and functionality. In this review, different biofunctionalization methods of chemically and electrochemically deposited PEDOT and PProDOT will be highlighted. We will discuss how these biofunctionalization methods have led to dramatic improvements for particular applications such as for interfacing with living cells, and for development of enzymatic sensors with improved sensitivity and stability. Additionally, the use of ionic liquids in combination with cross-linkable polymers as alternative solid state electrolytes is reviewed, principally as a means of improving the stability of recordings in electrophysiology, but with the added advantage of facilitating incorporation of a biorecognition element.

PEDOT poly(3,4-ethylenedioxythiophene)

PEDOT poly(3,4-ethylenedioxythiophene) is a semiconducting polymer that is degenerately p-type doped and rendered conducting with negative dopant ions, which stabilize positive holes on the conjugated backbone and balance the overall charge. Common dopants for PEDOT include poly(styrenesulfonate) (PSS) and the anions tosylate (TOS) and ClO_4 (Fig. 1c). Both PEDOT:PSS and PEDOT:TOS have been used for applications in biological interfacing. Although there are differences between PEDOT:PSS and PEDOT:TOS in terms of electrical, ionic and structural properties, the principal differences arise due to their variable amenity towards the polymerisation methods used, with PEDOT:PSS more likely to be used in the chemically polymerised form, and PEDOT:TOS more usually prepared *via* either electrochemical polymerization or vapor phase polymerization (VPP). In the vast majority of the applications that will be discussed in this review, the conducting polymers are used in thin films.

Chemically polymerized PEDOT:PSS

PEDOT:PSS is commercially available as a homogenous, dark, aqueous solution or suspension. The chemically polymerized PEDOT is kept solvated by PSS, which plays an important role as both a dopant and as an effective surfactant.[17] In PEDOT:PSS the negative charge of the PSS is compensated by a positively-charged hole in the PEDOT backbone (Fig. 1a and c). The commercial availability of PEDOT:PSS, along with its high conductivity, and its compatibility with photolithographic processing has led to the use of this material in a broad variety

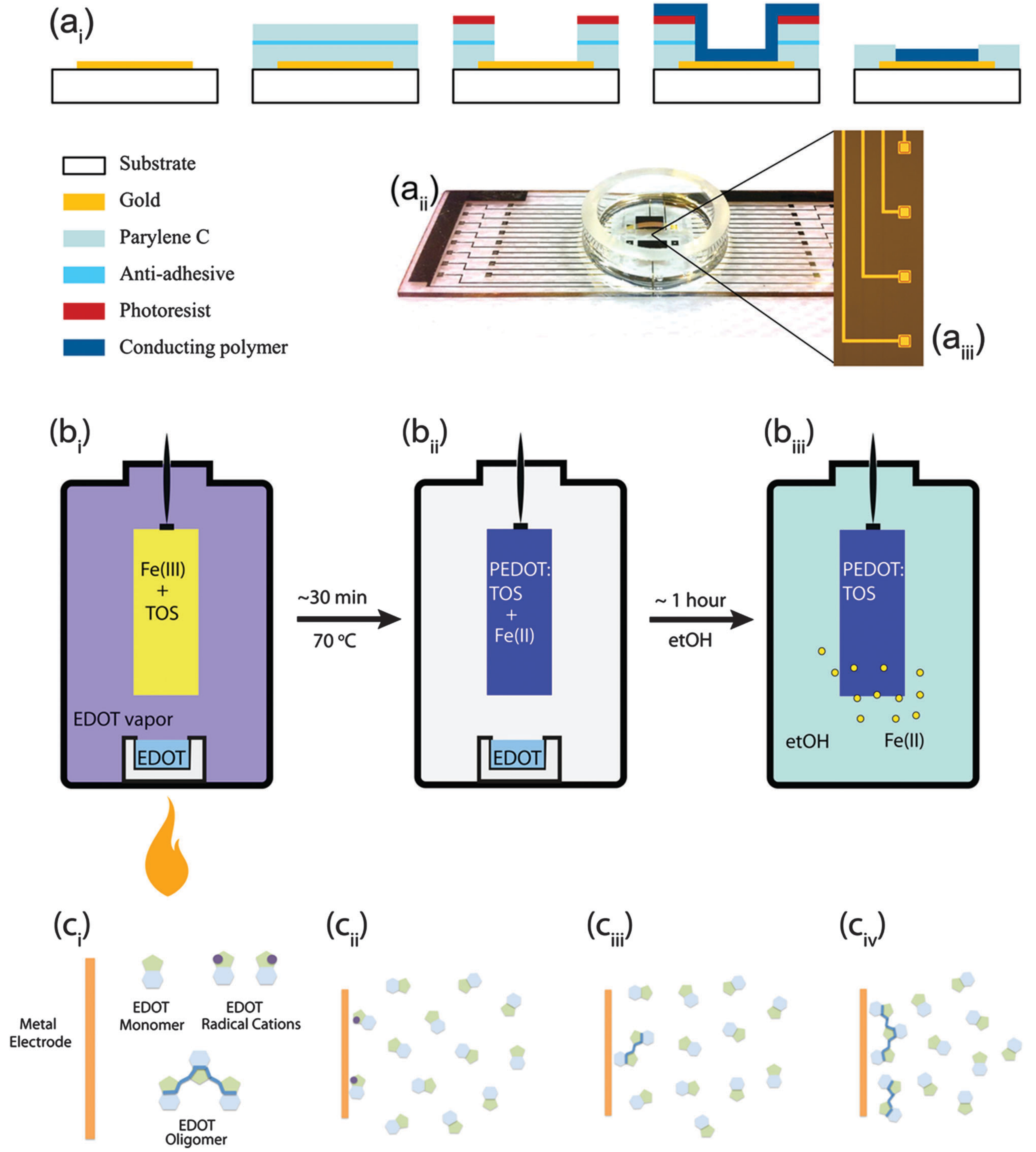

Fig. 2 Polydioxythiophene use in photolithography and polymerization processes: (a) chemically polymerised PEDOT:PSS is commercially available as a dispersion and is highly amenable to photolithographical processes.[7] (b) Vapor phase polymerization of PEDOT:TOS (b$_i$) a thin film of Fe(III):TOS is inserted into a polymerization chamber. The chamber containing EDOT monomer is heated at 70 °C and EDOT is evaporated creating a high pressure atmosphere. (b$_{ii}$) The EDOT polymerizes on the substrate creating a PEDOT:TOS film. (b$_{iii}$) A final rinsing step with ethanol removes the Fe(II):Fe(III) from the film. (c) Electrical polymerization process. (c$_{ii}$) The metal electrode oxidizes the EDOT monomers creating radical cations. (c$_{iii}$) These radical cations combine, creating dimers, trimers, and higher oligomers. (c$_{iv}$) As the molecular weight of the polymer chains increase they become insoluble, precipitating onto the metal electrode surface.

of biomedical applications (Fig. 2a).[18] To increase the electrical conductivity of PEDOT:PSS (~1000 S cm^{-1}) non polar solvants such as ethylene glycol,[19] sorbitol,[20,21] and dimethyl sulfoxide[22] may be used either by direct blending or subsequently spun cast on top of the pre-made film.[23] Dodecyl sulfonic acid has also been used to facilitate film formation.[24] To prevent delamination of PEDOT:PSS films from substrates and improve their long-term stability, 1 wt% (1 : 1 ratio with PEDOT : PSS) of GOPS (3-glycidoxypropyltrimethoxysilane) has been used as a cross-linker. This is especially important for devices that are expected to be operated in an aqueous milieu such as biological media or living tissue.[25] The resulting solid material should really be considered a PEDOT:PSS/crosslinked GOPS blend. Although the conductivity of PEDOT:PSS drops upon addition of GOPS (∼50%), it still remains adequate for most biological applications. As mentioned before, the cross-linked PEDOT:PSS films thus obtained are known to swell somewhat in aqueous solutions, although much less so than films with little or no GOPS crosslinker.[26] In addition to high electrical conductivity, PEDOT:PSS also displays high ionic conductivities, with the ionic mobilities for small ions migrating in PEDOT:PSS reported to be similar to those occurring in dilute electrolytes.[16] The combined high ionic and electronic mobilities along with the biocompatibility, chemical stability, and ease of use are most likely the principal reasons for PEDOT:PSS emerging as the preferred material for organic bioelectronics devices. Importantly, volumetric, rather than surface capacitance is emerging as the key parameter for description of electronic devices that use PEDOT:PSS as active material.[27]

