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REVIEW

Polypyrrole-based conducting polymers and interactions with biological tissues

D. D. Ateh[1,2,*], H. A. Navsaria[2] and P. Vadgama[1]

[1]*IRC in Biomedical Materials, Queen Mary University of London, London E14NS, UK*
[2]*Centre for Cutaneous Research, Institute of Cell and Molecular Sciences, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK*

Polypyrrole (PPy) is a conjugated polymer that displays particular electronic properties including conductivity. In biomedical applications, it is usually electrochemically generated with the incorporation of any anionic species including also negatively charged biological macromolecules such as proteins and polysaccharides to give composite materials. In biomedical research, it has mainly been assessed for its role as a reporting interface in biosensors. However, there is an increasing literature on the application of PPy as a potentially electrically addressable tissue/cell support substrate. Here, we review studies that have considered such PPy based conducting polymers in direct contact with biological tissues and conclude that due to its versatile functional properties, it could contribute to a new generation of biomaterials.

Keywords: biomaterials; conducting polymers; polypyrrole; tissue; cells

1. INTRODUCTION

Through new combined knowledge of molecular biology and the biophysical correlates of material surface properties (Kasemo 1998; Castner & Ratner 2002; Tiefenauer & Ros 2002), local interactions between cells and their immediate microenvironments are increasingly better understood and recapitulated for the design of practical biomaterials (Discher *et al.* 2005; Liu & Chen 2005; Stevens & George 2005). Tissue engineering (Langer & Vacanti 1993) is probably one of the most likely avenues for exploitation of such new generation materials along with other niche areas such as neuroprosthetics, biosensors and drug delivery.

In tissue engineering, especially with regard to bioreactors for optimal tissue growth in artificial constructs prior to implantation, the majority of cell-supporting scaffolds currently used are porous and degradable polymers (Seal *et al.* 2001). Such structures may be fabricated from natural materials, such as collagen or fibrin or synthetic polymers such as polyglycolide or polylactide. However, such scaffolds (or substrates) and their associated bio-functionality are now known to be important in tissue growth and guidance beyond any mesoscopic organization. For example, their topography (Curtis & Wilkinson 1997), mechanics (Wong *et al.* 2004) and incorporated controlled release growth factors and signal molecules (Saltzman & Olbricht 2002) can have profound effects on cell behaviour.

Tailoring specific material properties, bulk as well as surface, could provide novel solutions for tissue-engineered systems including controlled cell assembly (micro and nanopatterned surfaces), drug release (degradable polymers), tissue release (thermoresponsive polymers) and integrated biosensing (electroactive polymers). In addition, such materials provide a platform for the study of the fundamental underpinning science relating to tissue-material surface interactions. It is in recognition of these special requirements that researchers have engaged unique classes of materials for trial use in biological applications. Conducting polymers such as polypyrrole (PPy) offer a new class of material in this regard. This review presents research where PPy is in contact with biological tissue and outlines current achievements with an assessment of future opportunities.

2. POLYPYRROLE-BASED CONDUCTING POLYMERS

2.1. Conducting polymers

Letheby in 1862 first reported the anodic oxidation of aniline in dilute sulphuric acid, yielding an insoluble blue–black shiny powdered deposit on a platinum electrode. Further experiments led Goppelsroeder in 1876 to establish that oligomers were formed by the

*Author for correspondence (d.d.ateh@qmul.ac.uk).

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oxidation of aniline (Heinze 1989). Natta *et al.* (1958) synthesized polyacetylene and Dall'olio *et al.* (1968) discovered yet another compound, PPy, at the time called pyrrole black. However, it was not until 1977 that Shirakawa and his co-workers wrote their seminal paper showing that halogen doping of polyacetylene dramatically increased its conductivity (to around 10^3 s m^{-1} in the case of I-doped *trans*-polyacetylene). The major breakthrough with regard to the routine synthesis of conducting polymers, however, was achieved by Diaz and co-workers (Diaz & Kanazawa 1979; Kanazawa *et al.* 1979; Diaz 1981) when they reported the formation of a highly conductive, stable and manageable PPy film under controlled electrochemical conditions. Since then, in the context of cell-material studies, PPy has mainly been produced by electrochemical reaction. However, along with chemical synthesis, other polymerization methods have involved photochemistry, metathesis, concentrated emulsion, inclusion, solid-state, plasma, pyrolysis and soluble precursor polymer preparation (Kumar & Sharma 1998).

There are currently over 25 reported conducting polymer systems (Skotheim 1986) with the commonality that they have a conjugated structure of alternating carbon–carbon double bonds (figure 1). It is this peculiar structure that confers electronic properties, notably, low-energy optical transitions and ionization potentials as well as high-electron affinities. The most important aspect of a conjugated polymer from an electrochemical view is its ability to act as an electronic conductor. This property is further controlled by redox switching at specific potentials accompanied by the movement of dopant ions into or out of the material depending on net polymer charge.

Chemically synthesized conjugated polymers are initially insulators (i.e. in a neutral state) and it is only through oxidation (p-doping) and less frequently reduction (n-doping) by chemical or electrochemical means, that the necessary mobile charge carriers for conductivity are formed. In the case of PPy for instance, the backbone is neutral in the reduced state and positively charged in the oxidized state. Therefore, to maintain electroneutrality, some counterion is required to diffuse into the polymer during charging and out during neutralization. The oxidation process may also be accompanied by significant change in polymer volume upon ingress of the mobile anionic species, a characteristic exploited in actuator applications (Otero & Sansinena 1995). Overoxidation of conducting polymers, notably PPy, where the polymer is held above the standard oxidative potential, leads to loss of conductivity and de-doping (Gao *et al.* 1994; Farrington & Slater 1997; Shiigi *et al.* 2002).

