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Novel neural interface for implant electrodes: improving electroactivity of polypyrrole through MWNT incorporation

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Abstract Multi-walled carbon nanotubes (MWNTs) can be incorporated into conductive polymers to produce superior materials for neural interfaces with high interfacial areas, conductivity and electrochemical stability. This paper explores the addition of MWNTs to polypyrrole (PPy) through two methods, layering and codeposition. Conductivity of PPy doped with polystyrene sulfonate (PSS), a commonly used dopant, was improved by 50% when MWNTs were layered with PPy/PSS. The film electrochemical stability was improved from 38% activity to 66% activity after 400 cycles of oxidation and reduction. Growth inhibition assays indicated that MWNTs are not growth inhibitory. The electroactive polymer-MWNT composites produced demonstrate properties that suggest they are promising candidates for biomedical electrode coatings.

1 Introduction

Improvement of neuroprosthetic electrode interfaces is at the forefront of research into functional restoration of neural paths [1]. Current neuroprosthetic designs lack stability for long-term stimulation and recording. In vivo responses to stimulation have been observed to degrade over a period of 100 days or less [2] and in vivo recordings have been shown to be effective for a maximum of 7 months [3]. The use of conductive polymers, such as polypyrrole (PPy), aims to improve the electrode interface by decreasing the strain-mismatch that occurs between the electrode surface and the neural tissue. Additionally, biological factors such as cell adherence peptides and neurotrophins can be incorporated into the polymer films to provide an environment that will encourage cell interactions and incorporation into the electrode surface. This will reduce the currents required for cell stimulation and improve the long-term performance of neuroprosthetic implants.

Conductive polymers, most commonly PPy and 3,4-polyethylene dioxythiophene (PEDOT), have been investigated for both nerve regeneration scaffolds and bioactive electrode interface materials [4–6]. These polymers have the benefits of being conductive, biocompatible polymers that can be loaded with peptides for cell attachment [7] or neurotrophins for cell outgrowth stimulation [8]. However, as homogenous films conductive polymers have disadvantages that can affect their ability to perform as long-term electrode interfaces. PPy films lose considerable electroactivity over a short period, making them relatively insulative within hours. Studies by Yamato et al. [9] indicate PPy films can lose 95% of there original activity within 16 h of continual stimulation, greatly impeding their ability to function in a long-term implant. This is thought be primarily due to irreversible reactions occurring along the PPy backbone. Both Schlenhoff [10] and Beck [11] have investigated the time course degradation of PPy electroactivity and suggest that the formation of carbonyl groups on the PPy ring during oxidation lead to a loss of conjugation with the dopant. This research investigates the use of multi-walled carbon nanotubes (MWNTs) as a supplementary component of PPy electrode coatings. The inclusion of MWNTs aims to improve the electrochemical stability and conductivity of PPy films by providing

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conductive filler that is resistant to electrochemical degradation during the implant lifetime.

Carbon nanotubes are graphene sheets rolled into a cylinder with diameters ranging from 30 nm up to 500 nm [12] and lengths ranging from several hundred nanometres to micrometres. MWNTs are carbon nanotubes consisting of multiple graphene sheets arranged into concentric cylinders. MWNTs exhibit high mechanical strength (around 30 GPa) [13] and Young's modulus (up to 4.15 TPa) [14], making them the ideal candidate for reinforcing conductive polymers. Additionally they have a high surface area that is beneficial for electrical conductivity and exhibit substantial chemical and thermal stability. These properties have made MWNTs potentially a very attractive material for use in long-term implant devices, especially neural prostheses applications [15]. The aim of this study is to use MWNTs to improve the long-term performance and conductivity of PPy/PSS electrode coatings used in neural interfaces.

2 Experimental procedure

2.1 Fabrication of polymer films

Conductive polymer films were fabricated by electrodeposition onto either 100 μm thick platinum (Pt) foil or conductive ITO slides. Pyrrole was purchased from Sigma-Aldrich (Cat #131709) and doped with polystyrene sulfonate (PSS), (Sigma-Aldrich Cat. #641030). Immediately prior to all depositions the pyrrole was vacuum distilled to purify the monomer. The films were produced in wells formed by the adherence of a hydrophobic silicone gasket to the substrate. The silicone gasket provided a mask for the placement of the MWNT layer and electrodeposition.

