



## Review

## Functionalization of conducting polymers for biointerface applications

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## ABSTRACT

As the field of biomedical engineering grows, there is increasing demand for materials that can interface with biological materials – notably for use as biosensors, medical implants, neural interfaces and tissue engineering scaffolds. Conducting polymers are promising candidates for these purposes, owing to good biocompatibility and intrinsic conductivity with superior mechanical properties compared to traditional metal-based materials. Functionalization of the conducting polymers can further enhance these advantages, imparting properties such as improved solubility, antifouling behavior, stimuli-responsive switchability and the ability to modulate cell growth and differentiation. This review briefly discusses the incorporation of biodopants and direct attachment of (bio)molecules to the conducting polymers, before focussing on the rapidly-growing field of polymer grafting – with the aim of tailoring these materials towards a range of bioapplications.

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## 1. Introduction

A number of applications require materials to interface with cells, tissues or biomolecules. The specific interactions (or lack

*Abbreviations:* AFM, atomic force microscopy; ATRP, atom transfer radical polymerization; CP, conducting polymer; CS, chitosan; DEGMA/OEGMA/PEGMA, di-/oligo-/poly(ethylene glycol) methyl ether methacrylate; OEG/PEG, oligo-/poly(ethylene glycol); PANI, polyaniline; PBS, phosphate buffered saline; PEDOT, poly(3,4-ethylenedioxythiophene); PPy, polypyrrole; PS, polystyrene; PTH, polythiophene; QCM-D, quartz crystal microbalance with dissipation; RAFT, reversible addition-fragmentation chain-transfer polymerization.

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thereof) between proteins and interfaces are important for creating biosensors, as well as in the field of antifouling materials. Proteins also mediate interactions between cells and surfaces; the type of proteins, their orientation and their conformation are central to these interactions [1,2]. By creating smart surfaces that can enhance specific interactions between biomolecules and the material and promote certain cell behavior, great strides have been made in tissue engineering, biomaterials and cell culture [3]. Material properties such as mechanical properties [4,5] and degradation rate are important in these applications, as is porosity [6,7], surface chemistry and topography, the display of signals to cells (through proteins or peptides), and spatiotemporal modulation of surface properties and signals [8–11]. Many studies have been devoted to investigating and improving different aspects of this puzzle.

With better control and understanding of cell-material interactions, much effort is currently devoted to providing temporal control over cell-surface interactions through stimuli-responsive materials [12–14].

Conducting polymers have gained significant attention as biointerfaces due to their biocompatibility and polymeric nature, combined with their unique electric and optical properties [15–21]. Their relatively low cost, ease of synthesis and good biocompatibility has made CPs attractive candidates for a range of biomaterials, particularly in applications that require communication across interfaces. For instance, CPs can transduce chemical signals into optical or electronic signals, leading to their wide use as (bio)sensors [22]. Meanwhile, CP-based cell culture substrates are able to deliver electrical stimulation to cells to encourage growth or induce differentiation [23,24]. Since CPs are both electron and ion conductors, they have a unique advantage in exchanging signals with cells, transducing ionic to electric signals (and vice versa), which is particularly important for interfacing with the neural system [17,25–32]. As implants, CPs can function as recording devices to monitor neuronal activity, as stimulation devices to simulate nerve impulses in bionic organs or as a method of controlling symptoms in illnesses such as Parkinson's disease, as scaffolds for tissue regeneration, or as modulated drug delivery systems [33–37]. In these examples, CPs may outperform traditional metal implants due to their increased softness and flexibility, minimising inflammation and enhancing interactions with the surrounding tissues [33].

Conducting polymers are also very promising as stimuli-responsive materials, as they can be reversibly oxidised and reduced – a process leading to changes in charge, conductivity and dopant levels [38], as well as mechanical and other physical properties [39]. The stimuli-responsive properties of CPs have also been explored for materials displaying controlled drug delivery properties [37,40,41].

Functionalization of CPs can further improve their advantageous properties, providing the opportunity to tailor the materials to various applications. For example, improving water solubility/wettability of CPs can improve their performance in physiological (aqueous) environments, as well as potentially opening up new methods of processing into practical devices. The incorporation of suitable biomolecules or functional groups in biosensors is a common practice to introduce selectivity and sensitivity [22]. Similar and other functionalization methods have also been used to improve cell-surface interactions, and reduce fouling on the surfaces of tissue scaffolds or implants [36,42,43].

This review explores the use of conducting polymers as biointerfaces, focussing on examples where the conducting polymer has been functionalized to produce materials better suited for such applications. Ideally, one would like to easily be able to impart specific properties to the conducting polymers while retaining their unique electrical properties; key properties include antifouling, specific cell interactions, antimicrobial or biocidal effects, switchability, stability, processibility, solubility and desirable mechanical properties. Biointerfaces also benefit from the ability to specifically present active proteins or peptides on the surface to induce cell binding or cell differentiation. Here, we focus mainly on the new and developing field of functionalising CPs with polymer grafts to improve the surface properties, as our own work fits into that research field. We start however, by briefly discussing the use of biomolecules to add specific functionality to the CP biointerface.

## 2. Functionalized CPs AS biointerfaces

Research into using CPs as biointerfaces has expanded greatly since it was discovered that many CPs are biocompatible and can be

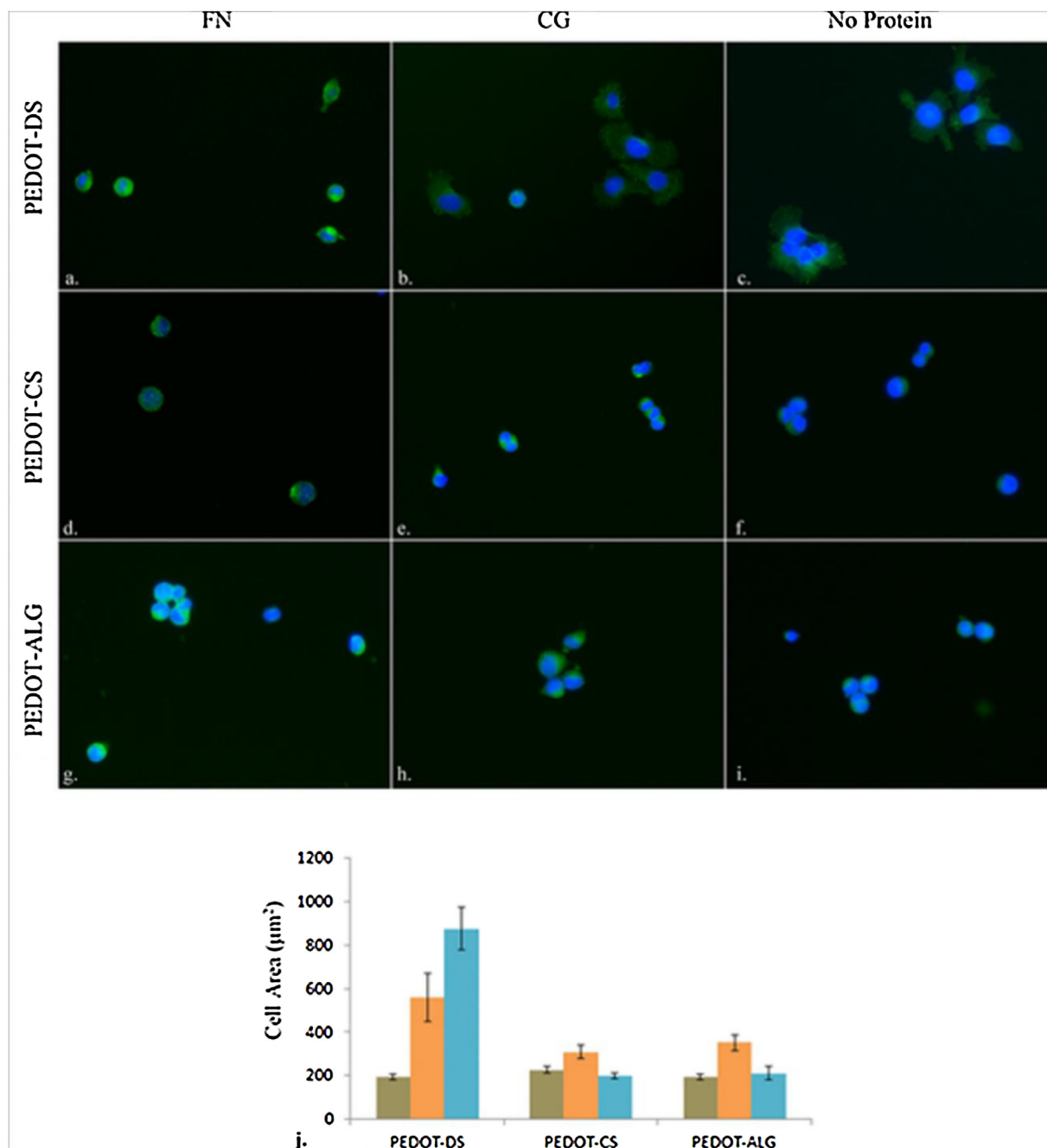
used to electrically stimulate cells, thereby altering cellular activities [44]. The electrical stimulation has mainly been explored using cells known to respond to electrical stimulation *in vivo*, such as nerve, bone and muscle cells [45,46]. However, CPs do not interact optimally with cells and tissues, and require further functionalization with biomolecules or biocompatible polymers. Thus, the development of methods to functionalize CPs opens up significant opportunities to produce materials more suited for various biointerface applications.