Vapor phase polymerized PEDOT:TOS

As mentioned above, polydioxythiophene conducting polymers can also be prepared *in situ* by either electropolymerization or vapor phase polymerization (VPP). Vapor phase polymerization was developed in the 1980s and has since been used widely as a method for synthesis of PEDOT:TOS (Fig. 2b). Indeed some of the highest conductivities of PEDOT derivatives have been reported using this method.[28,29] The process involves the deposition of an oxidant solution, typically Fe(III) Tosylate (Fe(TOS$_3$)), on the desired substrate. The substrate is then placed into a reaction chamber containing the EDOT monomer in vapor form. The EDOT monomer is oxidized by the Fe(III) and EDOT radicals are polymerized onto the substrate. Fe(III) is a strong oxidant and the polymer may become over-oxidized (and thus non-conducting) during the polymerization process. To avoid this, pyridine, a mild non-reactive base, is combined with Fe(III) to better control the polymerization process. The tosylate anion is used to dope the PEDOT in a manner analogous to PSS. In a final step, immersion of the polymerized substrates in a benign solvent such as ethanol is necessary to remove any remaining Fe(III). The films are said to collapse and form a final, relatively ordered polymer film; with the dopant ions forming distinct planes alternating with stacks of polymer chains.[30] To improve adhesion to substrates, a priming process can make the substrates

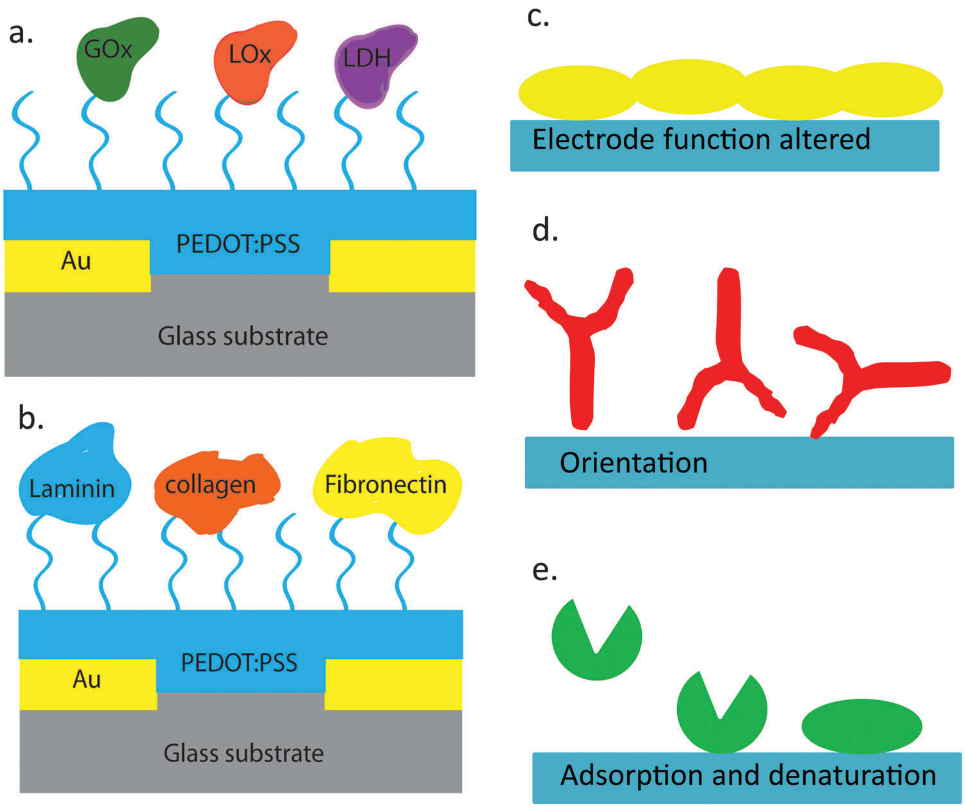

Fig. 3 Biofunctionalisation strategies of polydioxythiophene electrodes for either biosensing (a) or biocompatibility (b). (c) Biomolecules adsorbed onto active surfaces can result in loss or change of function for example through impedance of ion flow. (d) Orientation of the protein molecules is important. For antibodies, the antigen binding region (designated by the arms of the Y) should be oriented away from the surface. Other orientations result in non-functional antibodies. (e) Adsorption of molecules such as enzymes can result in loss of conformation and inaccessibility of active site.

hydrophobic, making it difficult for the water and electrolytes to penetrate underneath and lift the film. Again, along with the primary oxidant solution, other salts or molecules may be added during the polymerization, providing another means for incorporating active components that can improve properties or even biomolecules retaining their function.[31–33] However, the use of PEDOT:TOS in microfabricated devices has not been extensively explored and would require development of methods for controlling deposition at the micron scale. Additionally, the doping/de-doping process of PEDOT:TOS, that is to say, tosylate anion going in and out of the film, may raise toxicity issues when interfacing this material with sensitive cells or organs.

Electrochemically polymerized PEDOT

One of the most convenient and facile means for synthesizing conjugated polydioxythiophenes such as PEDOT and ProDOT is by electrochemical polymerization.[34] The EDOT or ProDOT monomers (Fig. 1a and b) are oxidized to the corresponding oligomers and polymers, forming connecting carbon–carbon bonds between monomers and losing the two hydrogens on either side of the thiophene rings in the process. During the reaction, the monomers are first converted into activated radical cations (Fig. 2c). These activated species react with each other and with other oligomers, increasing the molecular weight and eliminating two hydrogen atoms per monomer added. As the reaction continues, the higher order oligomers become more and more insoluble, and the polydioxythiophene product is precipitated onto the working electrode (cathode). The polymerizations are typically run at either constant voltage (potentiostatically), at constant current (galvanostatically), or in a cyclic sweeping mode.[35] The ability to control the extent of the reaction with an external current or voltage source makes it possible to precisely control the amount of material deposited onto the conductive surface. This process has even been used to polymerize PEDOT directly into the brains of rats, by using a microcannula to locally deliver the monomer solution directly adjacent to an electrode.[36,37] One drawback of the technique however, is that it must be carried out on an electrically conducting substrate.

Having provided a brief overview of the various methods for synthesis and preparation, we will now turn to specific examples from literature involving biofunctionalization of polydioxythiophene conducting polymers. As before, these examples are sorted by method of polymerization, since, as we will observe, the method of synthesis has profound implications on the route required for biofunctionalization.

Biofunctionalization of polydioxythiophene conducting polymers

Most biomolecules used for biofunctionalization fall into two categories; (i) a molecule used for biosensing such as an antibody or an enzyme (Fig. 3a) or (ii) a molecule used to generate a more biocompatible surface, *i.e.* for cell adhesion, examples being extracellular matrix proteins such as laminin, fibronectin and collagen (Fig. 3b). Glass and gold surfaces are some of the most commonly used transducer substrates and thus a wide variety of functionalization methods have been developed to link biomolecules more or less directly to these surfaces. These methods range from silanization to the often used EDC/NHS (EDC: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride/NHS: *N*-hydroxysuccinimide) chemistry for coupling of the commonly found biological amine and carboxyl functional groups.[38] For an excellent handbook the reader should see 'Bioconjugation techniques' by Greg Hermanson.[39] The basic principle of biofunctionalization is to find a method to stably link a biomolecule to a surface. From the device perspective it is important that the biofunctionalization method used does not significantly alter or hinder the device performance (Fig. 3c). Important considerations from a biological perspective include orientation of the molecule. In the case of antibodies it is important to insure that the antigen binding sites are pointing outwards, making the biomolecule available for interaction with a biological target (Fig. 3d). For enzymes, the active site must allow for transport of the reactants in and subsequent release of products. Most biological components and systems are complex macromolecules or macromolecular assemblies, whose three dimensional structure is a finely balanced ensemble of a multitude of energetically and entropically controlled effects including hydrophobic/hydrophilic forces, steric interactions, as well as intermolecular forces, such as Coulombic forces, van der Waals forces, and hydrogen bonding. In many cases, particularly for proteins which are amphipathic molecules, the problem is not how to achieve protein adsorption, but rather how to control it.[40] Often in the case where molecules are adsorbed electrostatically, the biomolecules are deformed to accommodate the electrostatic interactions (Fig. 3e). This may not matter so much in the case of structural proteins such as collagen or fibronectin, but for other protein types this can be very deleterious to function. Perhaps serendipitously, antibodies have been reported to adsorb to surfaces with the invariant Fc portion, leaving the reactive portion free to react. This is perhaps the reason that enzyme linked immunosorbent assays (ELISA) work so well despite the fact that antibodies are simply adsorbed to plastic supports. Indeed there is evidence that in some cases once adsorption occurs, the process is quasi-irreversible because of changes in the protein conformation which do not favor desorption.[40] A significant concern is that since CP devices are often prepared using solvents and curing temperatures that are incompatible with structural integrity of the majority of biomolecules, biofunctionalization should ideally take place after preparation of the films. Finally, since biofunctionalization generally is carried out in aqueous ionic solutions the charge of both the molecule and the surface must be considered to avoid repulsion of the biomolecule to be attached.

Before moving on to more directed biofunctionalization, it is worth saying a few words about adsorption of proteins on polydioxythiophene substrates.