An aqueous solution of 0.5 mg/ml short, thin MWNTs with carboxylic acid functionality, (Nanocyl Cat. #3151) was sonicated in DI water for 60 min to obtain a suspension. The MWNTs were incorporated into the conductive polymer films using two methods: (i) Codeposition and (ii) Layering.

For codeposited films, 0.1 M pyrrole and 0.05 M PSS was added to the sonicated MWNT solution. A 150 μl aliquot of the composite solution was then added to each well for electrodeposition. For the layered films, a 300 μl aliquot of the sonicated MWNT solution was placed in each well and dried on a hotplate at 60°C. Aqueous 0.1 M pyrrole and 0.05 M PSS was placed in wells containing the dry layer of nanotubes. Both conductive polymer solutions were galvanostatically electrodeposited at 1.5 mA/cm^2 for 15 min. All films were washed several times with water and left to leach overnight to remove any polymerisation contaminants. Control films of homogenous 0.1 MPPy/0.0PSS were produced using the same electrodeposition parameters. SEM was used to confirm the presence of the polymer deposition.

2.2 Cyclic voltammetry

Films deposited on Pt electrodes were analysed by cyclic voltammetry (CV) to determine electrochemical stability. An eDaq potentiostat unit using the EChem software package was used to apply a cycling voltage from −700 mV to 600 mV. This range was chosen to allow the oxidation and reduction of the polymer within the limits of the water window, to prevent hydrolysis occurring at the electrodes. The scan rate was set at 100 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 the film. The area contained within the oxidation–reduction curve was calculated to establish the current carrying capacity of the film. The area contained within the curve of successive cycles was calculated as a percentage of Cycle 1 to show the comparative loss of electroactivity over time. This process was repeated for three individually prepared samples and the mean curve for electroactivity loss was determined with standard error.

2.3 Four-point probe conductivity

Films were analysed using a Jandel four-point probe with tungsten carbide tips having radii of 40 μm, tip spacing, of 0.635 mm and set to a load of 60 g. A Jandel RM3 test unit was used as the current source and voltmeter. MWNT composite films and PPy homogenous films were manually removed from the ITO glass substrate and placed in a freeze-drier overnight. The standalone films were then subjected to currents ranging from 20 μA through to 2 mA with the linear four-point probe head. Current was applied in both directions and the average was taken. For each material sample three measurements were taken for each of the three samples, the mean and standard error were calculated.

2.4 Cell growth inhibition assay

PPy films have been shown to be biocompatible [9] in cell culture assays. Cytotoxicity studies were carried out on the functionalised MWNTs to determine their suitability as a component of PPy films for electrode interfaces. A media extraction was carried out by contacting 5.8 mg nanotubes per ml complete medium (EMEM + 10% FBS) and

incubated at 37°C for 24 h. Extract from the nanotubes was split into 3 and diluted with fresh media to make concentrations of undiluted, 1 in 2 dilution and 1 in 4 dilution. L929 mouse fibroblasts were used to assess the cytotoxicity of the materials. Extracts from the materials were applied to a sub-confluent monolayer of cells seeded 24 h earlier. The cells were then incubated for 48 ± 3 h at 37°C. After this time the cells were trypsinised and analysed using a fluorescence activated cell sorter (FACS). Cell counts determined by FACS were used to determine inhibitory or cytotoxic activity by the influence on normal cell proliferation. Results were compared to null plates (negative controls) and positive control extracts, ethanol and latex. Each FACS tube was analysed three times, the mean of these three readings was taken. For each material sample there were three FACS tubes analysed, taking the average of the means calculated previously. Consequently standard error was calculated for each material sample. Cell counts were plotted as a percentage of the null extract.

3 Results and discussion

PPy/PSS and PPy/PSS/MWNT composite films were galvanostatically produced on both Pt foil and ITO glass substrates for cyclic voltammetry and four-point probe assays. SEM was used to confirm the presence of both polymer and MWNT composite depositions as depicted in Fig. 1. An SEM of MWNTs (Fig. 1, Image b) dried in a film before layering with PPy/PSS was also produced.