This review is divided based on the type of functionalization. Firstly we briefly introduce and exemplify the use of biomolecules as dopants and as moieties attached to the CP surface, followed by a more detailed discussion of functionalization of CPs with grafted polymeric brushes. We focus on the latter as we believe the available methodologies for producing graft polymers offer a general approach to CP functionalization that has not been widely explored, compared to the other two techniques. We believe this approach offers a significant flexibility in the material design enabled by availability of a range of polymer synthetic methodologies, and advances in controlled polymerization techniques, such as ATRP and RAFT [47–49].

### 2.1. Bio-dopants

Conductivity in CPs rely on the incorporation of dopant ions to balance the charge introduced through oxidation (p-doping) or reduction (n-doping). Doping can be performed either chemically or electrochemically and a variety of charged species can be introduced into the polymer as dopants. The type of dopant has effects on not only the conductivity and electrochemical properties, but also on the structural and physical properties of the polymer. Often small inorganic salt ions are used, able to move in and out of the CP film in response to redox state. Larger polymeric dopants can be physically trapped in the CP film, commonly during the CP polymerization, and are therefore more stably integrated in the film [44]. The most obvious route to CP functionalization for biointerface applications is thus through varying the dopant ion. The type of molecular dopant used is known to affect the biocompatibility of CPs, which is a key factor in biointerfaces. Aside from the toxic or non-toxic nature of the dopant itself, the choice of dopant also affects the mechanical properties and surface topography of the CP biointerface, in turn affecting cellular behavior [23,25,50,51]. Further effects can be seen by using dopants with a direct impact on cellular interactions – such as biologically-derived dopant molecules [52,53]. Commonly used bio-dopants are glycosaminoglycans, such as chondroitin sulfate [54], hyaluronic acid [25,55], heparin [25,56], and dextran sulfate [53,57]. These dopants are all negatively charged large dopants expected to become trapped in the CP film. They are also known to have an affinity for binding to fibronectin, an important protein in the extracellular matrix that mediates cell binding. Most studies directly investigate cell adhesion and proliferation to the CP interfaces with various dopants, but detailed effects in protein surface interactions have also been investigated by Atomic Force Microscopy (AFM) [58], Quartz Crystal Microbalance with Dissipation (QCM-D) and fluorescence microscopy [59,60] (see Fig. 1).

A study by Gilmore et al. compares skeletal muscle cell proliferation and differentiation on polypyrrole substrates doped with several of these glycosaminoglycans [52]. Although the best dopant can be expected to vary with cell type, the study also provided a careful characterization of film morphology and roughness. Glycosaminoglycan dopants produced films with good biocompatibility, but are, in comparison to proteins or peptides, limited in the specificity of response that can be elicited in the cells or tissue that interface with the CP.



**Fig. 1.** a–i) Representative fluorescence microscopy images of PC12 cells adhered to PEDOT-dextran sulfate, chondroitin sulfate and alginate with or without a preadsorbed protein conditioning film. Image dimensions  $220 \times 180 \mu\text{m}$ ; j) Quantification of cell surface area for cells adhered to the biodoped PEDOT polymers with either fibronectin (green), collagen (orange) or no protein (blue) surface conditioning layer. [60], Copyright 2014. Reproduced with permission from John Wiley & Sons Inc. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

More specific effects may be seen when doping the CP with peptide sequences or proteins. If the peptide or protein is not charged enough, it can be entrapped in the film by polymerizing the CP with another dopant in the presence of the desired biomolecule [61]. One important example of a protein dopant is the inclusion of neurotrophic growth factors in PPy films [62–64]. Neurotrophic growth factors are normally added to the medium during cell culture to induce neurogenesis; by including it as a dopant, it can be released in response to electric stimuli, leading to an increased level of neurite outgrowth [62–64]. Proteins are generally not incorporated as the sole dopant, but are used in addition to a small molecular dopant. Peptide sequences on the other hand, have been used as sole dopants. Polypyrrole doped with various peptides

derived from laminin have shown enhanced interactions with neurons, such as enhanced neurite outgrowth and reduced astrocyte adhesion [65]. Zhang et al. doped PPy with two different peptide sequences (DCDPGYIGSR and DRNIAEIIKDIC) derived from laminin and a mixture of the two. The dopant affected cell adhesion, spreading and differentiation of human embryonic stem cells and adult rat neural stem cells. The former peptide was found to promote adhesion and spreading, while the latter peptide sequence increased neuronal differentiation and neurite outgrowth [66]. Another study investigated the simultaneous incorporation of several different biomolecules in PEDOT, namely nerve growth factor (NGF) and laminin-derived peptide sequences; the inclusion of both the peptide and protein was found to reduce the electrical and mechanical

properties of the polymer, and the accessibility of NGF was shown to be lower in films containing both dopants compared to NGF-only doped films [62]. This study demonstrates the need for further research on incorporating multiple biological factors within one polymer for optimal performance. One issue with biodopant incorporation as a means of functionalization is that both the topography and conductivity of the resulting film are generally significantly altered [44]. There is also a large variation between studies, with more systematic investigations of dopant ions still needed [67]. While initial cell adhesion can be promoted through other means, incorporation of growth factors or small signalling molecules that can be released to cells through electrical stimulation is particularly interesting. As the understanding of engineered biointerfaces is growing, more complex systems are being designed targeting the combined delivery of several signals to cells, such as growth factors and both electrical and mechanical stimuli [68].

## 2.2. Biomolecule attachment

Using the doping mechanism of CPs to incorporate bioactive material is rather straightforward, but is limited to highly charged molecules or molecular entrapment while polymerizing with a charged dopant. Dopant molecules have also been used to provide functional groups in the CP, which can be further coupled to biomolecules [69], or the (bio)molecules of choice can also be attached to the CP itself, meaning that a wider range of (bio)molecules can be targeted [70].

Biomolecule attachment to CPs can broadly be divided into adsorption and covalent binding to the CP. Covalent binding of biomolecules to CPs are commonly hampered by the lack of functional groups in CPs, which has led to the development of a range of CPs displaying functional groups such as hydroxyls, carboxylic acids and 'click' entities [20,71–73]. Direct attachment to a CP film ensures that the molecules are displayed at the interface. One particularly elegant example of surface attachment of biomolecules, which does not require synthesis of complex functionalized monomers, is that of Sanghvi et al., who developed a generic peptide functionalization approach based on a peptide sequence found to bind strongly to the PPy interface. The peptide was developed through phage display and binding to PPy was characterized by AFM. The chosen peptide could then be linked to a cell adhesion peptide, leading to increased cell adhesion to PPy in serum-free media [74]. The well-known fibronectin-derived cell adhesion peptide 'RGD' has also been directly covalently attached to PPy via an activated cysteine residue in the flanking peptide sequence, promoting adhesion of osteoblast cells compared to unmodified PPy controls [70,75]. It is worth noting that serum was present in the media, although at a low level (2%) to minimize the influence of serum protein adsorption.

As proteins adsorb onto most surfaces, specific protein components can also be simply physisorbed to CP interfaces prior to cell culture to improve cell adhesion to the material [59,76]. The simplicity of this approach makes it very valuable, but the pre-adsorbed protein layer will be degraded and replaced over time in presence of serum and as cells generate their own extracellular matrix. Even if no protein is pre-adsorbed, any CP film introduced into serum containing media will be rapidly coated by protein. The surface properties of the CP influence the adsorbed protein layer and downstream cellular behavior, and it has been shown that protein adsorption differs depending on CP oxidation state. These factors make simple protein adsorption somewhat unreliable, and to increase the stability of the protein coating, a covalent coupling may be desired. One such example is that of Liu et al., where collagen was covalently coupled via EDC/NHS to a glucosaminoglycan-doped PPy (Fig. 2). Rat nerve cells showed increased neurite outgrowth on the

fibrillar collagen, which was further enhanced through electrical stimulation of the underlying CP substrate [77].

Another recent, elegant and novel, approach to biofunctionalization was presented by Maione et al., who prepared PEDOT-RGD conjugates by using a peptide sequence with an exotic amino acid resembling the EDOT monomer. In this way, the cell adhesion ligand RGD could be attached directly to the ends of the PEDOT polymer. The electroactivity of the conjugates was found to be higher than that of PEDOT alone, attributed to the success of the chemically-similar peptide-polymer links [78].

There are several excellent review papers that discuss the biocompatibility of CPs and their functionalization using biomolecule attachment or doping and the interested reader is directed to those references for more details on such methodologies [20,44,46,71,79]. Clearly, the toolbox available through functionalising CPs using biomolecules is excellent. However, the basic limitation of CPs still remain, such as their relative crystalline and stiff nature, their poor processability and their lack of biodegradability [44,80]. This has led to large efforts in more extensive CP functionalization, which will be the focus of the remainder of this review.