Electrically controlled adsorption of proteins on polydioxythiophenes

In many cases where doped conjugated polymers have been used as substrates for cell growth, a mediating protein layer is added to the electrodes or active channels to encourage cell growth. This is particularly important for neural cell growth, where extracellular matrix proteins encourage cells not only to adhere but also to differentiate. Pioneering work in this field comes from Schmidt *et al.*, where they integrated cells on top of the conducting polymer polypyrrole (PPy).[10] Although Ppy was used in these early studies, later efforts focused on functionalized polydioxythiophenes such as PEDOT, because they proved to be more chemically stable.[41] The use of electroactive substrates for cell growth has also been used as a means to control and differentiate cells; early studies demonstrated that the ability of conducting polymers to be switched into different electrochemical states (reduced and oxidized), had dramatic effects on the adhesion and growth of neural stem cells.[12]

Salto *et al.* studied the growth of stem cells on PEDOT:TOS, finding significantly more cells on the oxidised surfaces. A significant difference in contact angle was observed on the bare PEDOT:TOS electrodes (red: 30.1° ± 5.1, ox: 58.1° ± 5.5), and the amount of adsorption of protein (HSA; human serum albumin) was found to be higher on the reduced PEDOT surfaces. It was postulated that the HSA was bound in a more optimal conformation for adhesion of the stem cells on oxidized surfaces (Fig. 4a).[12] Wan *et al.* showed subsequently that on a PEDOT:TOS device with oxidized and reduced pixels, local pH values of 7.1 and 7.7 were measured. This was hypothesized to result in conformational changes in the proteins on the respective pixel. FRET (fluorescence resonance energy transfer) analysis was used to confirm that the conformation of adhered fibronectin was more compact on the oxidized pixels and elongated on the reduced pixels (Fig. 4b).[42] An elegant embodiment of this effect was used by Herland *et al.*, who described a method for presentation of heparin-binding growth factors to adherent stem cells.[9] Electropolymerization of EDOT was done in a solution of

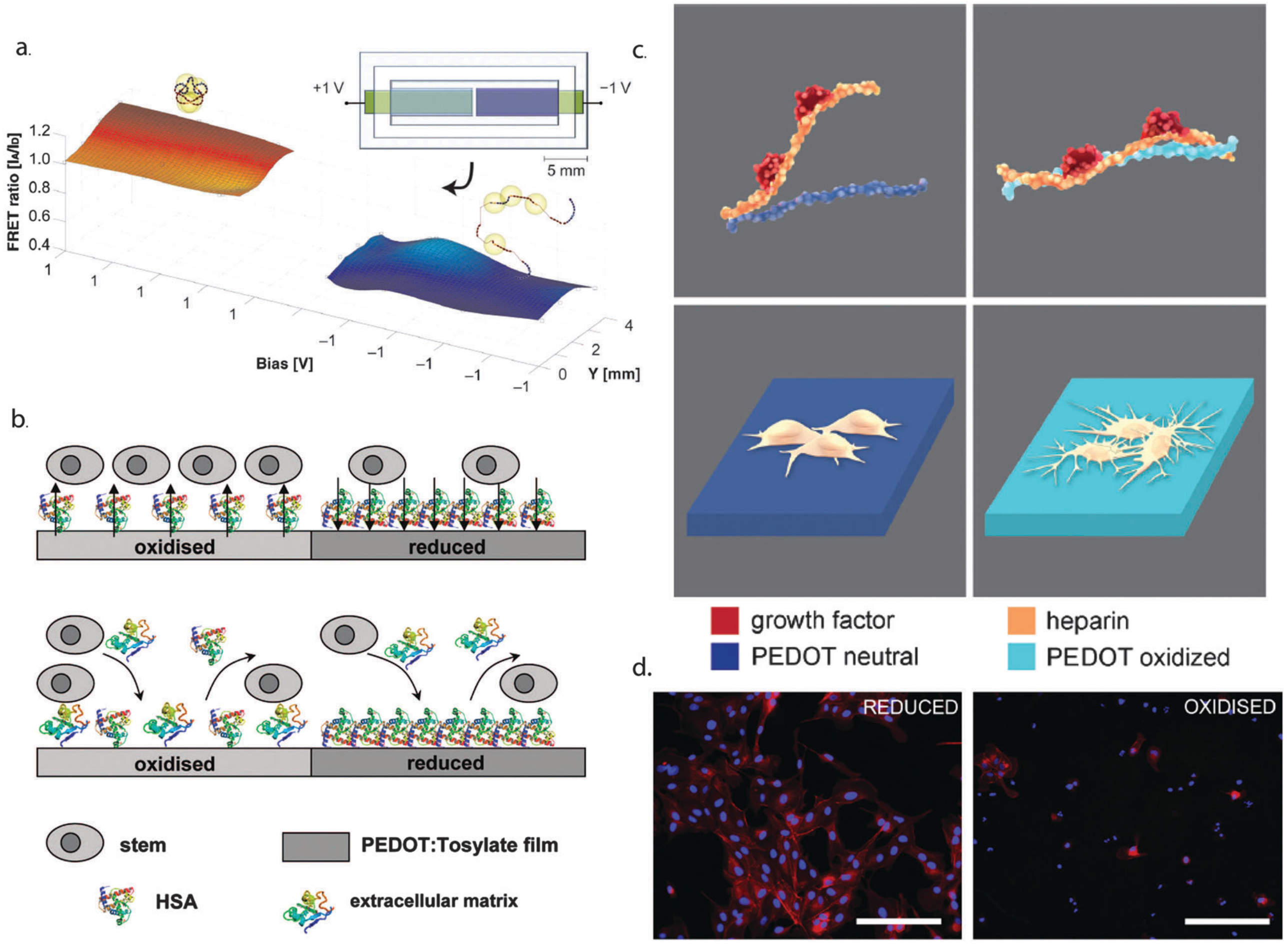

Fig. 4 Electrically controlled adsorption and function of proteins on PEDOT: (a) proposed mechanism explaining how changes in PEDOT surface chemistry alter protein binding and therefore affecting cell adhesion. From Salto *et al.*[12] Conformational changes of Fibronectin on top of oxidized and reduced PEDOT:TOS measured by FRET analysis. From Wan *et al.*[42] (b) and (c) PEDOT integrated with growth factors affecting stem cells differentiation. From Herland *et al.*[9] (d) MDCK cells stained for actin and nuclei on the biased PEDOT:TOS surface. Left – reduced surface; right – oxidized surface. From Svennersten *et al.*[43]

Clexane (a pharmaceutical grade, low-molecular weight heparin). As shown in Fig. 4c, the bioavailability (*i.e.* 3D protein conformation in correct orientation) of the anchored growth factors could be altered using the oxidation and reduction of PEDOT as an electrochemical switch, thus providing local control of the stem cell fate. A further example of this is shown in Fig. 4d, with differential growth of MDCK (Madin Darby canine kidney) epithelial cells observed on oxidized and reduced PEDOT surfaces.[43] Methods to elucidate these types of interactions between CPs and cells are emerging, including quartz crystal microbalance (QCM), single cell force microscopy, and electrochemical-AFM which may allow researchers to further fine tune biofunctionalization of CP electrodes, particularly with respect to understanding how to use electrical switching to the best advantage.[44,45]

Direct incorporation of biomolecules into/onto polydioxythiophenes

Oriented biofunctionalization may be preferred to achieve greater levels of control and stability, particularly in the case of sensitive proteins. Neither PEDOT nor PProDOT have convenient locations that allow them to be chemically functionalized without significant numbers of synthetic steps. This is also the case for the anions, both the polymeric anion PSS and the single molecule TOS. This situation has resulted in some more inventive approaches to biofunctionalization of polydioxythiophenes as will be detailed below.

Strategies avoiding biofunctionalization of polydioxythiophene

As noted earlier, several of the devices using PEDOT:PSS, in particular the organic electrochemical transistor (OECT), use contact/gate materials other than conducting polymers. Although it is possible to fabricate the OECT with conducting polymer electrodes, typically metal contacts are used as source and drain for defining a channel in the micrometer scale, while a choice is often made between silver/silver chloride (Ag/AgCl) or platinum (Pt) for the gate electrode. In other cases materials such as carbon nanotubes (CNTs) or graphene have been used as gate electrode. Highly sensitive biosensors using OECTs have been developed for sensing DNA, glucose, microorganisms and more, however many of the biofunctionalization methods involve modification of a non-polydioxythiophene based gate material. One example includes work by Tang *et al.* who used multi-walled carbon nanotubes with chitosan and electro-deposited platinum nanoparticles with immobilized glucose oxidase (GOx).[46]

In other cases, biosensing strategies have been developed without a biofunctionalization step. For example, Liao *et al.* presented an OECT for detection of diatoms, however the sensing mechanism relied on the binding of the diatoms to the negatively charged PEDOT:PSS channel, making specificity a significant challenge.[47] A review by Peng *et al.* focused on the use of CPs for electrochemical DNA detection, however although here only PANI and PPy were discussed, there was no mention of methods for immobilization of DNA onto PEDOT.[48] Another review by Lin *et al.*[49] focused on the use of organic thin film transistors for chemical and biological sensing, however again any functionalization strategies were not based on polydioxythiophenes but rather used other organic molecular semiconductors and CPs ranging from pentacene to polypyrrole. Similar approaches could be envisioned for PEDOT, and mechanisms of signal transduction might be expected to be similar.