There has been a large effort focusing on the development of conducting polymers for practical applications. Thus, these materials have been investigated for rechargeable batteries, electrochromic displays, information memory, anti-static materials, anti-corrosives, electrocatalysis, sensors, electromechanical devices, infra-red polarizers and radar (Stenger-Smith 1998). Biomedical applications have also been considered including biosensors (Lillie *et al.* 2001; Gerard *et al.* 2002) and as of late cell growth substrates. The later application is made more appealing by the possibility of dopant substitution with biologically functional macromolecules such as proteins, polysaccharides and even whole living cells, during the polymerization process (Adejolu & Wallace 1996). Furthermore, with recent evidence uncovering important physiological roles for *in vivo* electric fields as created by cell layers in order to provide wound healing or developmental cues for instance (Martindale 2004), conducting polymers may offer new advantages as biomaterials.

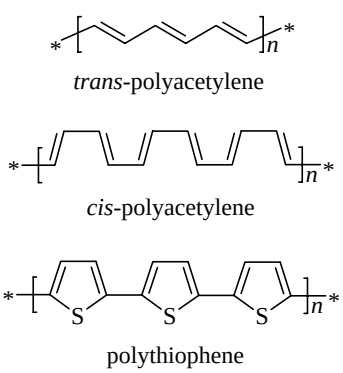

Figure 1. Examples of conducting polymer structures. The conjugated structure consisting of an alternating carbon–carbon double bond is common to all conducting polymers.

Certainly, in past experiments, small electrical currents have been shown to stimulate tissue responses such as bone re-growth and wound healing (Lindsey *et al.* 1987; Kohavi *et al.* 1992; Kloth & McCulloch 1996; Reger *et al.* 1999). These were achieved using metallic electrodes inherently incompatible with biological tissues. With organic conducting polymers, the possibility for a more intimate relationship with biological systems exists (Kane-Maguire & Wallace 2001). In particular, certain tissues such as those of the nervous system (Velasco 2000) or skeletal and smooth muscle (Grandjean *et al.* 1996) may be particularly susceptible to modulation via electrical stimulation. With these possibilities in mind, PPy has become by far the most studied conducting polymer and is therefore the focus of this review.

2.2. Polypyrrole

PPy is generally synthesized by chemical or electrochemical means. Chemical synthesis is used when large quantities of material are required and involves mixing a strong oxidizing agent (typically $FeCl_3$) with a monomer solution (Armes 1987; Duchet *et al.* 1998). Electrochemical synthesis is preferred for research purposes due to the simplicity of the technique, control over material thickness, geometry and location, the facility for doping during synthesis, the wide choice of available dopant ions and the generation of good quality films (Kumar & Sharma 1998; Inzelt *et al.* 2000). It leads to the development of adherent surface conformal deposits i.e. thin solid films, from the bulk solution phase of monomer units. The electrodeposition on the positively polarized working electrode proceeds