## 2.3. Grafting of polymer chains

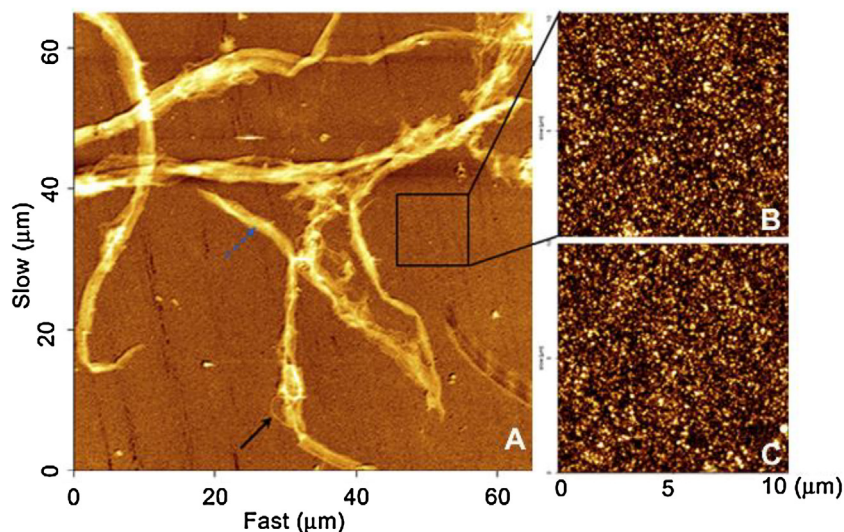
The concept of molecular attachment on to CPs can be extended further to polymer chains grafted covalently on to the CP backbone. The addition of polymer chains to CPs is a useful tool to alter the chemistry, stability, solubility and mechanical properties of the CP, and can be used to introduce functional groups for further functionalization. Polymer chains have been produced from a variety of substrates for a range of applications: to improve the solubility and processibility of polymers and nanoparticles, to tune the wettability and adhesiveness of surfaces, to prevent fouling of biosensors and coatings and to introduce stimuli-responsive behavior [81–83]. Many of these properties are of interest to CP-based biomaterials as well. While this area is currently not widely-researched, we believe that this approach offers exciting new tools for engineering functional electroactive biointerfaces.

There are three approaches to grafting polymer brushes from conducting polymers: 'grafting to', 'grafting from' and 'grafting through' (Fig. 3) [47,84]. 'Grafting through' involves the synthesis of macromonomers (macromolecules containing an end-group that can behave as a monomer); subsequent polymerization of the end-group monomeric functionalities gives the desired polymeric backbone with the rest of the macromolecule forming the polymer brush [85]. Alternatively, 'grafting to' involves chemical attachment of polymer chains to the substrate or previously-polymerized backbone [86]. The third case of 'grafting from' requires the functionalization of the polymer backbone or substrate with initiating sites from which the brushes are subsequently grown [87].

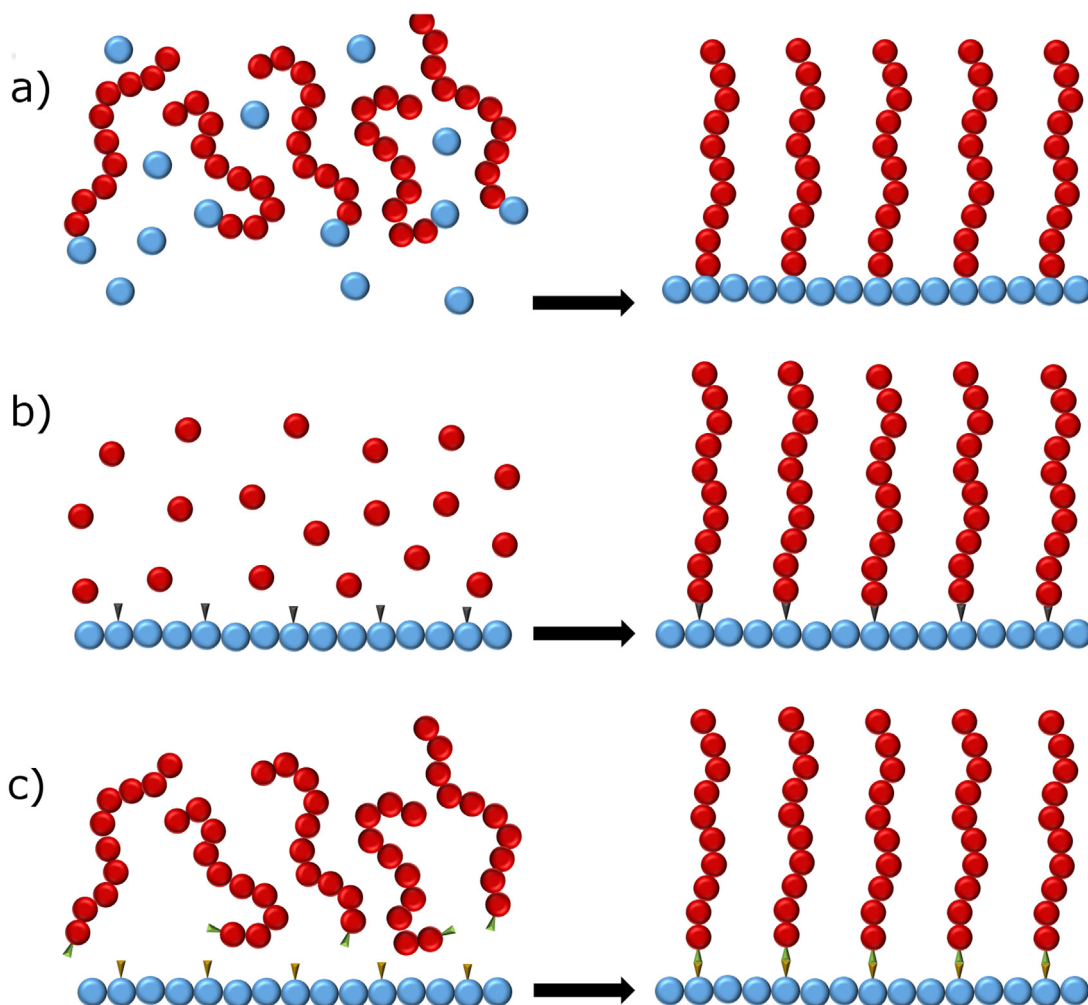
It should be noted, that as the field of grafting from CPs is still emerging, some of the studies listed below have not specifically targeted biointerface applications. However, we suggest that the chemistries developed in these exemplar studies can be easily extended to grafting of polymer chains on CPs to improve their biocompatibility, mechanical properties and biofunctionality.

### 2.3.1. 'Grafting from'

The 'grafting from' method is the most frequently reported of the three methodologies for grafting of CPs due to the commercial availability of functionalized monomers that can be readily converted to relevant initiating sites [88]. It has been also our own method of choice due to the generality of the approach [76,89–92]. The brush grafting post-polymerization of the CP avoids the brushes sterically hindering the CP polymerization, an important problem in polymerizing of functionalized CPs. The brushes can be grown



**Fig. 2.** AFM micrographs of PPy-CS-Col film (A), amplified area on PPy-CS-Col film without collagen fibres (B) and pristine PPy-CS film (C) in PBS. Blue arrow – large fibrous structure, Black arrow – smaller individual fibres. [77], Copyright 2011. Reproduced with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 3.** Generalized categories of graft copolymer synthesis: (a) grafting through (macromonomer approach), (b) grafting from (macroinitiator approach), and (c) grafting to. [38], Copyright 2016. Reproduced with permission from John Wiley & Sons Inc.

by controlled radical polymerization methods such as atomic radical transfer polymerization (ATRP) and reversible addition-

fragmentation chain-transfer polymerization (RAFT), allowing for control over the brush chain lengths and facile synthesis of block

copolymer brushes via ‘living’ chain ends [48,49,93,94]. Brushes can also be grown via other methods, such as ring opening metathesis polymerization (ROMP) [95] and UV-induced grafting from ozone-pretreated CPs [96]. The following paragraphs detail different types of grafted brushes from CPs grouped by their imparted properties and potential applications.

The ‘grafting from’ method has the advantage of being able to produce denser, longer brushes compared to other grafting methods, as the growing polymer brushes experience relatively less steric hindrance during polymerization. For example, Wang et al. attached an ATRP initiator to thiophene monomers pre-polymerization, then used the resulting PTh film as a backbone to graft poly(*N,N*-dimethylaminoethyl methacrylate) (PDMA) [97]. The resulting polymer was estimated to contain 2.6% polythiophene by mass, indicating the presence of large, densely grafted PDMA brushes (confirmed by AFM images of polymer adsorbed on mica, showing the presence of thick worm-like chains; Fig. 4).

One of the most important rationales for grafting brushes is to improve the solubility of the conducting polymer backbone, which in turn improves its processability and widens the range of environmental conditions that it can be employed in. Costanzo and Stokes were among the first to report the synthesis of a conducting polymer with an attached ATRP initiator for subsequent grafting [98]. In their study, 3-thiophene-ethanol was polymerized to give hydroxyl-functionalized polythiophene (PTh), followed by attachment of the initiator. They estimated that this procedure led to functionalization of 86% of the backbone, from which they were able to graft short poly(methyl acrylate) brushes with low polydispersities and predictable molecular weights (MWs). In a similar study, Massoumi et al. attached initiating groups to polyaniline (PANI) post-polymerization, which were used to graft poly(methyl methacrylate) brushes [99]. The grafted brushes improved solubility in polar solvents, and the grafted polymer showed defined electroactivity in aqueous H<sub>2</sub>SO<sub>4</sub>—both of which are of interest for potential bioapplications which are often carried out in aqueous conditions.

Aside from altering the solubility of the conducting polymers, grafted sidechains may also impart (bio-) erodibility, which can allow for controlled degradation of implants and injected biomedical materials. For example, Domagala et al. grafted short biocompatible oligo(3-hydroxybutyrate) (OHB) chains from a polypyrrole (PPy) backbone [100]; OHB degrades in phosphate buffered saline (PBS) to give (*R*)-3 hydroxybutyric acid, which is a non-toxic constituent of blood. The increasing acidification of the environment in turn contributed to chemical erosion and degradation of the polypyrrole backbone, as evidenced by reduced conductivity and shifted vibrational bands in FTIR spectra. The rate of OHB and subsequent PPy degradation could be controlled, with shorter and sparser OHB chains being more susceptible to erosion.