Functionalization using solid state electrolytes

Another strategy that circumvents the direct immobilization of enzymes onto the CP was used by Yang and co-workers.[50] OECTs for glucose sensing were fabricated using room temperature ionic liquids (RTILs) as the electrolyte. The reason for the use of the RTIL was twofold: to solve issues related to long-term stability of the OECTs and as a means of incorporating an enzyme. RTILs are salts that are molten at room temperature and are increasingly emerging as alternatives to aqueous electrolytes. RTILs avoid issues related to evaporation, and also changes in ionic concentration. In addition, they have been shown to stabilize biomolecules such as enzymes.[53,54]

Yang *et al.*, essentially used the RTIL as a biofunctionalization layer, restricting it in space *via* simple hydrophobic patterning. The RTIL formulation contained the enzyme glucose oxidase, and also a ferrocene mediator which was poorly soluble in water. Detection of glucose was demonstrated in the micromolar range (Fig. 5a). Subsequently, Khodagholy *et al.*[51] combined ionic liquids with crosslinkable polymers, to develop an OECT-based lactate sensor integrated with a solid state electrolyte (ionogel) (Fig. 5b). The resulting device was used to detect lactate in the physiological range for sweat. A significant advantage was the absence of liquid electrolyte which renders devices wearable, with the idea that sweat, or other physiological fluids could diffuse into the ionogel. This ‘dry electrode’ approach has been used to make electrodes of gold and PEDOT:PSS for non-invasive skin monitoring of human electrophysiological activity coupled with an ionogel (Fig. 5c). Improvements were shown in terms of wear time, stability and the biotic–abiotic interface.[52,55] Although a repertoire of biocompatible ionic liquids has been developed for use with proteins,[56,57] their biocompatibility is only beginning to be tested on skin.[58] Future work will need to focus on further development of biocompatibility and specifically cytocompatibility.

Silane-mediated biofunctionalization of PEDOT:PSS

In many cases it is desirable to functionalize the CP itself, which is generally used as the channel of the device. As mentioned before, silanization has been extensively used as a biofunctionalization method. This versatile family of molecules includes homo-bifunctional and hetero-bifunctional classes, which may consist of a variety of useful functional groups for reaction with biomolecules, including carboxyl, amine, thiol, and epoxy groups. One example of a silanization method is that of Kim *et al.* who developed an immunosensor and functionalized PEDOT:PSS films using an amino silane; l,3-aminopropyldiethoxymethylsilane (APTMS).[59] The functionalization was characterized by FT-IR spectroscopy (Fourier Transform Infrared), however few details were given on the exact nature of the reaction, so it was unclear if this was direct chemical bonding or absorption. An antibody for prostate specific antigen (PSA) was immobilized on the channel (Fig. 6a).

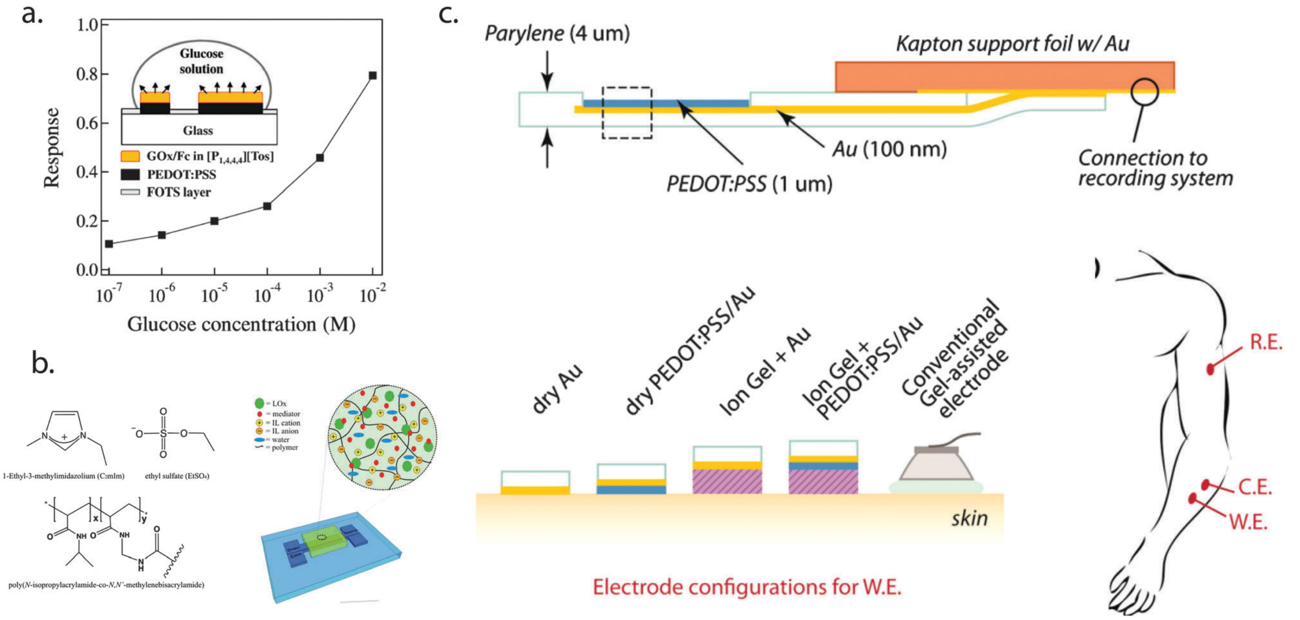

Fig. 5 Incorporation of biorecognition elements into Ionic liquid based electrolytes: (a) calibration curve of an OECT based glucose sensor with ionic liquid (IL) electrolyte. The inset shows the concept of device operation; the enzyme and the hydrophobic mediator are dissolved in the IL. From Yang *et al.*[50] Reproduced by permission of The Royal Society of Chemistry. (b) Similarly OECT based lactate sensor integrated with a solid state electrolyte. The IL enzyme and mediator are stuffed inside an iongel electrolyte with photo-croslinkable polymer. From Khodagholy *et al.*[51] Reproduced by permission of The Royal Society of Chemistry. (c) PEDOT:PSS based multielectrode array for cutaneous electrophysiology monitoring. The stability for long term measurements has been increased with the addition of an iongel electrolyte. Schematic of the electrode assembly, and cross-section of an electrode. As well as a schematic of the electrode positions on a subject's arm. The working electrode (W.E.) and counter electrode (C.E.) were placed on the forearm, 5 cm away from each other. The reference electrode (R.E.) was placed on the arm, 30 cm away from the W.E. From Leleux *et al.*[52]

The shift of the effective gate voltage was found to be proportional to the captured PSA antigen concentration. Detection was made possible using a secondary antibody conjugated with Au nanoparticles, in a typical sandwich-ELISA format, thereby resulting in an increased sensitivity, mostly likely due to the fact that Au–NPs are negatively charged in suspension.

He *et al.*, have also used a silane approach to fabricate an OECT that detects the presence of the pathogenic bacteria *E. coli*.[60] The immobilization step of the anti-*E. Coli* antibody took place through biofunctionalization of the PEDOT:PSS channel (Fig. 6b). Plasma treatment was used to create hydroxyl radicals for subsequent reaction with the silane GOPS (3-glycidoxypropyl-trimethoxysilane). Fig. 6b(ii) shows the effect of differing amounts of plasma treatment related to the capture of bacteria on the surface, characterized by quantitating the fluorescence of FITC-labeled antibodies binding to the bacteria. One drawback of the device however, was the low efficiency of detection in solutions with ionic concentrations greater than 10 mM.

Strakosas *et al.*, demonstrated an easy-to-use biofunctionalization method for PEDOT:PSS.[61] By incorporation of the polyalcohol PVA (poly vinyl alcohol) into the CP solution before casting, a synthetic 'handle' was provided for future biofunctionalization, as shown in Fig. 6c. The PVA was found not to interfere with the device performance; in fact alcohols have been reported to improve conductivity of polydioxythiophene films.[20] The PVA introduced alcohol groups into the PEDOT:PSS films which were subsequently reacted with silanes. The silane GOPS was used, which as mentioned is often also used as a crosslinker in the PEDOT film. This provided an epoxy group which formed a covalent bond with the amine groups of two different biomolecules tested, namely glucose oxidase (GOx) and poly-L-lysine. Quantification of the binding of PLL was carried out by fluorescence microscopy as before, along with demonstration of preferential binding to the PLL functionalized areas on the PEDOT:PSS film (Fig. 6c(ii)). Additionally, QCM was used to quantify the biofunctionalization of GOx on the surface before using the device to detect glucose in a solution of PBS (Fig. 6c(iii)). Biofunctionalization of GOx raised an important issue, which is the isoelectric point (pI) of the protein to be functionalized using such methods. At the pH used, GOx has a pI of 4.2, rendering it most likely negatively charged in solution. In contrast, PLL is highly positively charged with many free amines, perhaps explaining that PLL would be more densely functionalized on the PEDOT:PSS surface, since repulsion inevitably occurs between the GOx and the negatively charged PSS. Berezhetska *et al.* used a blending/silanization approach, mixing carboxymethyl dextran (CMD) with a PEDOT:PSS formulation, to achieve a surface with carboxyl group functionalization.[62] The optimal concentration of CMD was found to be 0.1% w/v, with no significant effects observed on film conductivity. This method was used to graft antibodies onto the surface of films *via* EDC/NHS chemistry and induce formation of an amide bond. An ELISA was then used to quantify the amount of a captured protein on the surface.