3.1 Cyclic voltammetry

Long-term electroactivity of the PPy/PSS films can be improved by up to 50% through the incorporation of MWNTs. Films that did not incorporate MWNTs were shown to lose 61% of activity over the course of 400 cycles as seen in Fig. 2. This concurs with results published by Yamato et al. who saw losses of 95% when PPy/PSS films were polarised for 16 h [9]. When MWNTs were incorporated by codeposition and layering, only 28.5% and 34% of electroactivity was lost respectively. While the MWNTs cannot prevent the oxidation of the PPy backbone and loss of dopant described by Schlenoff and Hong [10], they can provide a conductive path that will not be subject to oxidative degradation, there by increasing the overall current carrying capacity of the composite material.

3.2 Four-point probe conductivity

Film conductivity was shown to improve with MWNT incorporation through layering but not when incorporated during electrodeposition. As shown in Table 1 PPy/PSS control films were improved by 51% through layering with

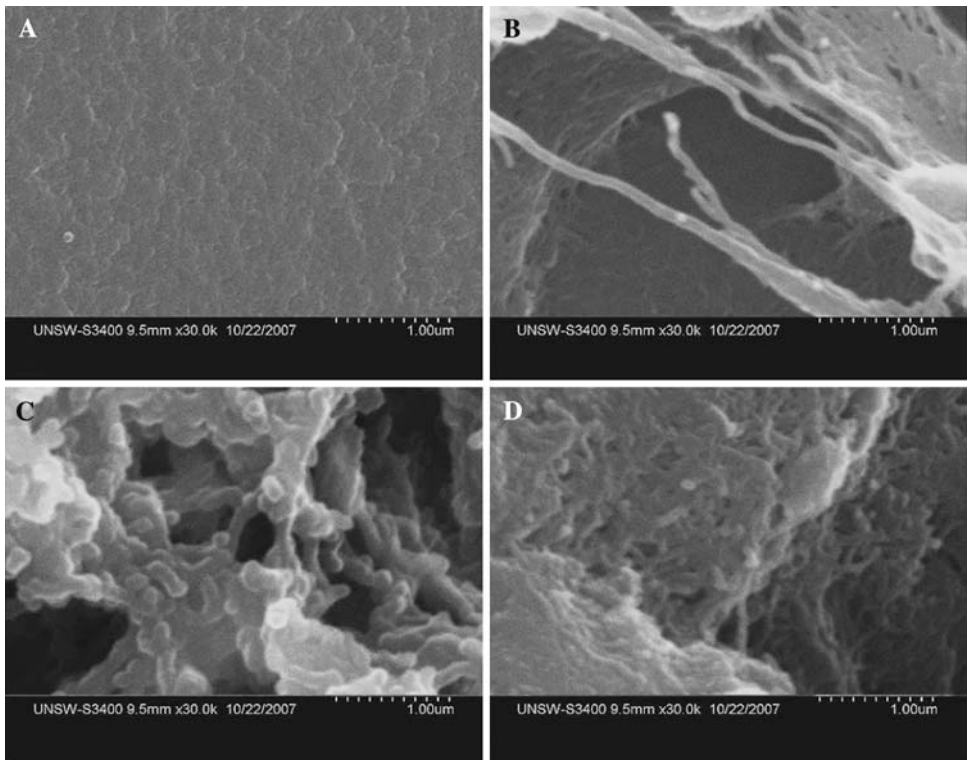

Fig. 1 SEM of polymer films. (**a**) PPy/PSS; (**b**) Standalone MWNT film; (**c**) PPy/PSS codeposited with MWNTs; and (**d**) PPy/PSS layered with MWNTs

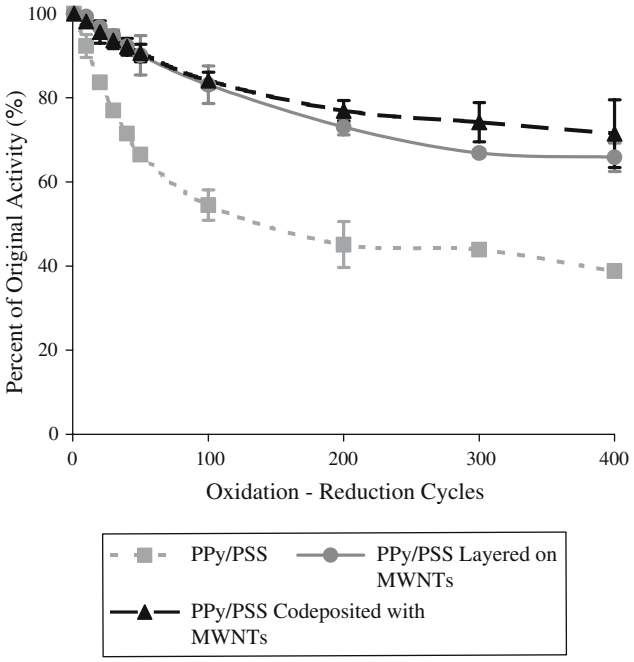

Fig. 2 Loss of electroactivity over 400 cycles of oxidation-reduction from −700 mV to 600 mV at 100 mV/s; $n = 3$