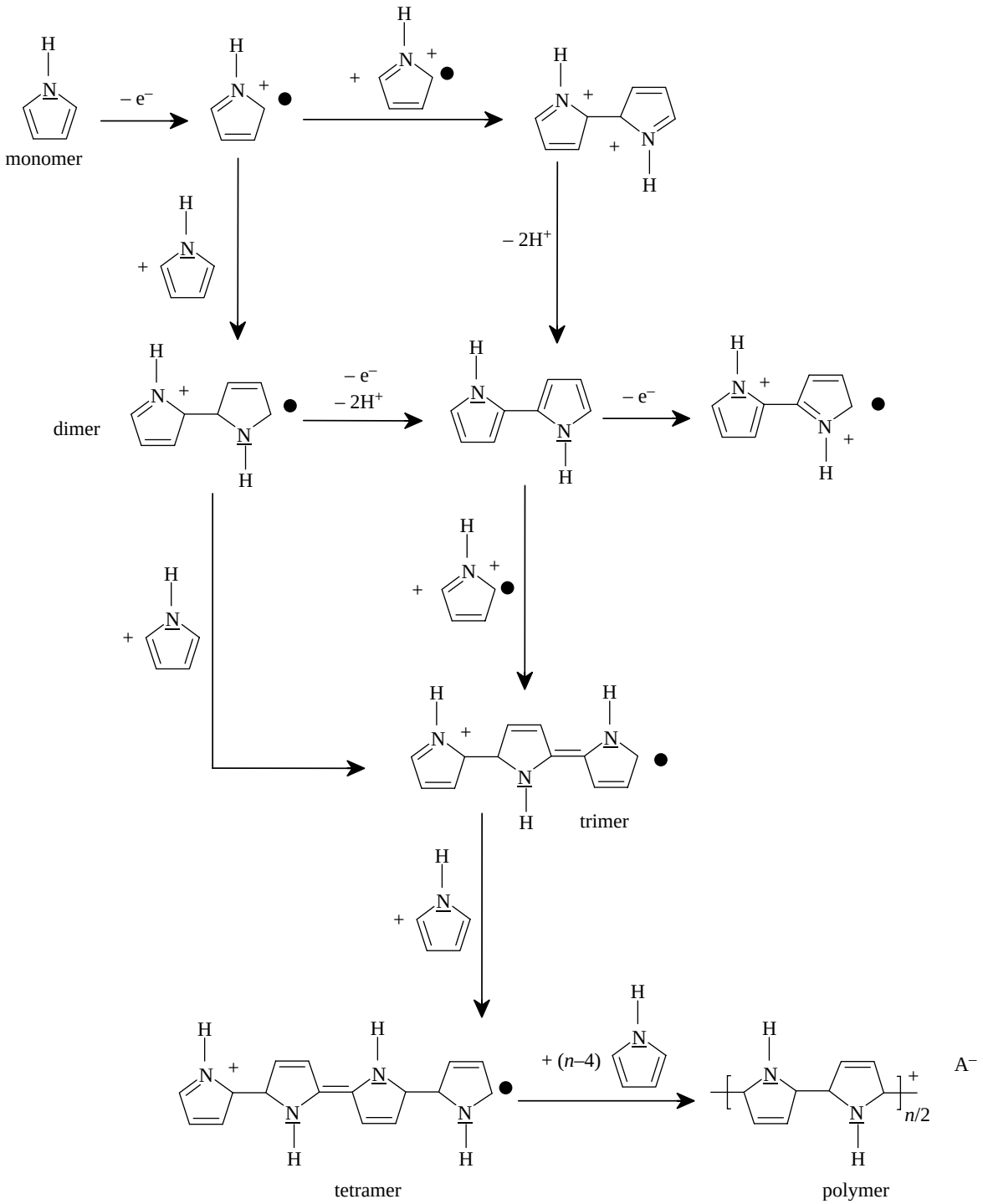

Figure 2. Electropolymerizaion mechanism of polypyrrole. Monomer units are adsorbed onto the surface of the working electrode resulting in one-electron oxidation to form a pyrrole cation radical. These cations then couple with themselves, with other cations or with neutral monomers from solution. In each case, this leads to the formation of a dimer dication, which undergoes a double deprotonation to give a neutral molecule. These more stable dimer radicals have a lower oxidation potential compared with the monomer units and chain growth then occurs by preferential coupling between the dimers and monomers (Skotheim 1986). Anion (A^-) is required to maintain electroneutrality.

via a condensation reaction between the monomer units of the five-membered heterocycle pyrrole (figure 2). Concomitantly, negatively charged counterions must be present in solution to maintain charge balance within the polymer since positive charges are developed along the PPy backbone. This latter process is referred to as doping and the choice of counterion, including biomolecules, affects formed polymer properties.

The growth of the PPy depends on its electrical characteristics; if it was non-conducting, its growth would be self-limiting, producing very thin films as in the case of polyphenol and its derivatives (Eddy *et al.* 1995). By contrast, PPy growth is virtually unlimited due to inherent conductivity and therefore charge connectivity to the subjacent anode. There are a large number of experimental formulations for the

preparation of PPy each of which significantly modify the phenomenological properties of the polymer. Generally, electrochemical polymerization is undertaken at potentials above +600 mV versus a Ag/AgCl reference. The morphology of the resulting film depends in particular on the nature of the supporting electrolyte, the crystallographic structure of the underlying anode, the kinetics of the process (related to the electrode material), the potential used for deposition, the nature of the dopant and the concentration of the original monomer solution. Temperature and pH also have an effect on the ensuing film. Figure 3 shows examples of how different surface topographies are generated when the counterion and synthesis duration are varied.

For formed films, conductivity arises from electronic transfer along the conjugated π-molecular orbital backbone coupled with the motion of charge carriers in the material. Upon oxidation, an electron is removed from the π-system of the backbone producing a cation and a local distortion due to a change in geometry every four pyrrole units. This radical cation coupled with the local deformation constitutes a polaron. Upon further oxidation, at higher charging levels, pairs of polarons combine to form bipolarons as these are energetically more favourable. Bipolarons are able to migrate along the conjugated polymer chain and provide the main charge transport mechanism within the conducting polymer (Heinze 1989; Inzelt *et al.* 2000). Final conductivity reflects the charge transfer between the dopant and the polymer segment, charge carrier mobility within the conjugated segments of a single polymer chain and charge transfer (or 'hopping') between individual chains (Bhattacharya *et al.* 1996). Essentially, it has been postulated that it is the least efficient of any of these mechanisms under any given condition of say temperature and pH that determines the final conductivity of the material at a macroscopic level. Research is still continuing with the aim of reaching a fuller, more detailed understanding of charge storage and transport mechanisms in conjugated polymers (Papathanassiou *et al.* 2005).

3. TISSUE AND CELL INTERACTIONS WITH POLYPYRROLE

3.1. Context

Since the early nineties, PPy has been substantially studied as a cell growth substrate within *in vitro* culture models. Furthermore, the effects of implantation *in vivo* have also been studied using animal models. These PPy films have usually been electrodeposited on an underlying electrode surface (e.g. indium tin oxide or gold) with simultaneous incorporation of inert counterions or biologically active molecules ready for cell culture or to be peeled off prior to use. These studies could be broadly divided into two categories, those that investigate interactions with tissue for PPy used after synthesis with specific loadings or conditions and those that additionally exploit conducting properties as a means of influencing cellular outcomes.

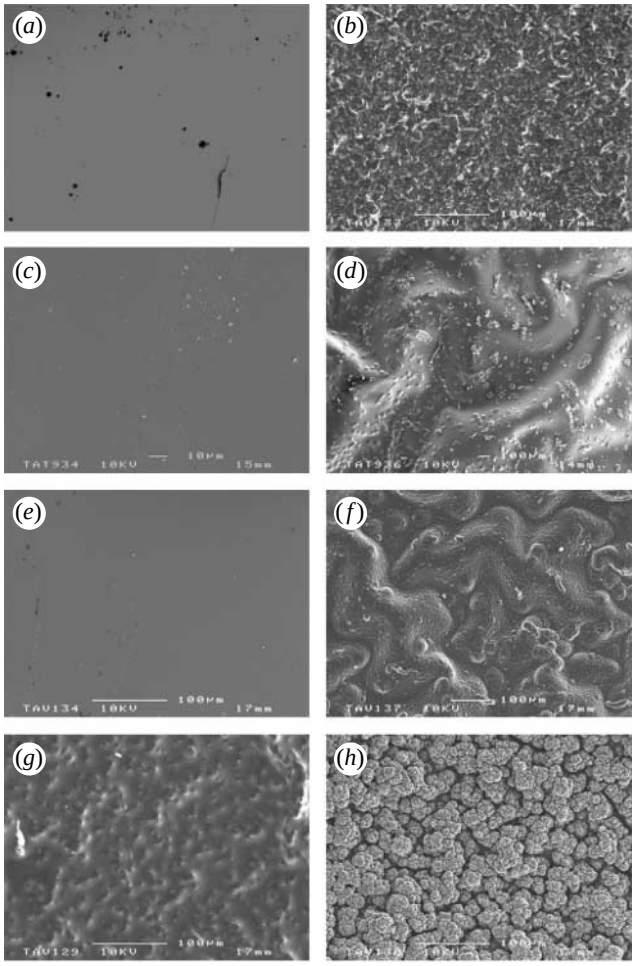

Figure 3. Scanning electron microscopy images of polypyrrole (PPy) surface topography generated for different counterions and electropolymerization durations (*a–b*) PPy/chloride, (*c–d*) PPy/polyvinyl sulphate, (*e–f*) PPy/dermatan and (*g–h*) PPy/collagen. The shorter times produced thin films (left column) with none or little surface features whereas at extended times, thicker films with distinct topography are seen (right column). More instances of counterion controlled topography may be found in the literature (Skotheim 1986).