Based on the aforementioned strategies of mixing polymers with the CPs in solution prior to film deposition, a large

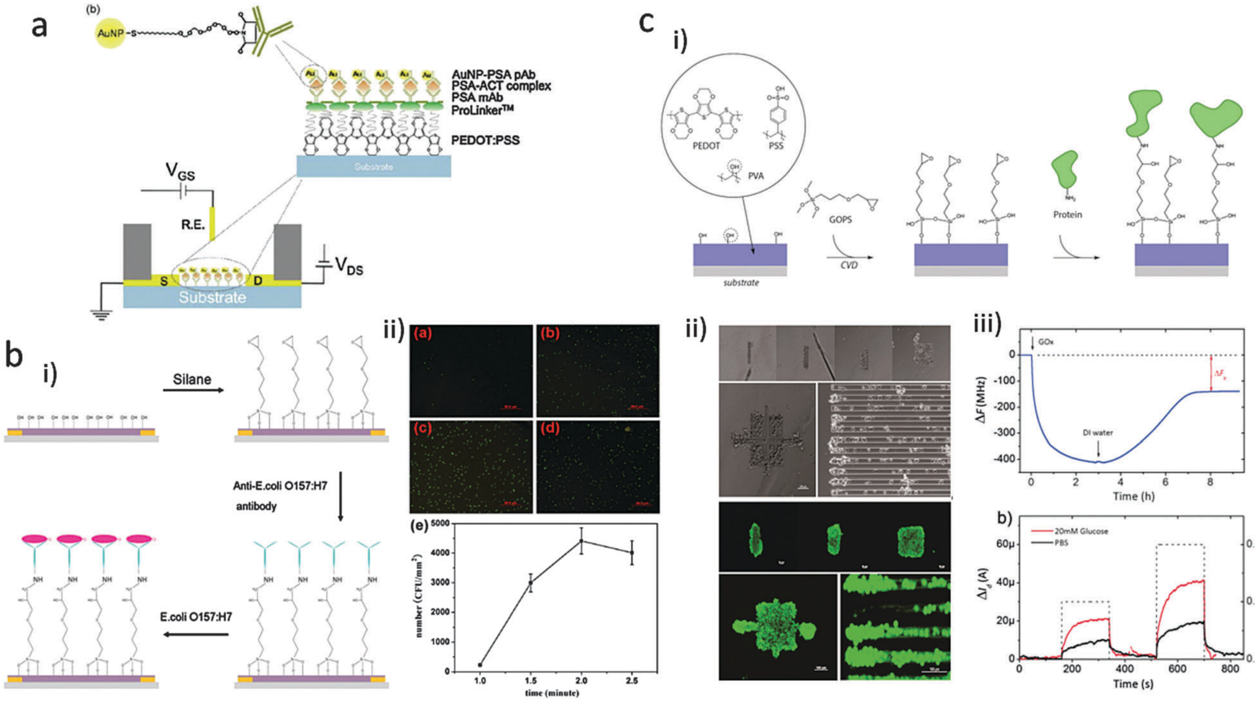

Fig. 6 Covalent surface functionalization of PEDOT:PSS: (a) OECT-based immunosensor for the detection of PSA–ACT complex utilizing gold nanoparticles for signal amplification. From Kim *et al.*[59] (b) (i) The schematic diagram for immobilizing anti-*E. coli* O157:H7 antibodies on the surface of PEDOT:PSS and subsequent capture of bacteria. (ii) The influence of oxygen plasma treatment on the efficiency of *E. coli* O157:H7 capture shown by Fluorescence imaging after FITC-labeled anti-*E. coli* O157:H7 antibodies were added. The PEDOT:PSS films were treated with oxygen plasma for different periods and correlated with numbers of bacteria captured. From He *et al.*[60] Reproduced by permission of The Royal Society of Chemistry. (c) (i) Reaction scheme for biofunctionalization of PEDOT:PSS by incorporation of PVA. (ii) Fluorescence intensity of patterned poly-L-lysine with FITC dye on PEDOT:PSS:PVA. (iii) QCM monitoring of GOx deposition on PEDOT:PSS:PVA films. Sensing of glucose with OECT solid line is I_d while dashed line represents V_g. From Strakosas *et al.*[61] Reproduced by permission of The Royal Society of Chemistry.

repertoire of methods are possible for the biofunctionalization of PEDOT:PSS for biological applications. Based on the application at hand, strategies can be devised that are specific for overcoming challenges. For example, Kergoat *et al.* crosslinked the enzymes (2-oxoglutarate, choline oxidase, and L-glutamate oxidase) with bovine serum albumin (BSA) *via* glutaraldehyde, for the detection of the neurotransmitters glutamate and acetylcholine. Glutaraldehyde, crosslinks to the amine groups of the enzymes and BSA, and BSA in turn adsorbs at the PEDOT:PSS surface. The catalytic activity of PEDOT was improved by blending it with Pt nanoparticles.[63] In addition, biofunctionalization strategies may be designed for repulsion or reduction of interfering species to reduce non-specific signals in biosensing. One example of this is recent work by Pappa *et al.*, where PEDOT:PSS/PVA films were functionalized *via* silanization by addition of chitosan ferrocene. This strategy lowers the effective gate operation through the use of the ferrocene mediator, thus reducing the number of electroactive species which would otherwise react at the gate, causing a high background signal. The amine groups on the chitosan were also used to form complexes with different enzymes *via* EDC/NHS chemistry to functionalize with a variety of redox enzymes for metabolite sensing in saliva.[64] Thus, the biofunctionalization strategy is designed to generate more sensitive and specific sensing in complex biological fluids.

Biofunctionalization of PEDOT:TOS prepared by vapor phase polymerization

Vapor phase polymerisation (VPP) (as described in the introduction) is an *in situ* polymerisation technique, in which an oxidant is used to coat a surface on which the EDOT monomer is then exposed to, leading to the formation of a conducting polymer film.[29] 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.[65] A significant amount of attention has been expended on improving the conductivity of PEDOT:TOS using molecules such as polyethylene glycol (PEG).[28] In this vein, Jimison *et al.*, demonstrated the addition of PEG to PEDOT:TOS, maintaining electroactivity and indeed improving conductivity (Fig. 7a).[31] Again, care was taken to ensure that, when devices such as OECTs were fabricated using this material, they had performance identical compared to neat PEDOT:TOS films. Further, since PEG is a biocompatible material, whose alcohol groups can be readily activated for subsequent functionalization of bioactive species, advantage was taken of the readily activated alcohol groups on the PEG. Composites comprising of PEDOT:TOS and a carboxylic acid functionalised PEG, referred to as PEG(COOH), were made and characterised to confirm the presence of both PEG-COOH and PEG. Subsequently, EDC/NHS chemistry was used to incorporate fluorescent proteins onto these surfaces *via* the carboxyl groups present in the functionalized PEG. To rule out electrostatic interactions, wash steps were performed both with detergent and high salt. Quantitation of bound fluorescent protein showed that low amounts of protein were bound covalently, the low yield postulated to be due to the paucity of reactive hydroxyl groups available for binding.

In a subsequent paper, Bongo *et al.*, incorporated gelatin, a derivative of collagen (one of the most well-known extracellular matrix proteins), into PEDOT:TOS by VPP as a substrate for endothelial cell growth.[32] In this study, the difficulty was that the use of Fe(III) as oxidant for PEDOT VPP in combination with a hydrophilic polymer with a large amount of active groups, could result in the formation of a gel during mixing of the VPP precursors, thus preventing the coordination of Fe(III) to the active groups. This challenge was overcome by changing the solvent system to a combination of water and acetic acid, the latter preferentially coordinating to Fe(III) and thereby preventing gelation. As acetic acid is a weak acid, it was thought to be a good choice to avoid denaturation of biomolecules included in the oxidant solution. Once again, the method was designed to not only maintain the electrical properties of the CP, but also to retain the functionality of the biomolecule. The presence of gelatin was confirmed in the bulk of the film by NanoSIMS (nanoscale secondary ion mass spectrometry) and surface exposed gelatin by an immunoassay using anti-gelatin antibodies. The PEDOT:TOS:gelatin composites were shown to support growth of bovine capillary endothelial cells (BBCEC) just as well as films onto which gelatin was drop cast.