Grafted brushes can also introduce controlled switching behavior to conducting polymers, leading to changes in conformation, wettability and solubility, electroactivity, and protein and cell rejecting properties. Induced changes can be triggered by a range of external stimuli, particularly temperature, salt concentration, pH, or applied electrical potential under physiological conditions. For instance, Malmstrom et al. reported the synthesis of (3,4-ethylenedioxythiophene)methyl 2-bromopropanoate (BrEDOT) monomer [90]. After polymerization, *tert*-butyl acrylate brushes were grafted by ATRP, followed by acid hydrolysis to give charged polyacrylic acid (PAA) brushes. Surface plasmon resonance and cyclic voltammetry measurements demonstrated the pH-responsiveness of the PAA brushes, which in turn led to changes in electrochemical behavior when switching between acidic and basic solutions.

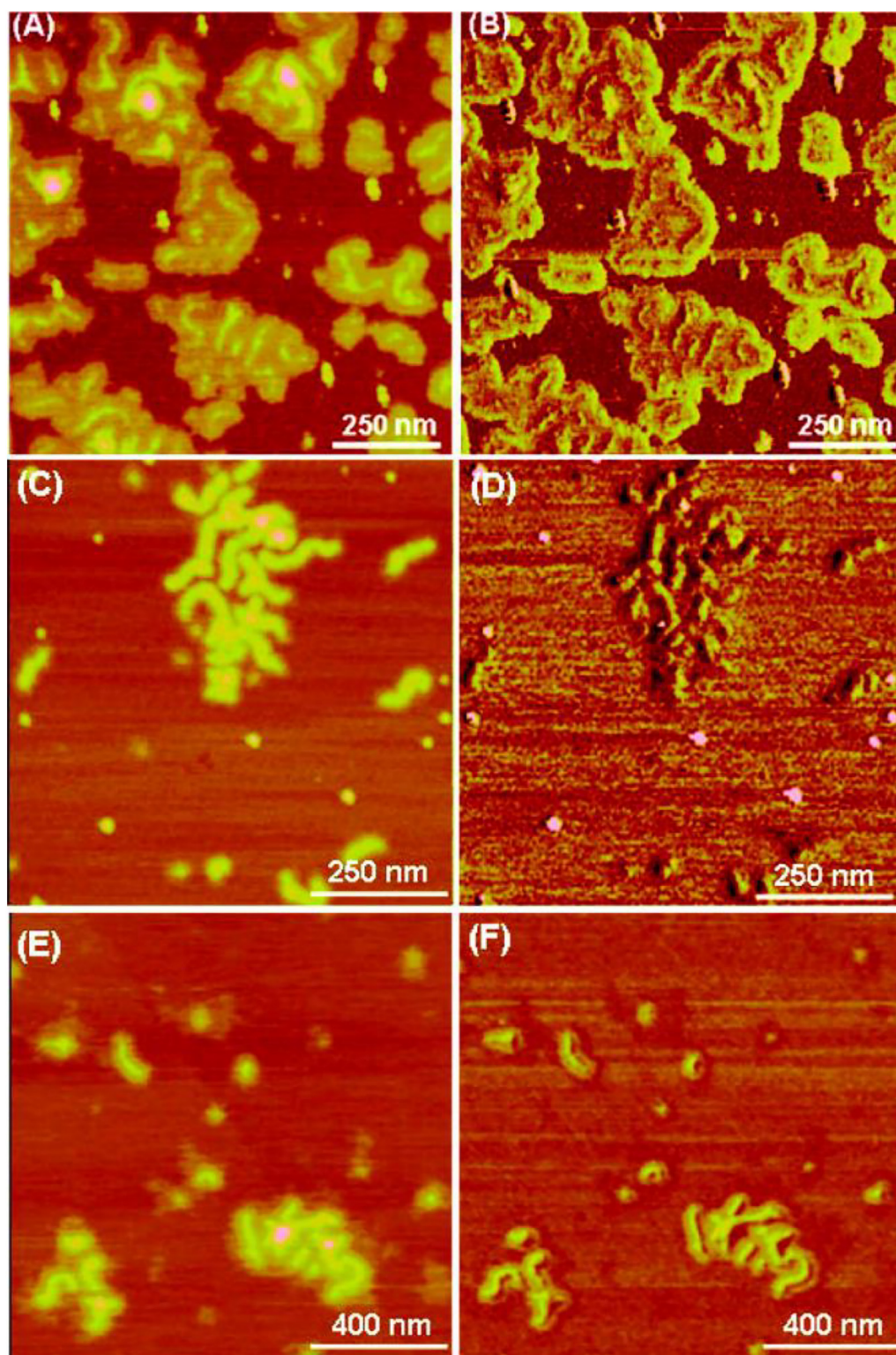
More commonly, thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm) brushes are exploited for

biomedical applications. The temperature-dependent collapse of PNIPAAm and subsequent change in hydrophilicity has been exploited to produce cell culture substrates capable of promoting protein and cell adhesion at 37 °C when the brushes are collapsed and dehydrated, but can cause them to detach at lower temperatures as the brushes hydrate and swell. An early example of PNIPAAm grafting from CPs was reported by Ghorbani et al., who attached initiating sites to PANI using chloroacetyl chloride (CAC) or 2-chloropropionyl chloride (CPC) [101]. The grafted PANI-g-PNIPAAm copolymers show improved solubility in polar solvents – up to 150 g/L for PANI-CAC-g-PNIPAAm in water or methanol, and 50 g/L in methanol for PANI-CPC-g-PNIPAAm due to decreased grafting density in the latter material (87% and 47% respectively). Cyclic voltammograms confirmed that the PANI-g-PNIPAAm retained electroactivity. In another example, Balamurugan et al. grafted PNIPAAm brushes from polythiophene to improve its water solubility; the grafted copolymers displayed reversible thermoresponsive behavior in solution [102].

Other researchers have also investigated brushes composed of poly(ethylene glycol) derivatives, which display similar thermoresponsive behavior to PNIPAAm – including reversible protein and cell adhesion based on hydration of the brushes. The advantage is that the transition temperature can be tuned by varying the length of the poly(ethylene glycol) chains [103]. Hackett et al. used PBrEDOT substrates as a backbone to graft brushes using poly(ethylene glycol)methyl ether methacrylate (PEGMMA) macromonomers [89]. The thermoresponsive behavior was tuned through copolymerizing two different PEGMMA monomers with differing molecular weights: di(ethylene glycol) methyl ether methacrylate (DEGMMA, MW=188.22) and PEGMMA-500 (MW=500). Addition of kaotropic or kosmotropic salts also raised or lowered the collapse temperature respectively, providing dual control over the brush conformation. The swollen brushes were demonstrated to be protein-rejecting, with effectiveness increasing when more hydrophilic PEGMMA-500 monomer was incorporated into the brush. The sample with 9:1 monomer ratio of DEGMMA:PEGMMA-500 displayed complete protein rejection after an hour of exposure to 20% serum (as determined by Quartz Crystal Microbalance with Dissipation measurements, Fig. 5a). Further studies revealed that the swollen brushes were also cell-repelling, with the extent of cell rejection dependent on the composition of the copolymer brushes (Fig. 5b, see also [76]).

Zhao et al. produced a similarly functionalized EDOT monomer, but used an initiator with a tertiary bromide, 2-bromo-2-methylpropionic acid-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methyl ester (EDOT-Br) [104]. After electropolymerization of the P(EDOT-Br), brushes of poly(oligo(ethylene glycol) methacrylate) (POEGMA) and zwitterionic poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide) (PSBMA) were grafted. Both brushes prevented the binding of bovine serum albumin (BSA) and fibrinogen (Fn). In contrast, protein adsorption on the ungrafted PEDOT control reached saturation after 15 min, reaching a maximum protein density of 529 ng/cm<sup>2</sup> for BSA and 1310 ng/cm<sup>2</sup> for Fn. The brushes also prevented adhesion of NIH3T3 fibroblast cells after incubation for 24 h, while the fibroblasts readily adhered to the ungrafted PEDOT film.