3.2. Unstimulated polypyrrole

The preferential maintenance of secretory function for chromaffin cells, neuroendocrine cells that secrete neurotransmitters, cultured on PPy modified indium tin oxide compared to the unmodified substrate was demonstrated by Aoki *et al.* (1995, 1996). Their research and that of others suggested early on that advantages over traditional electrode surfaces may be gained through such modifications. In other constructs, such as the commercially available PPy-coated woven polyester fabrics known as Contex, the response of various cell types has been studied (Jakubiec *et al.* 1998). Four different grades of increasing conductivity were compared with uncoated polyester fabrics and polydimethylsiloxane. Overall, it was found that for fabrics of highest conductivity, fibroblast and endothelial cell viability was impaired, polymorphonuclear cell activation increased and macrophage IL-6 expression reduced. Optimal responses were found for PPy-coated polyester fabrics of intermediate

conductivity and the authors, though not yet fully understanding the reason for this, speculated that local release of cations from PPy, which presumably varies between their compositions, affects cell behaviour due to modification of ionic transport across the neighbouring cell membrane. Similarly coated polyester fabrics have been shown to elicit less or comparable cellular reactive responses including inflammation as well as acid and alkaline phosphatase levels when implanted subcutaneously in rats over 3–90 days and compared with their uncoated counterparts (Jiang *et al.* 2002).

Garner *et al.* (1999*a*) studied human umbilical vein endothelial cells on PPy-heparin films. They chose the counterion heparin, as it is a component of the extracellular matrix of blood vessels as well as having anticoagulant properties. They established that the conditions for synthesis as well as polymer redox state led to variations in the level of surface exposed heparin. They then showed that the PPy-heparin composite supported the growth of endothelial cells with a reduction in the normal amount of heparin required as a medium supplement. Since cells did not grow on PPy-nitrate, this was attributed to the presence of heparin with attachment shown to be vitronectin dependent (Garner *et al.* 1999*b*). The work of Collier *et al.* (2000) considered composites of PPy and the ubiquitous glycosaminoglycan, hyaluronic acid (HA). They showed HA retained affinity properties on the surface of the formed polymer using biotinylated HA binding protein. *In vitro* compatibility studies using PC-12 cells (cell line derived from a transplantable rat phaechromocytoma that serves as a model for primary neuronal cells) confirmed that the PPy-HA composites supported cell attachment and viability. *In vivo* investigation using implantation in rat subcutaneous pouches for two and six weeks demonstrated various tissue responses (figure 4). It was found that compared with a PPy-polystyrene sulphate control implant, there was a statistically significant increase in vascularization around the HA containing polymer. In tissue, HA is a known angiogenesis promoter important during wound healing for instance; this study suggests it retains this ability whilst incorporated in PPy films. Others have showed good PC-12 growth and blood compatibility when PPy surfaces have been functionalized with HA and sulphated HA (Cen *et al.* 2004).

Neural recording microelectrodes have been coated with PPy doped with fibronectin and laminin fragments (Cui *et al.* 2001). In the study, preferential binding was reported for glial cells on films incorporating fibronectin fragments whereas neuroblastoma cells favoured films incorporating the laminin fragment with the CDPGYIGSR (p31) amino acid sequence. It was found that the coatings did not interfere with recordings when measurements were made in guinea pig cerebellum. PPy films incorporating another laminin sequence, RNIAEIIKDI (p20), and those incorporating both p20 and p31 were found to generate increased neuron densities in culture compared with polystyrene sulphonate loaded films (Stauffer & Cui 2006). Micropatterns produced through covalent attachment of polylysine and/or laminin to PPy-polyglutamic acid composites showed that neuron adhesion and neurite extension could be controlled in terms of spatial arrangement on these substrates (Song *et al.* 2006). These studies demonstrate that biomolecules pertinent to nervous system cell adhesion, migration and proliferation may be used in the provision of PPy-based neuroprosthetics since they enhance cell-material interactions. George *et al.* (2005) considered the response of rat cortical tissue both *in vitro* and *in vivo* to PPy formed under varying dopant compositions and electrodeposition temperatures (figure 5). They did not incorporate biomolecules, but nevertheless reported favourable responses compared to Teflon implants in terms of macrophage activity, gliosis and neuronal integration after implantation, although they presented pilot studies of PPy incorporating nerve growth factor (NGF), which suggested increased neural integration.