The process of polymerizing PEDOT in the presence of biomolecules *via* VPP is straight forward, avoiding further chemistry processes, however, it is not suitable for sensitive biomolecules, as high temperatures and strong acids can harm the function of these molecules. Instead of incorporating biomolecules during the polymerization process, another alternative is to entrap biomolecules into films. One highly desirable feature when immobilizing redox enzymes on/in electrodes is the ability to directly wire the active site of the enzyme to the electrode, as in the case of Gregg *et al.* who demonstrated electrical connection of the active site of the GOx with a redox active polymer based on osmium complexes.[67] Thomson *et al.* illustrated that glucose oxidase could be entrapped (stuffed) into VPP PEDOT:TOS films, and showed evidence of direct wiring between the CP and the enzyme active center (Fig. 7b).[66] In more detail, the enzyme was cast as a droplet over the unwashed PEDOT film. During the removal of Fe(II)–Fe(III), when the film collapsed it was hypothesized to entrap the enzyme into the bulk of the PEDOT:TOS film. This result is surprising because it implies that the active site is within the appropriate distance

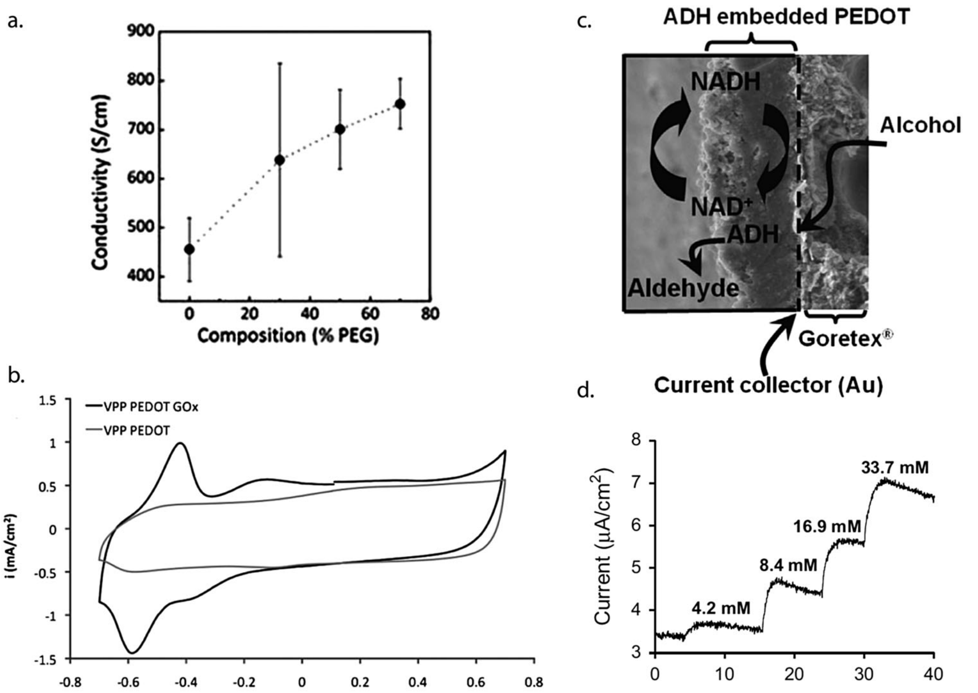

Fig. 7 Biofunctionalization of VPP PEDOT:TOS (a) conductivity of PEDOT:TOS as a function of PEG content. From Jimison *et al.*[31] Reproduced by permission of The Royal Society of Chemistry. (b) VPP-PEDOT/GOx shows characteristic peaks of GOx. Post-growth CVs of VPP-PEDOT with and without GOx incorporation (3rd scan shown, taken at 10 mV s^{-1}). VPP-PEDOT/GOx shows redox peaks characteristic of GOx at 0.58 to 0.43 V *versus* Ag/AgCl. From Thompson *et al.*[66] (c) Schematic of the three phase-interface reaction between PEDOT/ADH, alcohol vapour and NAD+/NADH redox couple in the electrolyte. (d) Current (*I*) modulation as a function of ethanol content. From Winther-Jensen *et al.*[33]

from the PEDOT to allow electron transfer (Fig. 7b). It is worth mentioning that in electropolymerized films the wiring of the enzyme with PEDOT:TOS has not yet been realized. Detection of glucose was not possible, most likely due to inactivation or denaturation of the enzyme. Winther-Jensen *et al.*, used a similar approach to prepare PEDOT:TOS films containing alcohol dehydrogenase (ADH), this time in a phosphate buffer, using basic conditions (Fig. 7c). The enzyme solution was allowed to soak into the films and then repeated to ensure sufficient enzyme capture. Characterization by SEM (scanning electron microscope) clearly shows enzyme globules and surface accumulation on the PEDOT/ADH films.[33] The stuffed films were combined with porous membranes, and in this case, a functional alcohol vapor sensor was demonstrated (Fig. 7d).

Biofunctionalization of polydioxythiophenes prepared by electropolymerization

Early work on incorporation of biomolecules into conducting polymers *via* electropolymerisation predominantly focused on biosensing of metabolites by either entrapping or attaching oxidoreductase enzymes onto CP electrodes.[68] The majority of this work was done on polypyrrole, however, a very limited amount was done with polydioxythiophenes. Increased biofunctionality was achieved by incorporating bioactive species into the conducting polymer, including peptides and proteins for encouraging cell adhesion,[69–71] or directing axonal growth and regeneration.[72] Two early examples include a study of the bioelectrocatalysed reduction of nitrate utilizing polythiophene bipyridium enzymes,[73] and amperometric bionsensors produced by immobilization of redox enzymes at polythiophene-modified electrode surfaces.[74] Further, Kanungo *et al.* showed the operation of a PEDOT-based conductometric sensor, where antibodies were either incorporated during electropolymerization or adsorbed onto the surface of films, showing efficient detection of an antigen, particularly in the case where the antibody is incorporated into the polymer during polymerisation.[75] However, no explanation was given for the mechanism of operation.

In recent years, much of the work done on biofunctionalization of PEDOT prepared *via* electropolymerization was motivated by the desire to increase the biocompatibility of this material for coating electrodes, used either *in vitro* or *in vivo*. PEDOT has been directly polymerized in the presence of neural tissue, and most of the neurons retained their function (Fig. 8a). PEDOT blended with hydrogels has been used to increase the soft nature of coatings in order to bridge mechanical mismatch with soft biological tissue (Fig. 8b) Neither the PEDOT nor PProDOT homopolymers have any functional organic groups that are expected to promote specific interactions with biological tissue. However, it is possible to incorporate biologically active molecules as counterions for conjugated polymers during the polymerization. Examples include peptides such as CDPGYIGSR, the functional sequence from laminin.[34,79] 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, as stated before, care must be taken that the introduced biological molecules do not drastically disrupt the electrical properties and also, that the biomolecule remains active post electropolymerization. The incorporation of neural growth factors with ligands into PEDOT films *via* electropolymerization has been shown to result in both decreased electroactivity and poor mechanical properties attributed to the change in polymerization rate upon the addition of the biomolecule.[80,81] A further example by Teixeira-Dias *et al.*, involved the incorporation of dextrin in both a cyclic and linear form to make biocomposites. Although it was noted that the electrostability/activity was decreased in the composite films, the films showed slightly increased performance with respect to cell growth and proliferation.[70]

Another method for creating biofunctional dioxythiophenes is to introduce chemically active groups during polymerization.[82] One example is the carboxylic-acid substituted EDOT monomer (EDOT-acid). EDOT-acid can be used to create EDOT/EDOT-acid copolymers with systematic variations in surface wetting properties.[83] Another area of utility for these functionalized monomers is in improving adhesion to solid substrates. EDOT-acid promotes the strength of interaction of PEDOT on metal surfaces such as ITO (Fig. 8c), resulting in highly mechanically stable films (Fig. 8d). Since the acid group does not disrupt the thiophene ring, the films remain highly electrically active (Fig. 8e).[77]

An added advantage is that the acid groups make it possible to readily attach various functional molecules, such as peptides, onto the PEDOT copolymer films (Fig. 8f). Povlich *et al.*, used this method to create GGGGRGDS-peptide functionalized PEDOT films.[78] This peptide contains the RGD sequence from the extracellular matrix protein fibronectin, found to be important and effective in promoting cell adhesion to a variety of substrates. The RGD-functionalized PEDOT films promoted adhesion and cell differentiation of primary rat motor neurons (Fig. 8g and h). Notably, films with only the hydrophilic EDOT-acid alone were even more resistant to cell adhesion than the unmodified EDOT controls.[78] As mentioned before, the EDOT acid has profound influence on the wetting properties of films. The mechanisms of the EDOT to PEDOT transition during electrochemical deposition have recently been imaged directly in the TEM (transmission electron microscope) using a liquid cell and low dose techniques, providing unprecedented local insight about the nature of the liquid-like domains that form near the electrode surface, presumably from the oligomers created during the reaction.[84] The size of the structures seen in dynamic TEM correlated well with the characteristic lumps seen on the sample surface in SEM and AFM.

