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The biological and electrical trade-offs related to the thickness of conducting polymers for neural applications

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ABSTRACT

Poly(3,4-ethylenedioxythiophene) (PEDOT) films have attracted substantial interest as coatings for platinum neuroprosthetic electrodes due to their excellent chemical stability and electrical properties. This study systematically examined PEDOT coatings formed with different amounts of charge and dopant ions, and investigated the combination of surface characteristics that were optimal for neural cell interactions. PEDOT samples were fabricated by varying the electrodeposition charge from 0.05 to 1 C cm^{-2}. Samples were doped with either poly(styrenesulfonate), tosylate (pTS) or perchlorate. Scanning electron micrographs revealed that both thickness and nodularity increased as the charge used to produce the sample was increased, and larger dopants produced smoother films across all thicknesses. X-ray photoelectron spectroscopy confirmed that the amount of charge directly corresponded to the thickness and amount of dopant in the samples. Additionally, with increased thickness and nodularity, the electrochemical properties of all PEDOT coatings improved. However, neural cell adhesion and outgrowth assays revealed that there is a direct biological tradeoff related to the thickness and nodularity. Cell attachment, growth and differentiation was poorer on the thicker, rougher samples, but thin, less nodular PEDOT films exhibited significant improvements over bare platinum. PEDOT/pTS fabricated with a charge density of <0.1 C cm^{-2} provided superior electrochemical and biological properties over conventional platinum electrodes and would be the most suitable conducting polymer for neural interface applications.

1. Introduction

Conducting polymers (CPs) are attracting increased attention as coating materials for biomedical electrodes. Key advantages include their ease of fabrication and excellent electrical properties. CPs can be electrochemically deposited onto electrodes for neural interface applications. This fabrication approach is rapid and efficient, and film thickness can be varied easily as it is a direct function of the charge used to electrodeposit the film. As the thickness of the film is increased, variation occurs in the physical features of the surface and consequently electrical properties also vary. Electrodeposited CP coatings have high surface areas compared with unmodified metal electrodes, and this feature leads to excellent electrochemical properties due to the larger area available for charge transfer. However, it is well known that the physical characteristics of surfaces can have a significant effect on cell behaviour [1,2]. This study aims to investigate the effect of film topography and thickness on the electrical and biological properties of poly(3,4-ethylenedioxythiophene) (PEDOT) CP films.

PEDOT is an ideal CP for biomedical applications due to its superior chemical stability in comparison with other CPs such as polypyrrole and its excellent electrical properties [3,4]. PEDOT films have characteristic nodular surface features which vary depending on electropolymerization parameters, film thickness (deposition charge), dopant chemistry and dopant size. Using large hydrophilic dopants such as poly(styrene sulphonate) (PSS), the surface roughness (rms) of PEDOT films has been reported to be of the order of 7 nm with nuclei from 50 to 250 nm in diameter forming on films deposited over longer time periods [5]. Systematic studies of the effects of electrodeposition parameters on the morphology and structure of PEDOT/PSS described the resulting morphology as having "fine-structured globular" features [6].

Dopant type and concentration can also significantly impact on surface characteristics. When PSS is used as a dopant, roughness was shown to increase as the PSS concentration in the electropolymerization solution was increased [6]. When PEDOT was doped with perchlorate (ClO_4), tosylate (pTS) and PSS, Baek et al. [7] showed that the nodularity increased with decreasing dopant size. In a study examining PEDOT using KNO_3 as the dopant, rougher, more globular surface features were described as progressively developing over 12 potentiodynamic cycles of polymerization [8].

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In the latter study, granularity was largely absent in thinner films formed after a single cycle, whereas large flower-like hierarchical structures occurred in thicker films formed after 12 cycles. Thus, the surface roughness and globular nature of PEDOT increases with film thickness, which is a function of charge delivered during electrodeposition. It is clear from previous research that surface roughness or nodularity affects both electrical impedance as well as cell interactions [3,6,7,11].

It is well established that the electrical impedance of CPs decreases with increasing surface roughness and nodularity. PEDOT doped with perchlorate has been used to demonstrate the direct relationship between electrical impedance and roughness [9]. More recently, Castagnola et al. [10] studied the effects of polymerization conditions on the morphology and electrical properties of PEDOT doped with PSS. Distinct "cauliflower-like" surface features were observed with rms roughness reported as varying between 150 and 300 nm depending on the polymerization mode [10]. Film thickness in these studies was of the order of 0.6 μm with negligible variation observed when the three polymerization modes were compared. In studies comparing the effect of dopant type, variations in nodularity of PEDOT imparted improvements in the electrochemical properties with increased charge transfer capability correlating with the surface roughness [7,11]. Although highly nodular films are preferred for optimal electrochemical properties, they may not be ideal for neuronal interaction.

