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Cell attachment functionality of bioactive conducting polymers for neural interfaces

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ABSTRACT

Bioactive coatings for neural electrodes that are tailored for cell interactions have the potential to produce superior implants with improved charge transfer capabilities. In this study synthetically produced anionically modified laminin peptides DEDEDYFQRYLI and DCDPGYIGSR were used to dope poly(3,4-ethylenedioxythiophene) (PEDOT) electrodeposited on platinum (Pt) electrodes. Performance of peptide doped films was compared to conventional polymer PEDOT/paratoluene sulfonate (pTS) films using SEM, XPS, cyclic voltammetry, impedance spectroscopy, mechanical hardness and adherence. Bioactivity of incorporated peptides and their affect on cell growth was assessed using a PC12 neurite outgrowth assay. It was demonstrated that large peptide dopants produced softer PEDOT films with a minimal decrease in electrochemical stability, compared to the conventional dopant, pTS. Cell studies revealed that the YFQRYLI ligand retained neurite outgrowth bioactivity when DEDEDYFQRYLI was used as a dopant, but the effect was strongly dependant on initial cell attachment. Alternate peptide dopant, DCDPGYIGSR was found to impart superior cell attachment properties when compared to DEDEDYFQRYLI, but attachment on both peptide doped polymers could be enhanced by coating with whole native laminin.

1. Introduction

Modification of conventional platinum (Pt), gold or iridium oxide electrodes with conducting polymer coatings has the potential to significantly improve the long-term performance of neural implants including the cochlear implant, vision prosthesis, neural regeneration devices and neural recording electrodes [1,2]. Conventional electrode materials are typically fabricated with smooth surfaces that are not conducive to tissue integration. As a result the interface between a metal electrode and neural tissue is associated with a significant fluid gap through which the electrical signal must be transduced. This distance is critical in determining the current amplitude required to activate the neural tissue and the quality of the perceived signal [3,4].

Conducting polymers such as polypyrrole (PPy) and polythiophene derivative poly(3,4-ethylene dioxythiophene) (PEDOT) have been used by several research groups to enhance the properties of neural interfaces [5–9]. Conducting polymer coatings have been shown to improve the charge transfer characteristics of conventional metal electrodes and biological assays have shown that cells preferentially adhere to coated electrodes [5,10,11]. The biological interaction has the potential to eliminate the fluid gap through intimate contact between the tissue and electrode. It is hypothesised that controlled interaction between the conducting polymer and surrounding tissue can be achieved through the incorporation of biological molecules tailored to produce a response from specific cell types [2].

Extracellular matrix molecules are known to support cell attachment and growth when incorporated into conducting polymers or used as a coating. Molecules that have been implicated in these roles for cell regeneration include laminin, chondroitin sulfates, other proteoglycans and hyaluronic acid (HA) [12–14]. Laminin, a multidomain basement membrane glycoprotein is known to provide a permissive substrate that binds to cell surface receptors and also can function to stimulate neurite extension [15,16]. However, the use of the full multidomain protein or even a single domain bears some disadvantages including the need for isolation and purification, the risk of degradation via immune attack and proteolysis [17]. Only specific sections of the laminin molecule contain the required receptors for cell adherence and studies have shown that partial functions of the large laminin molecule can be imitated by smaller fragmental components with specific functional binding [18]. Investigations by Huber et al. have found that synthetically prepared peptides have a greater stability than native laminin [19].

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Peptides of laminin can be synthetically manufactured and tailored for incorporation into conducting polymers. The peptide sequence is determined from the desired cell response. Laminin peptides have been reported as having specific cell response ligands with several domains having been identified for cell attachment or neural cell growth and development [15,20–24]. The addition of various amino acids to the end of a linear peptide chain can be used to control the overall ionic behaviour of the molecule. This ability to produce tailored laminin peptides as anions makes them ideal candidates for doping common conducting polymers.

While a number of cell attachment proteins have been incorporated into conducting polymers or onto the polymer surface, the effect of these molecules on polymer properties is not defined. In studies by Cui et al. synthetically manufactured DCDPGYIGSR was incorporated into PEDOT derivative PEDOT-MeOH films as a dopant, and showed good cell attachment properties, but was not characterised for electrochemical stability, mechanical hardness and adhesion to the electrode surface [13]. In order to produce a long-term durable implant, the impact of large biomolecules on polymer structures needs to be explored [2].

This study investigates the use of two different laminin peptide sequences in doping PEDOT. The resulting polymers are characterised across a range of properties integral to their long-term performance in a neuroprosthetic device. Two peptide sequences derived from laminin were synthetically manufactured with the addition of amino acids to produce anionic peptides. DCDPGYIGSR was chosen due to positive results reported by Cui et al. [10]. DEDEDYFQRYLI, a peptide which has not previously been incorporated into conducting polymers, was also assessed. This peptide contains the active sequence YFQRYLI. The YFQRYLI ligand was identified by Tashiro et al. and reported to mediate cell attachment and promote neurite outgrowth in both PC12 cells and cerebellar microexplant cultures [22]. In this study it was hypothesised that the addition of a DEDED anionic tail to YFQRYLI would produce a molecule capable of doping PEDOT, while providing increased cell adhesion and supporting neurite outgrowth.

The aim of this study was to produce conducting polymer coatings of PEDOT doped with synthetic anionic laminin peptides, DCDPGYIGSR and DEDEDYFQRYLI on model Pt electrodes. The effect of a large biomolecule dopant on conducting polymer physico-chemical properties was established with comparison to conventionally doped PEDOT/paratoluene sulfonate (pTS). The effect of peptide doped polymers on mammalian cell interactions and the cell response to peptides containing specific bioactive ligands was assessed using the neural-like PC12 cell line.