Other crosslinkers that have been examined for electrochemically deposited PEDOT films include a monomer with three EDOT units around a central benzene core (EPh) (Fig. 8a),[85] and a polyhedral oligomeric silsesquioxane (POSS) functionalized with 8 ProDOT moieties, one on each corner (Fig. 9a). It has been shown that the EPh significantly disrupts the conjugated of the PEDOT chain backbone when incorporated

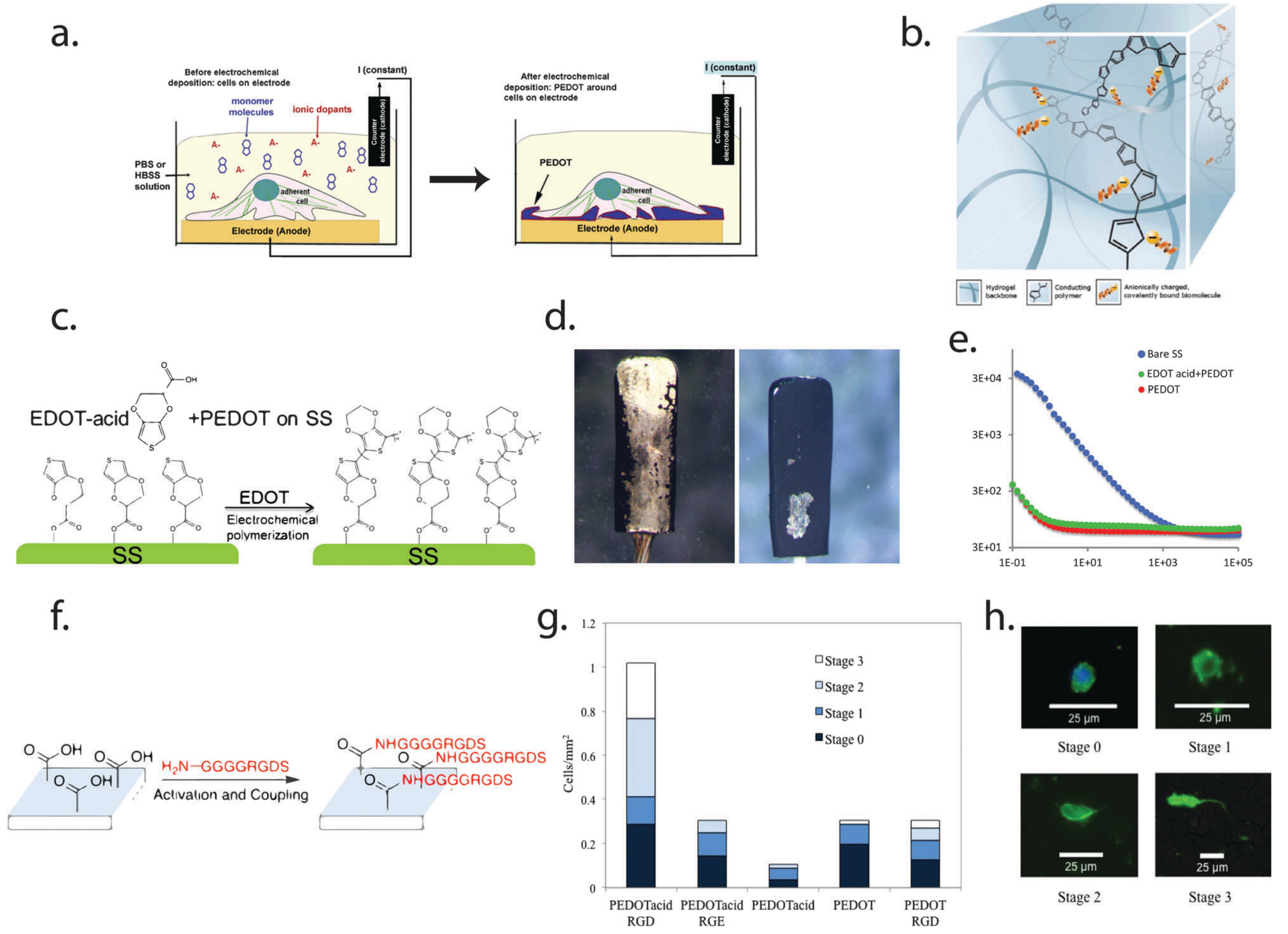

Fig. 8 Electrochemically polymerized PEDOT has been biofunctionalized using a variety of methods. (a) Schematic representing the polymerization of PEDOT around a living cell, prior to (left) and after (right) the polymerization. From Richardson Burn *et al.* (b) Schematic of an ideal hybrid configuration of a conductive hydrogel. From R. Green *et al.*[86] (c) A thin EDOT carboxylic acid was reacted with an indium tin oxide (ITO) surface to create an initial layer to promote adhesion; electrochemical deposition was then used to deposit PEDOT. (d) Pictures of electrodes with PEDOT after 2 min sonication, showing the mechanical stability of PEDOT film electropolymerized on top of EDOT carboxylic acid monolayer. (e) Impedance measurements for electrodes coated with EDOT and EDOT-acid *versus* bare stainless steel electrode. (c–e) From B. Wei.[77] (f) Biofunctionalization of P(EDOT)–P(EDOT-acid) copolymer films with peptides. (g) Number of primary motor neurons per mm^2 on conjugated polymer samples. Each column represents 2 samples of the same type divided by the total area (56 mm^2) and the cells are in various stages of development (0–3) as depicted in (h). (h) A larger number of cells attached to PEDOT–PEDOTacid–RGD and these samples also had more highly developed cells (stage 3). Cells were stained with DAPI (nuclei, blue) and Tuj1 (neuron-specific class III β-tubulin, green). DAPI staining did not fluoresce well on PEDOT–PEDOT acid films, possibly because of absorption by the polymer film. (f–h) From Povlich *et al.*[78]

as a co-monomer, leading to dramatic changes in color and a corresponding loss of charge transport performance.[85] POSS allows for large amounts of crosslinking, and since the molecule was designed with the thiophenes located well away from the crosslink center, there is minimal disruption to the conjugated thiophene backbone (B. Wei, PhD dissertation, University of Delaware).

Another convenient route towards biofunctionalization is the ene-functionalized ProDOT (Fig. 9b). This monomer can be created with either a single ene on the middle carbon[87] or with two enes.[77] A wide variety of functional groups can be added in a post-polymerization step through a thiol–ene "click" reaction.[86] The functionalized PProDOTs created in this manner show dramatic differences in charge transport performance depending on whether the thiol substituent used was a charge-blocking alkyl group, a hydrophilic alkoxy moiety, or an electroactive ferrocene (Fig. 9c).[86] Similar synthetic strategies could be easily used with biologically functionalized thiols to make appropriately modified conjugated polythiophenes.

One important consideration for electrochemical polymerization is that to successfully precipitate solid films onto substrates, it is necessary to retain the insoluble nature of the polymer being synthesized. With large substituents, the monomers and resulting polymers may become too soluble in the deposition solvent. This can be mediated by finding alternative solvents such as propylene carbonate or ionic liquids. Ionic liquid groups can be added to the EDOT monomer as well.[88] Another strategy is to deposit the films first, and then functionalize them only after the solid films have been formed on the electrode. This has the added advantage of limiting the