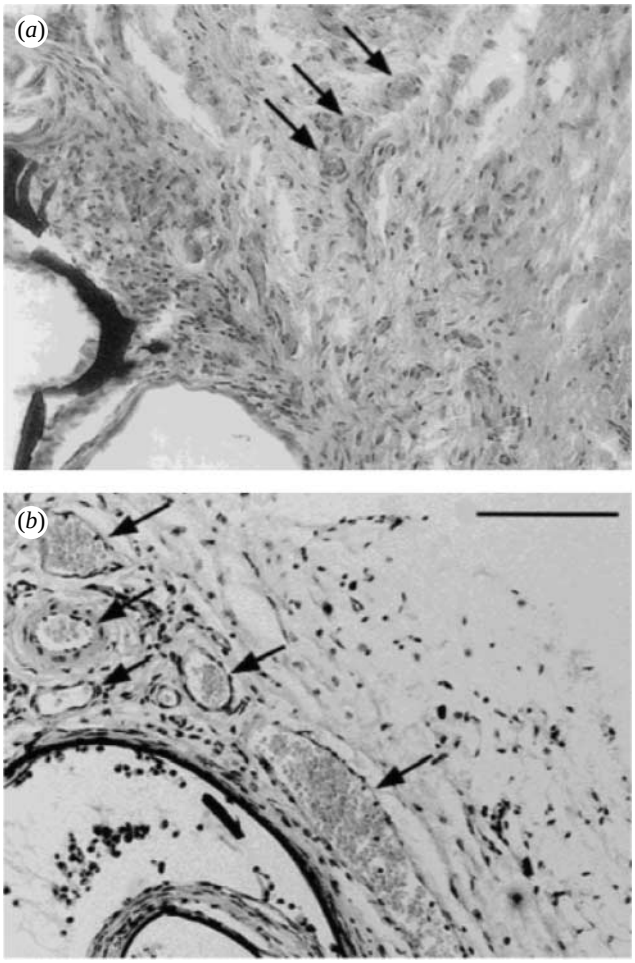

Figure 4. *In vivo* tissue response to polypyrrole-hyaluronic acid (PPy/HA) bilayer films. (*a*) Polypyrrole-polystyrene sulphonate (PPy/PSS) films and (*b*) PPy/HA bilayer films were implanted into subcutaneous pouches in rats. Tissue surrounding the material was harvested after two weeks, fixed, imbedded and stained with hematoxylin and eosin. The heavy black lines in both images are the PPy/PSS and PPy/HA bilayer films. Blood vessels are denoted by arrows. Scale bar, 100 µm (both images are at the same magnification). This figure shows that HA retains its angiogenesis properties whilst incorporated in PPy since more blood vessels were seen around this implant compared to the PPy/PSS control. Reprinted from Collier *et al.* (2000) with permission from John Wiley & Sons, Inc.

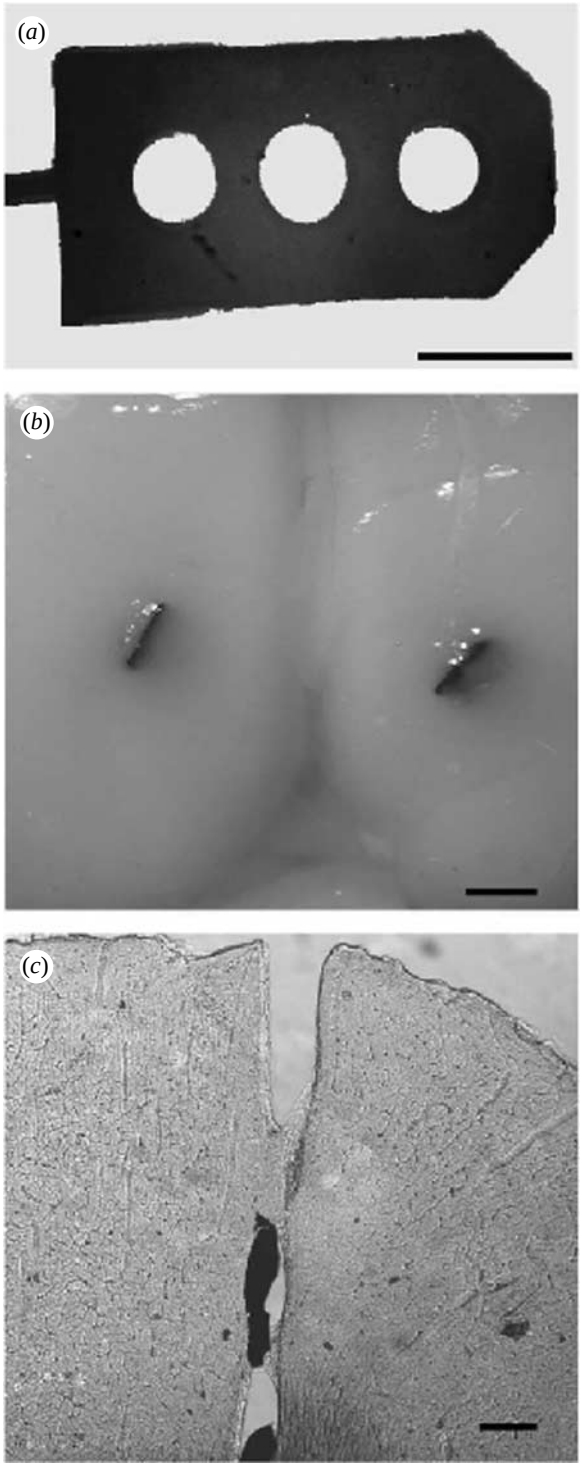

Figure 5. Polypyrrole (PPy) implants. (*a*) An example of a typical implant; scale bar, 1 mm (*b*) two PPy implants placed in the rat's cortex; scale bar, 2 mm (*c*) a histological slice at six weeks post-implantation with the remnants of the Polypyrrole implant; scale bar, 200 µm. Favourable responses for PPy compared to Teflon implants in terms of macrophage activity, gliosis and neuronal integration were found after implantation. Reprinted from George *et al.* (2005) with permission from Elsevier.

They thought that improvements for the PPy neuroprosthetics compared with the inert Teflon were due to less physical integration and higher inflammatory responses in the latter whereas PPy presents a more favourable surface chemistry.