One of the key issues of the ‘grafting from’ procedure is the need to control brush grafting density to produce homogeneous surfaces. Steric hindrance between the brushes during polymerization can lead to low grafting densities and inhomogeneous brush growth. However, spacing out the initiating sites can lead to brushes with more well-defined lengths and controlled densities. Both Malmstrom et al. [90] and Zhao et al. [104] investigated electrochemical copolymerization of the ATRP-functionalized EDOT with unfunctionalized ‘spacer’ EDOT. In both cases, the calculated

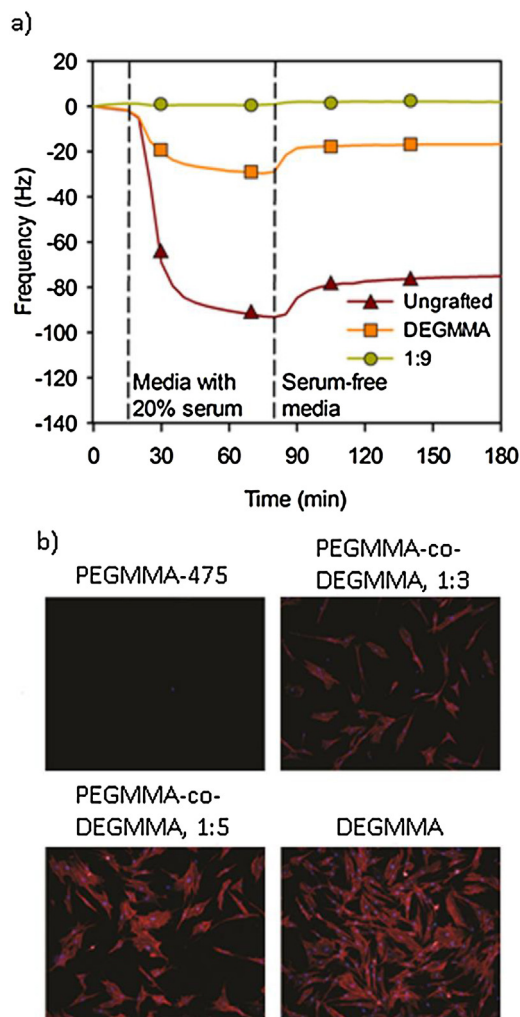


**Fig. 4.** AFM images (A, C, E: height; B, D, F: phase) of PTh-g-PDMA adsorbed on mica from dilute solution ( $2 \times 10^{-3} \text{ g L}^{-1}$ ) of toluene (A, B), THF (C, D), and  $\text{CH}_2\text{Cl}_2$  (E, F). Z range (height): 0–10 nm, from dark to bright. [97], Copyright 2008, reprinted with permission from the American Chemical Society.

ratio of BrEDOT in the polymer was lower than that in the monomer feed, which was attributed to the slightly higher onset potential of BrEDOT polymerization during cyclic voltammetry. Zhao et al. showed that the level of EDOT-Br incorporation was linear with the monomer feed ratio, demonstrating that this was a valid method of controlling initiator density, and by extension, brush density [104]. Increasing the amount of EDOT-Br in the monomer feed by

5% decreased protein adsorption after grafting by up to 40%; at feed ratios consisting of over 50% EDOT-Br, the brushes were dense enough to be fully protein rejecting.

Other groups have attempted to tune the initiator density on the CP backbone by synthesising oligomeric repeating units. Aside from greater control over the initiator density, oligomers also allow statistical control over copolymer composition where the singu-



**Fig. 5.** a) Frequency response demonstrating the relative protein resistance of ungrafted PBrEDOT, and PBrEDOT grafted with PDEGMMA and P(PEGMMA-co-DEGMMA, monomer ratio 1:9) brushes in serum-free media and 20% serum, measured using Quartz Crystal Microbalance with Dissipation. [89], Copyright 2015. Reproduced with permission from the Royal Society of Chemistry; b) Fibroblast adhesion on PBrEDOT grafted with P(PEGMMA-co-DEGMMA) brushes of different compositions, showing variable cell adhesion based on brush composition. Blue = DAPI (nucleus), red = Phalloidin (actin).

lar monomers have differing polymerization rates. Oligomers may also be electropolymerized at lower potentials compared to corresponding singular monomers, reducing the risk of over-oxidation of the conducting polymer.

Grande et al. synthesised a terthiophene (3Th) macromonomer containing a chain transfer agent (CTA) for reversible addition-fragmentation chain-transfer (RAFT) polymerization [105]. The 3Th-CTA termonomer was electrochemically polymerized, and used as a macro-RAFT CTA to graft brushes of poly(methyl methacrylate) (PMMA), polystyrene (PS), poly(*tert*-butylacrylate) (PtBA), and poly(carbazole ethyl methacrylate), as well as block copolymer brushes of PMMA-*b*-PS and PMMA-*b*-poly(pentafluorostyrene). Strover et al. also synthesised a terthiophene macromonomer BrTTh, containing an ATRP initiator on the central thiophene rather than a RAFT chain transfer agent [106]. The BrTTh monomer was easily polymerized both chemically and electrochemically. PS brushes were successfully grafted from both PBrTTh and PBrTTh-*g*-(PS-*b*-PAA) showed significant changes in water contact angle upon oxidation and reduction ( $>20^\circ$ ),

demonstrating electrochemical switchability. Deposited films of chemically oxidised PBrTTh-*g*-(PS-*b*-PAA) demonstrated reduced electroactivity compared to the electrochemically polymerized films, likely due reduced contact between the conducting polymer and electrode due to the steric effect of the brushes.

In an effort to produce films with greater electroactivity in water, Strover et al. also synthesised a termonomer containing a central ATRP initiator-functionalized thiophene, flanked by more hydrophilic pyrrole units, which they dubbed PyThon [107]. Grafting of PS, poly(pentafluorostyrene) and poly((ethylene glycol) methyl ether acrylate) brushes appeared to give the films some capacitive switching behavior when conducting cyclic voltammetry in water, although the effect was not fully investigated. Electrografting was also attempted with poly(2-hydroxyethyl methacrylate) via eATRP, in an attempt to improve the oxygen tolerance and lower the amount of cytotoxic copper catalyst present during ATRP grafting by electrochemically regenerating the active catalyst; however, the process was not well controlled [108]. Alternatively, Chams et al. synthesised an ATRP-functionalized termonomer containing a central pyrrole ring and two thiophene units, which they called SNS-Init [109]. The SNS-Init termonomer was able to polymerize at lower potentials ( $<0.80$  V) and produced films that were more adherent to the substrate than homopolymer films produced from an initiator-functionalized pyrrole without the flanking thiophene groups. The films displayed smoother morphology under scanning electron microscopy (SEM) imaging than the typical 'cauliflower' morphology of electrodeposited PTh and PPy. Brushes of dimethylaminoethylmethacrylate (DMAEMA) were successfully grown from the P(SNS-Init) films, appearing as dense 'cotton-like' spheres distributed over the films surface.

Chan et al. also synthesised termonomers containing two thiophene units, but with a central phenylene unit instead [92]. A range of functionalities were attached to the phenylene ring, including short PEG chains, ATRP initiating sites, and azides that could be used to 'click' chains with terminal alkyne groups. Copolymerizing these termonomers allowed for films with dual functionality, as demonstrated by the successful 'clicking' of hex-1-yne after grafting short PS brushes. These films could possibly be extended to produce surfaces with mixed stimuli-responsive brushes for precise control over the surface properties, or to easily attach small molecules such as peptides on to the CP backbone while utilising polymer brushes to reduce non-specific binding.

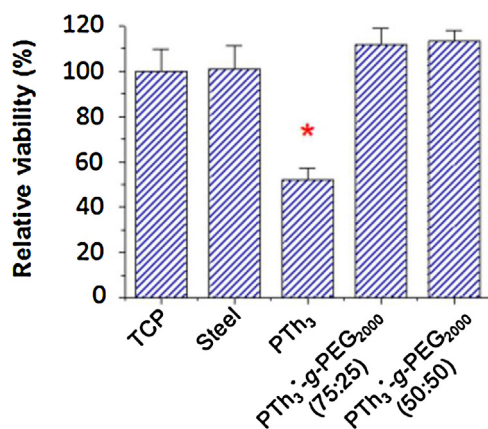
### 2.3.2. 'Grafting through'

'Grafting through' was one of the earliest methods adopted to produce polymer chains from CP backbones. An early example is the polymerization of a thiophene-capped poly(methyl methacrylate) to give PTh-*g*-PMMA, as reported by Alkan et al. in 1999 [110]. As the 'brushes' are synthesised first, this approach provides very good control over brush composition, length and polydispersity. However, the steric bulk of the macromonomers can hinder polymerization of the CP backbones; thus, this method is best suited to short brush lengths and lower grafting densities.

In a study by Zhao et al. the authors reported the polymerization of dibromothiophenes that had been modified with short oligo(ethylene glycol) chains (OEG) (2 or 4 repeating units) capped with either methyl or benzyl groups [111]. As dropcast films, the OEG-modified PTh demonstrated good electrochemical stability in water and the UV-vis spectra were unchanged after 4 days of immersion in PBS buffer. Even though poly(ethylene glycol) is typically used to prevent protein and cell adhesion, the short chains in this study did not hinder cell adhesion; spincoated films were able to support the growth and proliferation of NIH3T3 fibroblast cells to confluence within 84 h.

In a more recent study, Bendrea et al. synthesised pentathiophene macromonomers, with methyl-capped poly(ethylene glycol)





**Fig. 6.** Cytotoxicity of PTh<sub>3</sub>-g-PEG2000 (75:25 and 50:50 Th<sub>3</sub>-g-PEG2000:Th<sub>3</sub>) and PTh<sub>3</sub> doped with ClO<sub>4</sub><sup>-</sup>. Three samples were analysed for each group. Bars represent the mean standard deviation. The relative viability of Cos-7 cells was established in relation to the TCP control (tissue culture polystyrene). Steel was also considered as a control substrate because PTh<sub>3</sub>-g-PEG2000 and PTh<sub>3</sub> were deposited on this material. The asterisk (\*) indicates a significant difference with the control, Tukey's test ( $p < 0.05$ ). [113], Copyright 2015, Reproduced with permission from John Wiley & Sons Inc.

chains attached to the central thiophene [112]. The authors used longer PEG chains (22 or 44 repeat units) compared to Zhao et al. [111]. The pentathiophene macromonomers were easily electropolymerized to give smooth films, which were used to culture epithelial Vero cells. After seven days, both PTh-g-PEG films displayed greater cell viability than both steel and culture plate controls, and PEDOT films. Cell adhesion also appeared to enhance the electrochemical properties of the films.