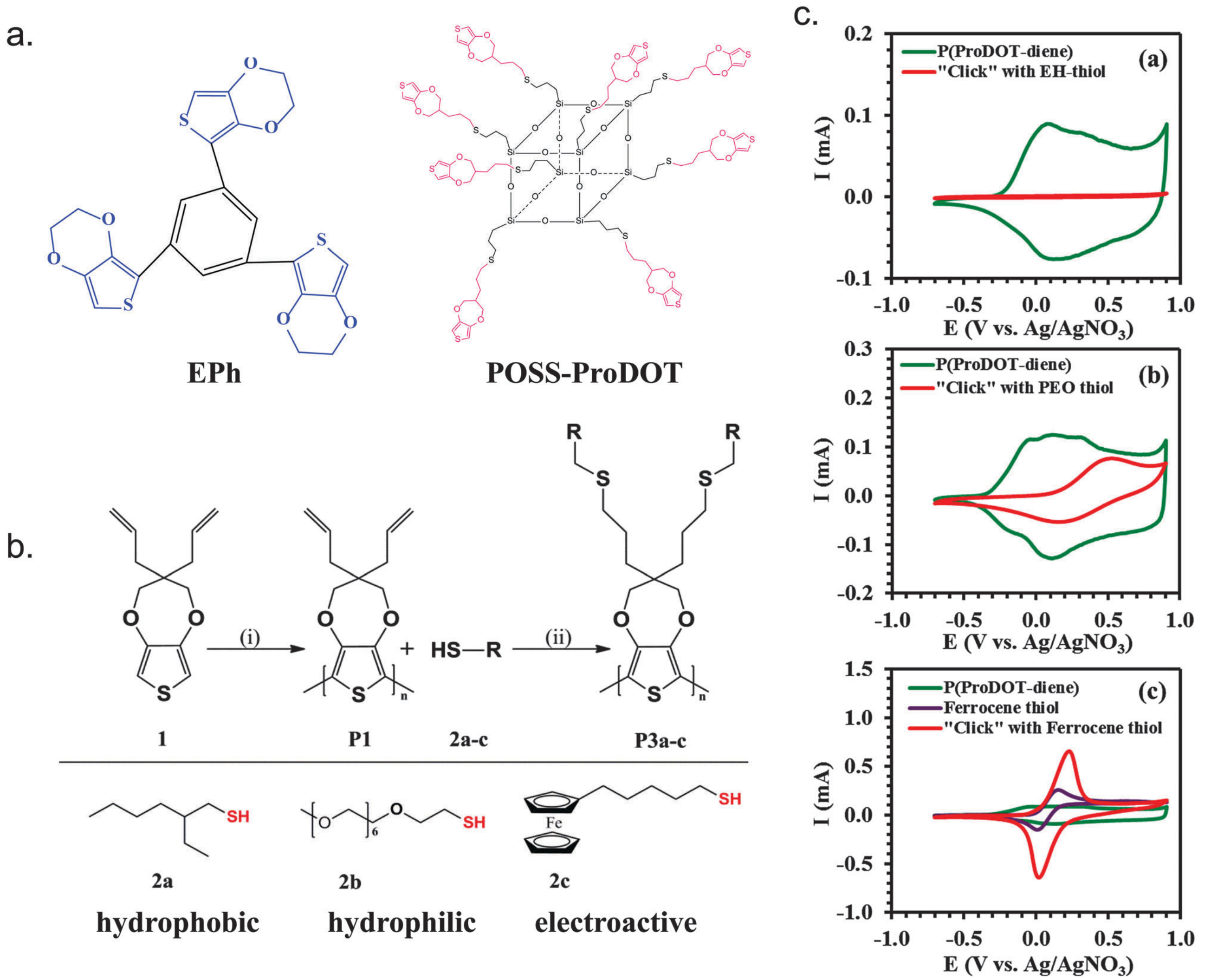

Fig. 9 (a) Structures of EPh and POSS ProDOT. (b) Post-polymerization functionalization of P(ProDOT-diene) thin films; electrochemical polymerization of ProDOT-diene and subsequent post polymerization functionalization with three different functional thiols (i–iii) *via* thiol–ene "click" chemistry. (c) Cyclic voltammograms of P(ProDOT-diene) films after functionalization with various terminal thiols (a–c) in acetonitrile containing 0.1 M TBAP as electrolyte. From Wei *et al.*[86] Reproduced by permission of The Royal Society of Chemistry.

amount of the usually more valuable and limited in quantity functionalized molecule necessary to create the film.

Conclusions

The polydioxythiophenes PEDOT, and more recently ProDOT, have emerged as champion materials in the field of organic bioelectronics, both in the domain of biosensing and also for integration with living cells (both *in vitro* and *in vivo*). Although polydioxythiophenes in their pristine forms have shown great promise for bioelectronics, in order to broaden the spectrum of applications, a biofunctionalization step is essential.

We have attempted to summarise the methods that have been used thus far to biofunctionalize polydioxythiophenes. As we discuss above, polydioxythiophenes can be synthesized in many versatile ways, such as chemical oxidative polymerization, vapor phase polymerization or direct electrochemical polymerization. For use in bioelectronic and biosensing applications, due to the common requirement of a thin film geometry, these polymers are mostly prepared through chemical oxidative polymerization (PEDOT:PSS) and direct electrochemical polymerization (PEDOT:ClO_4^- or PEDOT:TOS).

Different biofunctionalization techniques can be employed according to the needs of each specific application. A facile approach is to drop-cast biomolecules and proteins directly on top of the CP where they will adsorb to the surface. However, biomolecules may desorb from the surface or denature, resulting in unwanted changes in device performance. We anticipate that the field of bioelectronics will see many more applications where long term stability is a prerequisite, meaning that bio-species must be covalently bound onto the surface of the polymer. One example is the immobilization of enzymes on implantable biosensors for continuous measurements of brain metabolites, currently ongoing in the Owens group.

A significant advantage of PEDOT and ProDOT films is the ability to tailor their composition by either blending together different materials during processing, or by manipulating the structure of the monomers used during synthesis. As a result, a repertoire of different biofunctionalization techniques is available. Robust proteins blended with PEDOT can result in entangled biocompatible conducting polymers that will maintain stability and enhance the properties of both the proteins and the conducting polymers. For sensitive proteins, where the functionalization needs to take place after the formation of the polymer, functional groups can be provided either by blending PEDOT with an intermediate polymer with appropriate functional groups, or by modified EDOT and ProDOT monomers. In the future, we expect to see more blends and composites, where materials with high biocompatibility and good mechanical properties such as PEG or natural/synthetic elastomers can be incorporated into the polymer chain backbones (alternating copolymers) or side chains (graft copolymer) to improve the biocompatibility and long term performance of the devices. In addition to synthetic conjugated polymers, natural molecules, such as melanin,[89] indigo,[90] chitin[91] could also be of great interest. Indeed, a whole family of naturally occurring polymers and small molecules exist, besides the more obvious extracellular matrix and connective tissue materials such as glycosaminoglycans, polysaccharides *etc.*, which could be blended into polydioxythiophenes. Blends and hydrogels containing polydioxythiophenes may also play an important role in tissue engineering, where the ability to create electroactive 3D structures to host tissues is a key attribute.[76,92,93]

Direct functionalization or copolymerization with synthetic polymers could be beneficial for biosensing applications. Recently, it was found that the toughness and the stretchability of PEDOT:PSS films can be greatly improved *via* blending with PEG.[94] This mixture will be also interesting for bioelectronics if it could form a network structure *via* crosslinking. As more data is accumulated on the effect of biomolecules on electrical properties of polythiophenes, a better understanding will arise on which biofunctionalization method is most appropriate for the application at hand. For example in the case of P3HT, a semi-conductor frequently used in organic field effect transistors,[8] a significant body of work has arisen to describe the effect of biofunctionalization on the charge mobility.[95] Understanding the effects of different formulations of polymer (*e.g.* regioregular *vs.* regiorandom in the case of P3HT) on the denaturation of proteins adsorbed on the surface may also be important. Future studies will likely provide additional details about similar effects for PEDOT and ProDOT as well.

As a clear understanding emerges of the desired properties of electronic materials to improve signal transduction, an improved ability to characterise the biotic/abiotic interface will become essential. A potential drawback of polydioxythiophenes, prepared *via* the methods shown here, is the lack of information on the structural characteristics of the bulk films. In fact, since the majority of characterisation is done on dry films using classical tribology type methods, very little is understood about how structural properties of these films change in aqueous milieu. This opens the door to characterisation techniques that will allow operation in aqueous solutions to probe not only the interface but also the bulk. Early attempts using AFM, SCFS (single cell force spectroscopy) and QCM have made some progress here, but alternative methods are also required.[45] Label-free surface sensitive optical techniques such as SPR[96] (surface plasmon resonance) and DPI[97] (dual polarization interferometry) could also be adapted for use with biofunctionalized, polymer coated substrates to probe biomolecular interactions thanks to the (mostly) optical transparency of polydioxythiophenes.

Future work on generation of electrodes or devices using these functionalized polymers will necessarily focus on better understanding of the exact nature of the biotic/abiotic interface, and more precise tuning of the surface, both with respect to the conducting polymer, but also the biomolecule immobilised in or on the film. Taking advantage of the properties of conducting polymers in combination with different functionalization techniques can further improve current technologies in the area of bioelectronics and healthcare, by enhancing signal transduction. Furthermore, state of the art devices will emerge that will combine diagnostics and treatment, for example by monitoring a metabolite and generating therapy based on feedback to the device.[98] Recently, PEDOT was integrated with plants creating "cyborg plants" that conduct electricity. This potential could be used in future for harvesting electrical energy through the plants or improving their properties.[99] It may not be such a stretch to imagine biofunctionalised CPs integrated with the human body. There is still an ongoing interest in developing new materials with higher biocompatibility and longer stability for the next generation of conjugated polymer based bioelectronics or biosensors. These new materials should help enable the direct biointegration engineering technologies that have been proposed to improve the sensitivity of biosensors and for brain machine interfaces as envisaged in many science fiction films.

Acknowledgements

We gratefully acknowledge funding from the European Research Council ERC-2010-StG Proposal No. 258966 (IONOSENSE).

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