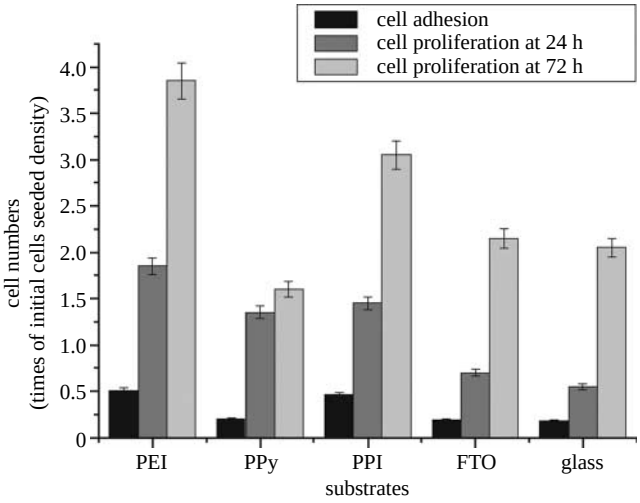

Figure 6. Neuronal cell line adhesion and proliferation on different substrates after 8, 24 and 72 h of culture. The numbers of cells were normalized to initial density of seeded cells (200 000 cells ml^{-1}) ($n=3$ per substrates). The volume of the cell suspension used is 100 µl. All results are given at $\pm$ 5%. PEI is polyethyleneimine, PPy is polypyrrole, PPI is polypropyleneimine and FTO is fluorine-doped tin oxide. This study illustrates that under some conditions poor interactions may occur between polypyrrole and cells compared to other materials. Reprinted from Lakard *et al.* (2004) with permission from Elsevier.

Mattioli-Belmonte *et al.* (2003) studied the tissue and cellular tolerance of non-resorbable and resorbable materials. While they reported the absence of necrosis or degeneration around implants inserted subcutaneously in rats, the extent of fibrous encapsulation and the number of surrounding inflammatory cells reduced in the order, poly(lactide-b-1,5-dioxepan-2-one-b-L-lactide) < polyaniline < polypyrrole < polyimide for the former response and polyaniline < polypyrrole < poly(lactide-b-1,5-dioxepan-2-one-b-L-lactide) < polyimide for the latter. They also studied the *in vitro* growth of a human keratinocyte cell line on PPy films and found poor adhesion compared with the other substrates including the resorbable triblock polymer based on poly-L-lactide. In another study, from Mattioli-Belmonte *et al.* (2005), it was shown that behaviour of the same keratinocyte cell line was modulated by redox state and morphology of PPy-tosylate films with poor growth occurring on oxidized substrates but none on those that were overoxidized. They assigned this limited cell growth to surface tension and irregular roughness in the oxidized films, with the possibility that tosylate diffusion into the culture media worsened the outcome on overoxidized films. Poor proliferation was also reported by Lakard *et al.* (2004, 2005) for a rat neuronal cell line on PPy when compared to other substrates including those that were electrodeposited (figure 6). Furthermore, Castano *et al.* (2004) showed a dependence on film thickness as controlled by monomer concentration during admicellar polymerization, where a surfactant, monomer and initiator are used to form the polymer, for the viability and differentiation of mesenchymal stem cells towards the osteoblastic phenotype. Thin films prepared from lower monomer concentrations were excellent for cell

adhesion and subsequent growth in contrast to thicker films and those made by the standard chemical polymerization method i.e. without surfactant. They thought poor results for increased monomer concentrations could be due to toxic leachables or surface properties that impede cell attachment.

The biocompatibility of PPy prepared from both chemical and electrochemical means was thoroughly evaluated by Wang *et al.* (2004). They carried out a series of systemic toxicity tests according to ISO 10993 and ASTM F1748-82 standards by applying a solution of extracts from PPy powder to cell cultures and animal models. They found that extract solutions did not have adverse effects on cell cultures or on the animals tested. In the case of the animal models, this included the absence of body temperature change, red cell haemolysis, allergic response or mutagenesis. In addition, good growth of Schawnn cells cultured on electrochemically polymerized PPy compared with bare glass substrates was observed. The report also demonstrated the novel electrochemical deposition of PPy on the inner surface of a silicone tube, used to bridge gaps created in the sciatic nerve of rats. Slightly improved nerve regeneration with only mild inflammation after six months was seen compared to uncoated silicone tubes. Li *et al.* (2005) showed PC12 cell attachment and growth to be superior on their porous PPy-tetraethylammonium perchlorate-polyvinyl alcohol composites compared with tissue culture polystyrene. They thought this was due to the presence of pores in the PPy composite, which enhanced signal triggering and acted as nutrient reservoirs below the cells.

In our studies, we have shown that variously loaded PPy films including those incorporating proteins are feasible for producing coherent membranes and are able to support keratinocyte growth (Ateh *et al.* 2006). However, we found that this was strictly dependent on thorough washing of the films prior to culture so as to eliminate toxic remnants such as monomers or oligomers from the synthesis step. In addition, we found that for thicker films which have a rough topography (figure 3), characteristic of each dopant, cleaning becomes more difficult presumably due to higher levels of remnants from the decrease in polymerization efficiency as films grew. This could help explain poor cell interactions with PPy for some of the studies discussed thus far since they did not mention a washing step for their polymers prior to use. However, it is also accepted that tissue and cell reactions to PPy-based conducting polymers are likely to be modulated by a variety of factors including synthesis conditions and dopant choice, which affect resulting surface chemistries and topography, as well as the tissue or cell type considered. Due to the endless combinations possible, outcomes reported with these factors vary widely in the literature.

3.3. Stimulated polypyrrole

The main attraction of PPy in the role of biomaterial stems from its electrochemical properties, essentially the ability to conduct charge coupled with the polymeric nature. Much work was carried out in the Late Eighties by Aizawa's group at the Tokyo Institute of Technology. They found that electrochemical oxidation of PPy caused a large local pH change along with incorporation of anions from solution (Shinohara *et al.* 1989*a*) and explored the rupture of erythrocyte cell membranes by an applied potential (Shinohara *et al.* 1989*b*). They attributed the pH change to local OH^- transfer between the electrolyte and the PPy film. In their work with erythrocytes, they found that cell lysis occurred on PPy-coated electrodes at *ca* +400 mV versus Ag/AgCl whereas a potential higher than +1400 mV was required to do the same on bare electrodes.