In another study, Maione et al. investigated the effect of graft density on the PTh-g-PEG films, using PEG-grafted terthiophenes instead of the previous pentathiophenes [113]. These were copolymerized with ungrafted terthiophenes in different ratios, and investigated for potential cytotoxicity using Cos-7 fibroblast-like cells. After 24 h, ungrafted PTh<sub>3</sub> showed ca. 50% reduction in cell viability compared to steel and culture plate controls, while grafted PTh<sub>3</sub>-g-PEG films demonstrated slightly increased cell viability compared to the control substrates (Fig. 6). Likewise, the grafted films also showed significantly greater cell adhesion and proliferation compared to the steel control. Both PTh<sub>3</sub> and PTh-g-PEG substrates also displayed improved electroactivity when coated in cells.

Akbulut et al. attached PEG-2000 chains to single 2,5-dibromothiophene units and copolymerized them with amine-functionalized 2,5-dibromothiophenes via Suzuki polycondensation with 2,5-thiophenediboric acid [114]. The result was a polythiophene film with amine- or PEG-functionalized units alternating with unfunctionalized 'spacer' thiophene units. Laccase (Lac) was immobilised via the amine groups, and the system was tested as an electrochemical catechol biosensor. The system demonstrated improved sensitivity compared to previously reported Lac biosensors [115,116], with a linear range from 2.5–500  $\mu$ M. The PEG brushes improved the activity of the PTh backbone in aqueous solutions, which was important as the addition of Lac enzyme appeared to hinder electron transfer between the electrode and solution. The authors have also used the similar methodologies to produce poly(*p*-phenylene)s (PPPs) with PEG and covalently-bound poly-L-lysine [117], demonstrating that their techniques can be applied to produce functionalized CPs other than PTh.

### 2.3.3. 'Grafting to'

'Grafting to' is a method of grafting polymer brushes post-CP polymerization. As the polymer brushes are synthesised prior

to attachment to the CP backbone, this method provides good control over brush characteristics such as molecular weight and composition. This avoids situations where the brush polymerization conditions are incompatible with the CP backbone or vice versa. It can also facilitate the synthesis of patterned or mixed brushes, by selective functionalization of the surface with appropriate attachment sites [86]. Although historically not widely utilised, the 'grafting to' method is nevertheless growing in popularity due to the advent of 'click chemistry' [118].

A disadvantage of the method is that 'grafting to' does not produce high grafting densities compared to other methods, due to steric hindrance from the brushes. This issue can be partly mitigated by using short brushes or using poor solvent during the grafting reaction to ensure the brushes are coiled up [119]. 'Cloud-point grafting' of PEG derivatives, for instance, relies on highly concentrated salt solutions to lower the solubility of the PEG chains in water causing them to contract and hence reducing chain repulsion during grafting [120]. However, this technique does not appear to have been applied to graft to CPs.

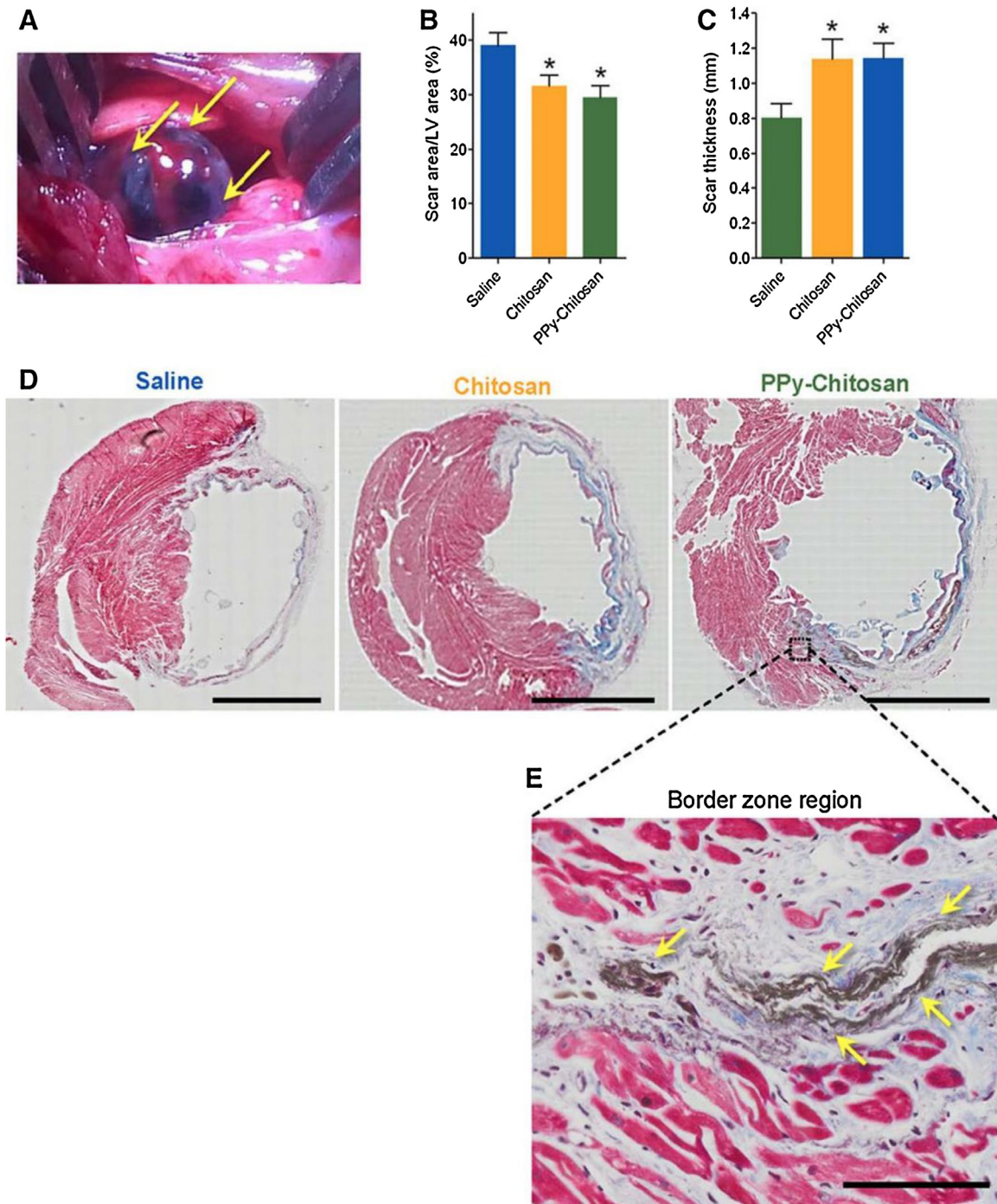
In one example of the 'grafting to' approach, Molino et al. used a thiol-ene reaction to attach protein-repelling PEG chains to PPy, leading to complete rejection of mouse myoblast adhesion [121]. Martin et al. similarly attached thiolated PEG to poly(3,4-propylenedioxythiophene) (PProDOT), allowing the previously insoluble polymer to dissolve in several organic solvents [122].

Selective reactivity of 'click' graft sites allows versatile difunctionalized polymer backbones to be synthesised. Chan et al. synthesised conducting polymer films containing both ATRP initiators and azide groups for 'click' coupling with alkyne-functionalized polymer chains, with the potential to produce mixed polymer brushes [92].

### 2.3.4. CPs as polymer brushes

An alternative approach to CP grafting is to grow the CP from a 'conventional' polymeric backbone. In these materials, advantageous properties of the CP can be exploited even though the bulk material itself is not conductive. Chitosan (CS) is a good example of these materials, as it is a natural polymer with good biocompatibility and biodegradability [123]. It has been extensively used in a range of applications, particularly as antimicrobial packaging in the food industry, as cell culture scaffolds and as a drug delivery carrier. Cabuk et al. grafted conducting PPy from chitosan, producing stable colloidal suspensions of CS-g-PPy [124]. These particles demonstrated improved antibacterial activity against five strains of common bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium*, *Enterococcus faecalis*, *Staphylococcus aureus*) compared to CS and PPy. Such properties were attributed to the synergistic effect of positive charges from protonated amino groups on the CS backbone and from oxidised PPy chains, resulting in electrostatic disruption of the negatively-charged microbial cell walls. Compared to reference antibiotics, 75  $\mu$ L of CS-g-PPy hydrogel showed greater activity compared to *Penicillin* (10 mg), *Trimethoprim* (25 mg) and *Rifampicin* (5 mg), and similar activity to *Erythromycin* (15 mg) and *Amikacin* (30 mg). In a follow-up study, the authors compared the CS-g-PPy grafts to CS-g-PANI and CS-g-PTh. CS-g-PPy was determined to exhibit the greatest antibacterial activity of the three composites, while CS-g-PTh and CS-g-PANI performed similar to each other [125]. CS-g-PPy again demonstrated equipotent or greater activity compared to the five reference antibiotics. CS-g-PANI and CS-g-PTh performed better than *Penicillin*, *Trimethoprim* and *Rifampicin* when added at 100  $\mu$ g/mL, but exhibited less activity than *Erythromycin* and *Amikacin*.