2. Materials and methods

2.1. Electropolymerisation

For each electrodeposition 0.1 M EDOT (Cat # 483028 Sigma–Aldrich) doped with 5 mg/mL of synthetic peptide was made up in a 1 part acetonitrile: 1 part deionised (DI) water solution. DCDPGYIGSR (10-mer, 1 kDa) and DEDEDYFQRYLI (12-mer, 1.6 kDa) custom peptides were produced by Invitrogen. Control films of 0.1 M EDOT doped with 0.05 M pTS (Cat # 152536 Sigma–Aldrich) were produced under identical conditions. Polymers were electrodeposited onto Pt electrodes using an in-house manufactured galvanostat at 1 mA/cm^2 for 10 min versus a Pt counter electrode. Following deposition each polymer was washed and films were incubated in DI water for 24 h at 37 °C to leach any excess monomer, dopant or process contaminants.

2.2. Scanning electron microscopy (SEM)

SEM was performed to obtain qualitative data on the film surface morphology. Following deposition on Pt, conducting polymers were air dried overnight and coated with an 8–12 nm layer of chromium using an EmiTech K575x high resolution coater. The samples were placed in a Hitachi S3400 SEM under vacuum with an accelerating potential of 20 kV. Images were captured at 15,000 times magnification.

2.3. X-ray photoelectron spectroscopy (XPS)

XPS analysis was carried out on a Kratos XSAM800 XPS and peaks were analysed to determine the presence and relative amount of monomer units and dopant . Since the number of nitrogen (N) atoms can be calculated for each peptide from their molecular composition, the XPS spectra was used to calculate the degree of peptide incorporation in the polymer relative to the EDOT monomer. Usually this is referred to as the doping ratio, however, when doping with peptides, the number of available anions used in the polymerisation is variable and dependent on a number of factors. Effectively DEDEDYFQRYLI should have five available electrons and each DCDPGYIGSR molecule should have a single anionic charge available to balance the PEDOT backbone. However, this does not take into account the molecule polarity, folding or pH of the electrolyte. As such XPS was used to quantify the presence of peptide at the surface, but was not defined as the doping ratio since it was not possible to tell how many monomer units were charge balanced per dopant molecule.

2.4. Electrochemical stability

Cyclic voltammetry (CV) was used to determine the electrochemical stability of all PEDOT electrode coatings. An eDAQ potentiostat and eCorder unit coupled with the supplied EChem software package (eDAQ Pty Ltd., Australia) was used to apply a cycling voltage from −800 mV to 600 mV. This range was chosen to allow the oxidation and reduction of the polymer within the limits of the water window. The scan rate was set at 120 mV/s for 400 continuous cycles and measurements were performed in 0.9% saline. The recordings were made with an isolated Ag/AgCl reference electrode and Pt counter electrode. The first stable curve was considered Cycle 1 and used to determine the original electroactivity of each polymer. The area contained within successive CV curves was calculated for Cycles 10–50 in increments of 10 then Cycles 100–400 in increments of 100. These areas were normalised to Cycle 1 to show the comparative loss of electroactivity over time. The area contained within the oxidation–reduction curve at Cycle 400 was calculated to establish the final current carrying capacity of the film. This process was repeated for three individually prepared samples and the mean curve for electroactivity loss was determined with standard error.

2.5. Impedance

Electrochemical Impedance Spectroscopy (EIS) was used to determine the impedance characteristics of the electrode system. The EIS system consisted of a National Instruments (NIs) function generator controlling a house-made potentiostat with the input and output signals recorded on a NI oscilloscope. The signal was applied to the working electrode, comprised of the Pt substrate with polymer coating, in 0.9% NaCl controlled by a Ag/AgCl reference and returned via a Pt counter electrode. The impedance magnitude and phase angle were calculated. For all samples an AC sine wave with 40 mV amplitude was applied with 0 V DC offset. Two data points were recorded at 10, 100, 500, 1000, 5000, 10,000, 50,000 and 100,000 Hz, proceeding from low frequency to high frequency and then returning to low frequency. The values taken in each direction were averaged to give a single data point for each frequency. Values were presented on a Bode plot and compared to bare Pt foil. Three individually prepared films were sampled and the average curve plotted for both magnitude and phase with error presented as one standard deviation.

2.6. Film hardness

The use of softer more pliant materials in implant interfacing aims to reduce the strain mismatch between the electrode and tissue that can lead to the formation of scar tissue through inflammation and foreign body reactions. Few research groups assess the mechanical properties of conductive polymers, other than to indicate that they are softer than metals and hence have a greater compliance with neural tissue. During this research the polymer hardness was determined through the use of ASTM hardness assays. The standard was developed as a procedure for rapid, inexpensive determination of the film hardness of an organic coating on a substrate in terms of pencil leads of known hardness [25]. The test was modified to account for small areas of polymers deposited on Pt foil. Calibrated Staedtler pencils were prepared according to the standard.

Starting with the hardest lead, the pencil was held at a 45° angle and pushed away from the operator across the polymer. Sufficient pressure was exerted to either cut or scratch the film or cause the pencil tip to crumble. The process was repeated for each hardness and results were recorded on a LYNX magnifier with camera attachment (Vision Engineering Ltd., UK). The National Institute of Health (NIH) software Image J was used to assess results. The gouge hardness and scratch hardness were recorded as stipulated by the standard. The gouge hardness was scaled down from the ASTM guidelines to suit small area films and was defined as the hardest pencil that left the film uncut for 46% of the stroke length. The scratch hardness was the hardest pencil that did not rupture or scratch the film. Each test was conducted across six films (each sample was prepared from a separate batch) and standard error was given.