Wong *et al.* (1994) studied the viability of bovine aortic endothelial cells on PPy substrates, in some cases pre-coated with fibronectin, since cell attachment and spreading were found to be poor on uncoated films. Further experiments, where cell-seeded films were switched from oxidized to reduced states by applying a small negative electrical potential (−0.5 V versus saturated calomel electrode), revealed cellular responses within an hour. The reactions involved cell rounding (where the cell tends to detach from the substrate) and appeared to be determined by the electrochemical state of the PPy film. The authors suggested that this might be due to the local removal of fibronectin anchors or to mechanical changes in the reduced film. They further showed that these responses could be used to exert control over cell cycle progression since DNA synthesis was reduced to near zero on PPy in the case of an applied potential compared to over 70% for the control.

Williams & Doherty (1994) demonstrated the possibility of providing electric fields to neuroblastoma cells cultured on PPy. The work of Schmidt *et al.* (1997) built on these foundations and showed that positive electrical stimulation of polystyrene sulphonate-loaded PPy films during the culture of rat PC-12 cells and primary chicken sciatic nerve explants enhanced attachment and neurite extension (figure 7). In the case of PC-12 cells, no significant difference in neurite extension was recorded for cells grown on tissue culture polystyrene and unstimulated PPy. For the electrically stimulated PPy substrate, there was however, a doubling in median neurite length. They could not explain the exact mechanism for enhanced neurite extension in culture, but proposed the electrophoretic redistribution of molecular components involved in growth cone formation, favourable protein conformational changes, direct polarization of nerves, enhanced protein synthesis and field-induced ionic and molecular gradients in the culture medium as possible factors. Kotwal & Schmidt (2001) used PC-12 cells to investigate the effect of protein adsorption on PPy and the subsequent outcome of this on neurite extension. Their study discovered that electrical stimulation increased the adsorption of fibronectin from solution onto the PPy film prior to cell seeding. They postulated that this was due to fibronectin adopting a favourable conformation during electrical stimulation. Upon cell culture on the PPy substrates after the protein adsorption regimen, they reported increased neurite extension for electrically driven

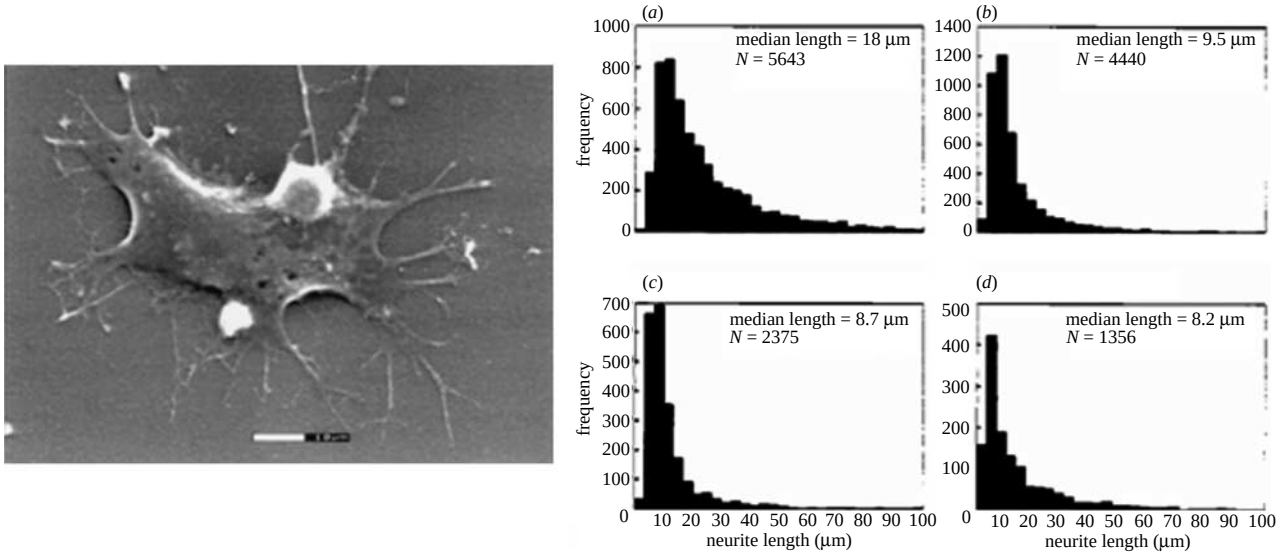

Figure 7. (Left) Scanning electron microscopy of a PC-12 cell on polypyrrole (PPy). PC-12 cells were cultured in NGF-supplemented medium for 48 h on thick disks of PPy, then processed for scanning electron microscopy. Bar = 10 µm. (Right) Neurite length histograms. Shown are histograms of neurite lengths for cells on PPy (*a*) with electrical stimulation (S) and (*b*) without (NS) potential applied through the PPy film. Histograms for cells on PPy with (*c*) potential applied through the solution and on (*d*) tissue culture polystyrene (TCPS) are also shown. This study demonstrates the potential of electrically stimulating PPy in order to affect cell behaviour. In this case, enhanced neurite outgrowth has implications in nerve regeneration therapies. Reprinted from Schmidt *et al.* (1997) with permission from National Academy of Sciences, USA (Copyright 1997).

fibronectin adsorption compared with the unstimulated material.