Mihic et al. investigated CS-g-PPy hydrogel composites as implants to treat damaged cardiac tissues and improve heart functioning after a myocardial infarction [126]. The composite material



**Fig. 7.** Hydrogel-injected hearts had smaller scars. Saline, chitosan, or PPy-chitosan was injected into the border zone of rats 1 week post-MI. A, Intraoperative photograph, showing 3 darker areas of PPy-chitosan injected into the border zone (arrows). Eight weeks after injection, hearts were excised and scar area and thickness were measured (saline,  $n = 6$ ; chitosan,  $n = 8$ ; PPy-chitosan,  $n = 8$ ). PPy-chitosan- and chitosan-treated hearts had similar scar areas (B) and scar thicknesses (C) and were significantly different than saline-treated hearts ( $*P < 0.05$ ). Scale bar = 3 mm. D, Representative heart sections from each experimental group from a comparable transverse section landmarked just below the papillary muscles. E, Masson trichrome staining of a representative PPy-chitosan-injected heart 8 weeks post-injection. PPy-chitosan particles can be seen in the scar and border zone (arrows). Scale bar, 100  $\mu\text{m}$ . [126], Copyright 2015, Reproduced with permission from Wolters Kluwer Health, Inc.

was first investigated *ex vivo*, demonstrating the ability to support proliferation and metabolism of rat smooth muscle cells and to promote electric conduction between skeletal muscle cells, as well as improving the propagation of transient  $\text{Ca}^{2+}$  signals across neonatal cardiomyocyte tissue. Following these results, the composite materials were injected into rats after myocardial infarction. One week after injection, echocardiograms of the rats injected with CS-g-PPy resembled those recorded prior to the myocardial infarction. In

contrast, rats injected with CS or saline controls showed prolonged depolarisation of the left and right ventricles, suggesting reduced conduction across the heart due to scar tissue. Excised hearts at the end of the study confirmed that the CS-g-PPy-injected hearts demonstrated faster transverse conduction velocities than the control injections, similar to rats that had not experienced myocardial infarctions. The shape of the scar tissue was also affected, producing elongated scar tissue instead of the rounded scar tissue seen

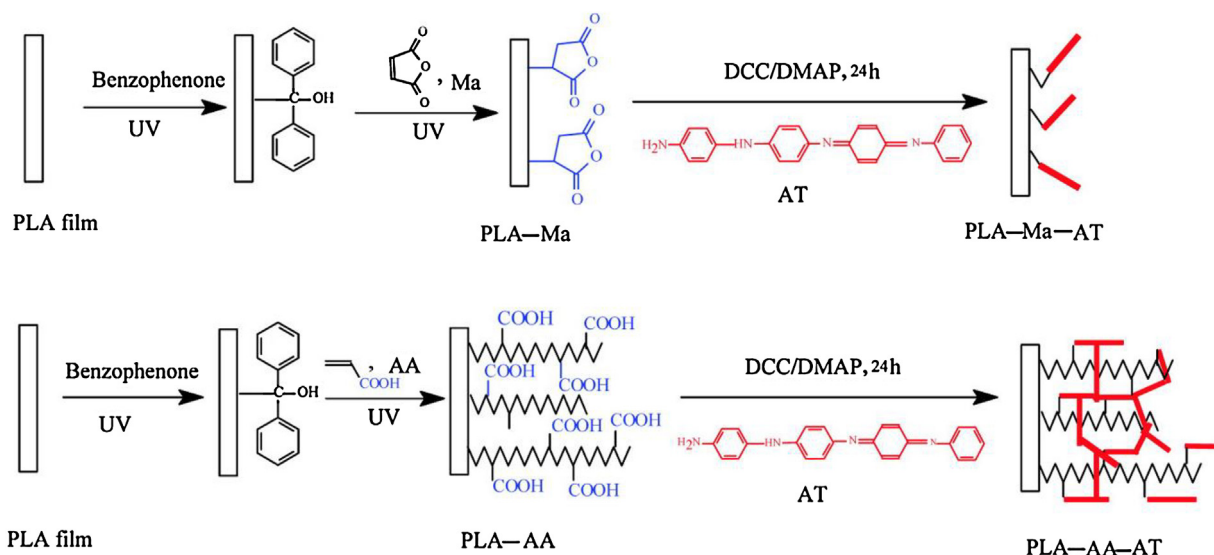


Fig. 8. Surface grafting of poly(lactide) (PLA) films with tetraaniline tetramer (ATA). [127], Copyright 2012, Reproduced with permission from the American Chemical Society.

in the control samples (Fig. 7). Both CS and CS-g-PPy reduced the size of the scar compared to saline injections, and improved cardiac function across multiple parameters.

Aside from chitosan, a range of synthetic polymers can also be used as a backbone for grafting CP brushes. Guo et al. investigated poly(lactide) (PLA) films, which have excellent mechanical properties, biocompatibility and biodegradability for biointerfacial applications, but are too hydrophobic to induce cell adhesion [127]. The PLA films were functionalized with either PAA brushes or a monolayer of maleic anhydride, followed by coupling of tetraaniline (TA) to the carboxyl groups (Fig. 8). At each step, the water contact angle of the films decreased, from  $84^\circ$  for PLA to  $50^\circ$  for HCl-doped PLA-maleic anhydride-TA, and between  $30\text{--}45^\circ$  for doped PLA-PAA-TA depending on the PAA chain length; the greater decrease in hydrophobicity for PLA-PAA-TA was attributed to the larger number of carboxyl groups present in the PAA chains, and hence greater density of coupled TA oligomers. The doped films also retained their conductivity. Although no cell studies were undertaken, the authors hypothesised that the increased hydrophilicity would improve cell adhesion to the films, and electrical stimulation from the AT brushes could induce cell growth and differentiation. This was confirmed by a later study by Cui et al., who clicked tetraaniline brushes onto biodegradable poly(ester amide) by EDC/NHS chemistry to give PEA-g-TA films [128]. Higher content of TA (denser grafting) slowed film degradation; however, the grafted copolymers films showed higher cell viability ( $>90\%$ ) of preosteoblastic MC3T3-E1 cells compared to TA alone, and higher cell average areas than ungrafted PEA. Some films were electrically stimulated periodically during cell incubation; after two weeks, the cells growing on these films showed higher levels of markers for osteogenic differentiation (free  $\text{Ca}^{2+}$  concentration and ALP enzyme activity) compared to unstimulated cells, demonstrating the effectiveness of electrical stimulation in inducing cell differentiation.

Hardy et al. also investigated electrical stimulation of differentiating bone tissue cells – in this case, human mesenchymal stem cells [129]. The authors attached pyrrole initiating sites on to polycaprolactone backbones, followed by chemical polymerization of amine-functionalized PPy-NH<sub>2</sub> or carboxylate-functionalized PEDOT-CO<sub>2</sub>H brushes. The solution-cast films were biomineralized with either silica (PPy-NH<sub>2</sub>) or calcium phosphate (PEDOT-CO<sub>2</sub>H), and used as substrates to culture mesenchymal stem cells in osteogenic media. As with the previous study by Cui et al. [128], some cells were electrically stimulated periodically during cul-

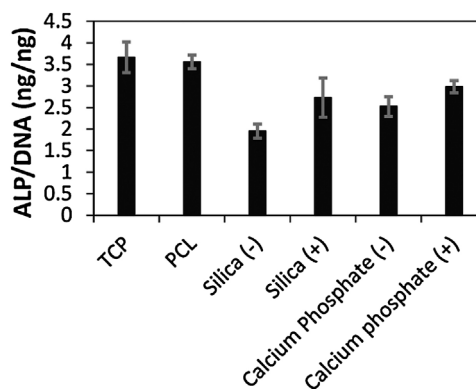
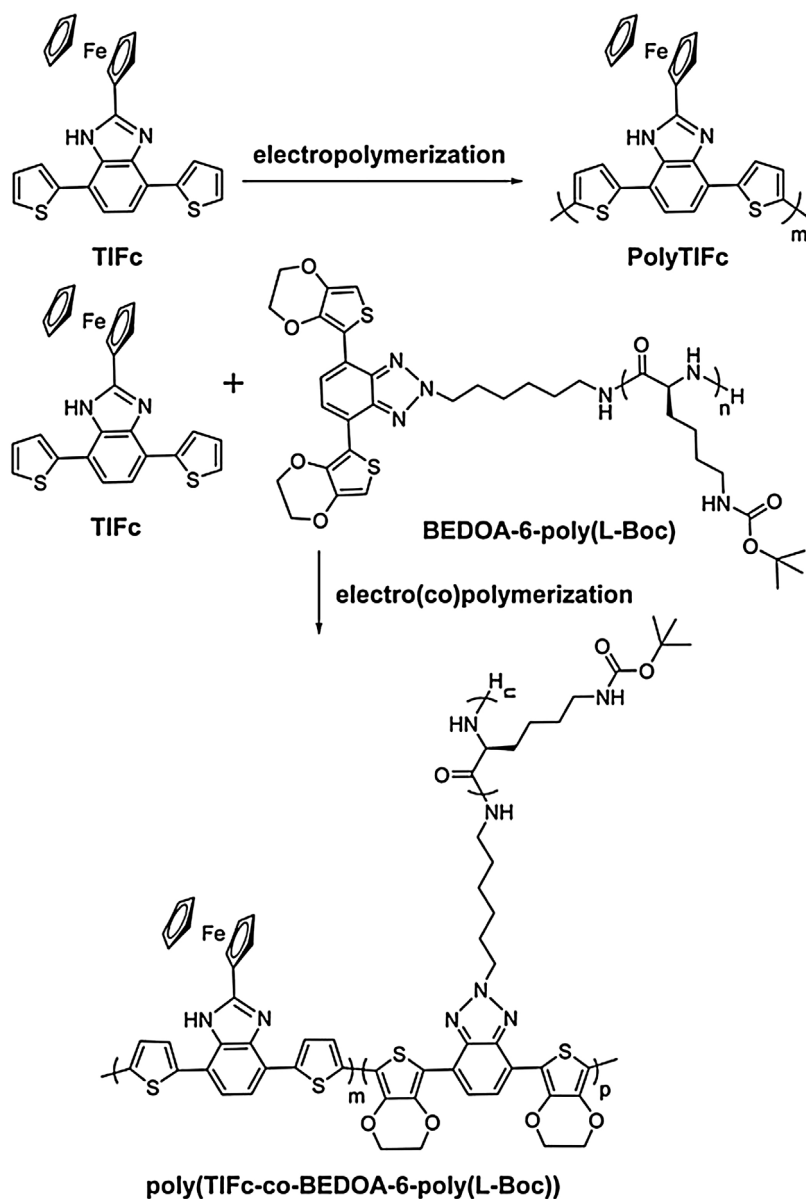