Cell adhesion and proliferation are likely to be affected by the thickness and nodularity of CP films. Previous research has demonstrated a general relationship between spacing and depth of polymer surface features on cell adhesion behaviour [1,12]. Seminal research by Clark demonstrated that by increasing the height and depth of patterned surface features, cell alignment could be controlled [1]. The effect of random roughness has been extensively studied in particular in the field of orthopaedic and dental biomaterials. Hydroxyapatite and titanium with rougher surfaces were more conducive to cell adhesion and resistance to detachment than smooth surfaces of the same materials [2,13]. Despite the wealth of information on the effect of physical surface characteristics on cell behaviour, there have been few studies examining the relationship between CP surface characteristics and neural cell adhesion and neurite outgrowth [14–17].

In addition to topography and surface roughness of substrates influencing the adhesion, differentiation and proliferation of neurons, the chemistry of films may also have a significant impact. Green et al. [14] reviewed the challenges associated with developing CPs for neural applications. The review concluded that there is necessarily a trade-off that must occur between electrical, mechanical, chemical and biological properties for development of an optimal biomedical CP. Thicker films inherently expose cells to greater concentrations of chemicals, in particular unreacted monomers and mobile dopant species. In a study by Fonner et al. [18], pheochromocytoma (PC12) and neurolemmocytes (Schwann cells) were shown to have higher viability on thin (0.01 C cm^{-2}) films than on thick (0.08 C cm^{-2}) films after 24 h in culture. The chemicals used to produce CPs have been shown to produce cytotoxic responses when they are not fully polymerized or remain mobile within the CP matrix [7] and it is likely that physical and chemical factors have a synergistic effect on cell adhesion.

Baek et al. [7] showed that the adhesion and neurite outgrowth of rat pheochromocytoma (PC12) cells were strongly influenced by the topography, and this appeared to be dominant over any cytotoxic effect of increased mobile chemical species. In the latter study the dopant pTS was shown to be minimally cytotoxic, but lower cell numbers adhered to the thick PEDOT/pTS films than on comparable films doped with the more cytotoxic PSS. This result was likely to be influenced by the topography of PEDOT/pTS which was shown to be substantially more nodular than the PEDOT/PSS. In the current study, the hypothesis was that the biological performance of PEDOT can be improved by reducing the film thickness, and thereby reducing the surface nodularity as well as chemical cytotoxicity. To test this hypothesis, PEDOT films were fabricated across four different thicknesses through variation of the charge used for polymerization. Films were produced through delivery of a total charge of 1, 0.5, 0.1 and 0.05 C cm^{-2}. Additionally, three dopant types, namely pTS, ClO_4 and PSS, were used in this study. Samples were directly compared in terms of topography, chemical content, electrical performance, and neural cell attachment and differentiation.

2. Materials and methods

2.1. Preparation of PEDOT samples

Three types of PEDOT films were prepared using the dopants ClO_4, pTS and PSS. Monomer solutions containing 0.05 M EDOT and 0.1 M dopant were prepared in a 50/50 vol.% mixture of acetonitrile and water. The amounts of each chemical used to make 2 ml solutions are shown in Table 1a. Electrodeposition was carried out in a silicone well system (FlexiPERM micro12) connected to a galvanostat made in-house. A platinum foil and a platinum probe array were used as the working and counter electrodes, respectively. Constant current electrodeposition was performed with 0.166 mA, providing a current density of 0.5 mA cm^{-2}. Films with four different thicknesses were fabricated by varying the deposition time, as shown in Table 1b. When the deposition was completed, each film was flushed with 150 μl deionized (DI) water three times and then soaked in 300 μl DI water for 24 h to remove excess electrolyte. Films were dried in a sealed chamber at room temperature for a further 24 h.

2.2. Physical properties of PEDOT

2.2.1. Macroscopic images

The optical changes (i.e. colour and transparency) associated with the film thickness were observed from stereoscopic images. PEDOT samples deposited with 0.05, 0.1, 0.5 and 1 C cm^{-2} were imaged at 7.5× magnification using a Modular Routine Stereo Microscope (Leica M80, Germany).

2.2.2. Surface topography and thickness measurement

Scanning electron microscopy (SEM) was performed with a Hitachi S3400 scanning electron microscope (Hitachi High-Technologies Co., Japan) using an acceleration potential of 5 kV. Samples were tested in a dry state without further sputter coating. To measure the thickness of films, a platinum foil supporting CP films was mounted vertically on an L-shaped metal bar with double-sided carbon tape. The thickness of the 0.05 C cm^{-2} films could not be measured by SEM due to the limited resolution. Cross-sectional SEM of the 0.1, 0.5 and 1 C cm^{-2} films was performed at magnifications of 15,000×, 6500× and 3000×, respectively. Morphological SEM images of each film were taken at magnifications of 5000× and 1000×. Additionally, the macroscopic structure of the 1 C cm^{-2} films was examined at 100× magnification. The theoretical thickness was also calculated as outlined in Section 2.3 below.

2.3. Theoretical thickness and doping ratio analysis

The chemical composition of material surfaces was measured by X-ray photoelectron spectrometry (XPS) using an ESCALAB220i-XL with Al K_α radiation (photon energy = 1486.6 eV). The take-off angle was 90°. Survey scans were obtained using a

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