Hodgson *et al.* (1994, 1996) included NGF among other bio-components within a PPy-sulphated polysaccharide composite and achieved controlled release by electrical stimulation. Upon reduction of the PPy backbone at a suitable potential, they registered a release of NGF and subsequent differentiation of PC-12 cells growing on the polymer surface. More recently, Wadhwa *et al.* (2006) showed that the ionic form of the steroid dexamethasone could be incorporated into PPy and delivered by cycling the film between fixed potentials. They found that the delivered drug was as active as the non-incorporated counterpart in reducing the number of reactive astrocytes/microglia as well as in supporting normal neuronal growth and suggested this could help minimize gliosis around neuroprosthetics. However, delivery after implantation has yet to be achieved, and it is possible that the high cycling potentials required may cause undesired outcomes within the surrounding tissue. Thus, while there are numerous examples of PPy-based drug delivery platforms in the literature, *in vivo* efficacy needs to be established.

In studies extending beyond macromolecular counterions, Campbell *et al.* (1999) demonstrated the possibility of incorporating whole cells into the PPy matrix. In this study, it was shown possible to grow PPy films galvanostatically from an aqueous electrolyte solution of 0.27 M sucrose, 0.1 M pyrrole, 1 g l^{-1} polyvinyl sulphonate and erythrocytes. Most of the erythrocytes incorporated were intact disks with strong red staining suggesting normal haemoglobin content. A few cells were pale with the presence of inclusion bodies and this was attributed to haemoglobin loss either as a result of electroporation or other oxidative

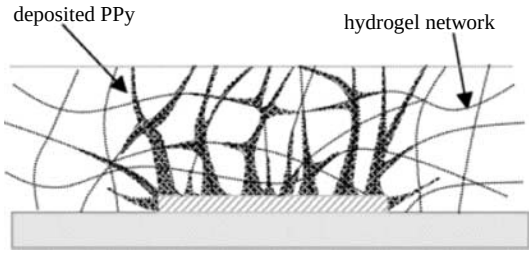

Figure 8. Schematic of the cloud-like conducting polymer on the gold electrode polymerized through the hydrogel matrix. The flexibility and usefulness of polypyrrole may be further enhanced with such composites. Reprinted from Kim *et al.* (2004) with permission from John Wiley & Sons, Inc.

damage during synthesis. It was also found that Rh (D) antigens on erythrocytes remained intact after inclusion in PPy and in the presence of antibody, a resistometric signal (related to changes in electrical resistance) indicated antigen/antibody binding. This suggested that erythrocyte-loaded polymers could be used as the basis of novel blood group immunobiosensors. Cui *et al.* (2003) reported good recordings of electrical activity with PPy-synthetic peptide coated electrodes when implanted in guinea pig brain over two weeks with better nerve cell integration than uncoated electrodes. When PPy was grown in a hydrogel scaffold (figure 8) coated on neural electrodes, it was also possible to make recordings (Kim *et al.* 2004). We have recently worked on impedimetric methods, where a small oscillating voltage is applied and the resulting current response analysed, to monitor cell behaviour on PPy-based substrates (Ateh *et al.* submitted). In fact, advantages over bare gold were found for the PPy modification including preferential cell growth and

resolution at lower cell numbers presumably due to better cell-material integration as well as a reduction in overall impedance due to the increased surface area generated by the PPy coating.

Researchers at the southeast University of China have looked at the effect of PPy electrical stimulation in relation to rat primary keratinocytes cultures (Pu *et al.* 2001). They electropolymerized PPy films on porous (120 μm pore size) stainless steel filters and through a series of experiments, optimized cell culture media composition and PPy electrical stimulation potential. The key finding was that under optimal conditions, including electrical stimulation for 2 h at 100 mV, there was over a 20% increase in cell viability, as measured by the MTT assay 3 days later, compared to standard culture methods. However, the method was unusual as the keratinocytes were firstly seeded onto tissue culture plates and after 5–6 h, the PPy films on their stainless steel supports were placed over the adhered cells. Therefore, unlike other accounts, the cells were not directly grown on the conducting polymer. Shi *et al.* (2004) developed a conductive biodegradable composite made from polylactide and PPy nanoparticles. Upon fibroblast culture and the application of a DC current for 4 days, an up-regulation in growth was also seen at optimal currents.

4. PROSPECTS

To the same extent as any potential biomaterial, the interactions of PPy with tissue depend on its unique, intrinsic properties notably surface chemistry, topography and micro-mechanics. The reported studies highlight the versatile nature of PPy whose properties may be tailored depending on the chosen synthesis conditions including choice of dopant ion and modification through electrical stimulation post-synthesis. They also show that there are distinct outcomes of interactions with tissue depending on choice of synthesis conditions and the precise tissue or cell type considered. Interpretation is further complicated by a lack of understanding of mechanisms or a consensus on the effect of electric fields and charged surfaces on biological tissue, although this prospect seems to be improved with new discoveries in biological electric field physiology (Martindale 2004). This means there are no clear rules as yet, such as a defined correlation between hydophilicity, redox state or applied current/potential with improved cell growth for instance, but with more research these should emerge.

It is likely that surface modification post-synthesis such as the novel strategy that developed a PPy-chloride binding peptide from a bacteriophage library (Sanghvi *et al.* 2005) will prevail over dopant approaches since the latter can often result in a loss of electrochemical properties including conductivity. Other advances in PPy synthesis methods, such as preparation routes for biodegradable conducting PPys synthesized with degradable ester linkages (Rivers *et al.* 2002) or from β-substituted pyrrole monomers with ionizable/hydrolysable side groups (Zelikin *et al.* 2002) and stretchable PPy films made with dopants that act as plasticizers (Oh *et al.* 2001) also enhance their potential as an adaptable biomaterial. Research in this area is still in its infancy but PPy-based conducting polymers have the potential to be coatings or stand alone substrates that could be tailored to support various tissue/cell types. They could also electrically modulate behaviour through induced drug delivery, property changes or just electric field provision. Films could also be used to report on tissue/cell interactions through induced impedance changes. Future research should aim to go beyond our current understanding by linking advances in surface science with cell biology to improved PPy substrates and focusing on biological interactions at the molecular level, including gene expression changes and proteomics. This would further clarify cause and effect during PPy-tissue interactions so as to help achieve 'real world' biomaterials.

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