Fig. 9. Biochemical analysis of in vitro cell culture experiments. ALP activity. TCP, tissue-culture treated Corning® Costar® tissue culture plate controls. PCL, PCL control. Silica (-), conducting silica-biomineralized film without electrical stimulation. Silica (+), conducting silica-biomineralized film with electrical stimulation. Calcium phosphate (-), conducting calcium phosphate-biomineralized film without electrical stimulation. Calcium phosphate (+), conducting calcium phosphate-biomineralized film with electrical stimulation. [129], Copyright 2015, Reproduced with permission from the Royal Society of Chemistry.

ture. After 3 weeks, the cells had penetrated the biomineralized substrates, but cell growth was hindered compared to PCL and commercial tissue culture plate controls. ALP activity was also reduced for all modified films compared to the controls, but the electrically stimulated cells showed significantly higher activities compared to unstimulated substrates – indicating that electrical stimulation helped to induce osteogenic differentiation of the mesenchymal cells (Fig. 9).

### 2.3.5. CPs with polypeptide brushes

Polypeptide-based materials exhibit many desirable properties for bioapplications, including excellent biodegradability, the ability to form 3D structures such as  $\alpha$ -helices and  $\beta$ -sheets, potential switchability depending on amino acid composition and the ability to influence the binding and activity of cells and biomolecules, such as promoting cell adhesion through the use of the integrin-binding RGD peptide motifs [130–132]. Covalent grafting of polypeptide chains overcomes issues of leaching that may occur in materials fabricated using physisorption or entrapment of the polypeptides. The use of longer polypeptide brushes also provide more functional



**Fig. 10.** Synthesis of poly(TIFc) and poly(BEDOA-6-poly(L-Boc)) by electropolymerization. [134], Copyright 2014, Reproduced with permission from the Royal Society of Chemistry.

groups per surface area to the material compared to the shorter peptide attachments covered in Section 2.2.

One important use of CPs grafted with polypeptides is in the development of electrodes for biosensing. Polypeptide brushes are able to covalently bind with enzymes; in turn, the enzyme recognises and binds to the target analyte, changing the electrochemical properties of the electrode which can be monitored by electrical read-out [133]. An interesting example of this methodology was reported by Kesik et al. [134], who attached poly(L-Boc) to amino-functionalized bis-EDOT, and copolymerized them electrochemically with ferrocene imidazole derivatives of dithiophene (Fig. 10), with the ferrocene acting as an electron transfer mediator. Alcohol oxidase (AOx) was covalently immobilised on the terminal of the polypeptide chain, and the resulting electrode was used as an ethanol sensor. Under optimal conditions the sensor showed a linear response between 0.17–4.25 mM ethanol, with a limit of detection (LoD) of 0.28 mM. Compared to other studies of similar biosensors [135–137], AOx showed higher affinity for ethanol, due to effective immobilization and excellent microenvironment pro-

vided by the CP. The biosensor also showed no response to common interferences such as glucose, urea, and ascorbic acid, and provided accurate and reliable results for alcoholic beverages sampled without pretreatment [134].

Glucose oxidase (GOx) is another common enzyme used in biosensors, particularly in polypeptide-based biosensors. Akbulut et al. used the 'grafting through' method to synthesise polythiophene-g-polyalanine (PTh-g-PAla), followed by covalent attachment of GOx [138]. This biosensor showed a linear response to glucose from 0.01–1.0 mM, similar to other biosensors based on covalent immobilization of GOx [139,140], but with a higher sensitivity and better repeatability compared to physisorption methods [141,142]. Thus, this method produces more robust biosensors due to the lack of enzyme leaching. A similar biosensor was fabricated by Guler et al. [143], who immobilised GOx on polythiophene-g-polyphenylalanine (PTh-g-PPhe) films. The resulting glucose biosensor showed a linear response from 0.025–1.0 mM, similar to the work by Akbulut et al. [117]. The PTh-g-PPhe/GOx biosensor showed no interference with common interferants such as ascor-

bic acid, ethanol and uric acid, and measurements in real samples without pre-treatment were in good agreement with the results from a commercial assay kit [143].

Both Akbulut et al. [117] and Guler et al. [143] briefly investigated other applications of their respective PTh-g-polypeptide films. Akbulut et al. tested the antimicrobial properties of both Th-g-PAla monomer and PTh-g-PAla polymer using the disc diffusion method [117]. The monomer showed no antimicrobial activity, but a PTh-g-PAla-coated graphite disc demonstrated moderate antimicrobial activity against Gram-positive *Staphylococcus aureus* with a 7 mm zone of inhibition around the disc, compared to 20–30+ mm for standard antibiotic controls. PTh-g-PAla also showed no antimicrobial activity against Gram-negative *E. coli*. Meanwhile, Guler et al. used their PTh-g-PPhe films to culture HaCaT (human keratinocyte) and U87-MG (human glioblastoma) cell lines [143]. The films showed a significant decrease in cell adhesion after 72 h compared to PS control plates. Attaching RGD peptides to the PPhe chains greatly improved U87-MG growth until it was almost equivalent to the PS control, while little difference was observed for the HaCaT cells; this was attributed to the overexpression of integrin in U87-MG cells, which binds to RGD and promotes cell adhesion and growth.

### 3. Summary and outlook

Today, materials are able to interface with biological molecules and tissues in ways we never thought possible a decade or two ago. Organs are grown in petri-dishes, implants can help repair nerve damage, even in vivo electrical stimulation using flexible polymeric electrodes are routine. Many of these advances are due to developments in materials chemistry, particularly regarding the tailored functionalization of polymers.

Conducting polymers are prime candidates for a range of biomedical applications that take advantage of their intrinsic conductivity and superior mechanical properties over metal-based devices. As discussed, CPs are used as biosensors, biomedical implants and microelectrodes, cell culture substrates, and controlled drug delivery devices. Through functionalization, researchers have improved the biocompatibility and environmental degradability of CP-based materials, tuned solubility, wettability and antifouling properties, incorporated switching behavior, and induced cell growth and differentiation.

'Traditional' functionalization of CPs through doping, particularly with bio-dopants, is an effective and easy approach, and is indeed necessary for many applications, such as drug delivery systems. Dopant-induced cell growth and differentiation, or switchable wettability to control cell adhesion, have been extensively demonstrated with that approach. However, it is important to consider the accessibility of biodopants buried in the CP film or, alternatively, the risk of them leaching out. In other applications, long-term stability of the functionalization is important. An obvious example is biomolecule attachment (via covalent bonding or physisorption) for sensing applications, such as oligonucleotide sensors for genetic disease markers.

However, more extensive derivatization of CPs for biointerfaces (as opposed to other fields such as photovoltaics) with covalently-bound functional moieties has been still sparsely reported in the literature, in spite of obvious great opportunities – not just to impart desired biological functionality, but also to tune the physicochemical properties of the CPs. Making these materials (solution-) processable and with tailored mechanical properties (e.g. soft, elastomeric, adhesive, antifouling) is a highly desirable goal.

We suggest here that functionalization of CPs with polymeric side chains (brushes) provides an array of options in tuning the properties of electroactive CP-based biointerfaces. Compared to

other methods of CP functionalization, grafting with polymers is a relatively new and unexplored field. This approach could capitalize on significant recent advances in the field of synthetic macromolecular chemistry, to provide tailored antifouling, stimuli-responsive or cross-linked conductive biointerfaces. When grafting, it is important to consider the surface presentation and density of the brushes; synthesis methods may also allow for the attachment of multiple brush types or indeed other functionalities. Polymer chains can easily be utilised to introduce further functional moieties; obvious examples include the attachment of cell adhesion peptides or growth factors. Thus, with the flexibility offered by grafting methods, we anticipate a rise in tailored CP-based grafted materials and devices to be used in a range of biological and medical settings.

One exciting new development is that of optically exciting the electrical activity of living cells by using exogenous absorbers. It has been suggested that functionalized CPs can be used as such photoactive biointerfaces due to their superior biocompatibility and processability compared to the traditionally silicon based light harvesting interfaces [144]. Further developments in this field open up the possibility of wireless communication with biological tissues. The potential to not only excite, but also to inhibit neuronal activity will open up interesting opportunities for the use of conjugated polymers in neuroscience.

The future prospects of bioelectronics are rapidly expanding. With the increasing focus on CP functionalization, it's likely that we will soon see a rise in bioelectronics devices designed to be integrated with human bodies to monitor, modulate and repair biological functions.

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