Table 1 Conductivity of composite films as determined by four-point probe analysis; $n = 3$

Film	Conductivity (S/cm)
PPy/PSS	19.84 ± 4.11
PPy/PPS Layered with MWNTs	30.01 ± 4.23
PPy/PSS and MWNTs Codeposited	12.75 ± 0.95

MWNTs. Codeposition had a negative effect, reducing the conductivity by 36%. It is possible that this is due to the MWNTs interfering with the electrodeposition process, reducing the efficiency of the polymer formation. Theoretically, pyrrole monomer should conjugate the with COOH functionalised groups on MWNTs [16]. This should result in a coherent polymer film with MWNTs conjugated along the polymer backbone. However it is possible that the time in which the MWNTs were combined with the pyrrole solution was not sufficient for conjugation to occur to a significant extent. Future experiments will look at improving the conjugation through increased exposure of distilled pyrrole with COOH-MWNTs prior to co-electrodeposition.

3.3 Cell growth inhibition assay

MWNTs were determined to be non inhibitive of cell growth when extractions were exposed to L929 monolayers

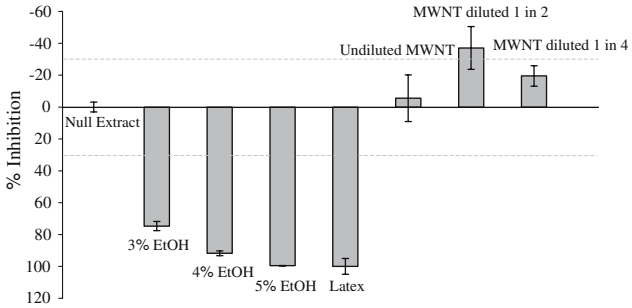

Fig. 3 Cell growth inhibition assay: Percentage of inhibition caused by MWNTs, positive controls and null extracts at 48 h of exposure on L929 monolayers

for a 48 h period. Figure 3 shows the undiluted MWNT extract had no significant difference in cell inhibition when compared to the null extract. The subsequent dilutions of the MWNT extract were shown to be growth promotive by approximately 20% when compared to the null extract.

Carbon nanotubes are essentially pure carbon and as such may be thought to be biocompatible, however research suggests that due to their nanoscale sizing they may have cytotoxic capabilities. It is important to note that no studies have thoroughly investigated the use of implanted CNTs in biomedical applications. MWNTs are of a size that would allow them to infiltrate single cells within the body and possibly accumulate, having a detrimental effect similar to that of asbestos. Investigations by Muller et al. [17] and Lam et al. [18] indicate that carbon nanotubes (CNTs) induce significant adverse effects in the lungs of rats. Additionally mobile MWNTs in biomedical applications may be harmful through alternate mechanisms such as being undetected by the normal phagocytic defences allowing them access other tissues through the blood or like haptens to modify protein structures, possibly altering their function or rendering them antigenic [19]. An important feature of this design is the constraint of the MWNTs within the polymer matrix preventing accumulation or mobilisation of MWNTs within the surrounding cellular tissues. In this way the polymer-MWNT composite film maintains the superior electronic properties of the MWNTs without compromising surrounding cellular environment.

4 Conclusions

Layering of PPy with MWNTs produce a film with far superior electrical properties than PPy alone. The addition of COOH functionalised MWNTs significantly improves the electrochemical stability of PPy/PSS films for electrode coatings. Conductivity increases with the use of a layered fabrication method. However codeposition techniques require optimisation of MWNT and pyrrole conjugation.

Additionally, the inclusion of functionalised MWNTs into a biological system should not have a significant impact on cell growth. The incorporation of MWNTs into conductive polymer electrode coatings shows promise for neural interfaces with improved electrical properties.

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