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A feasible way for the fabrication of single walled carbon nanotube/polypyrrole composite film with controlled pore size for neural interface

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ABSTRACT

Single walled carbon nanotube (SWNT)/polypyrrole (PPy) composite films with controlled pore size and strong adhesive force was prepared as electrode material for improving the performance of neural electrodes. SWNT film with controlled pore size was first fabricated through electrophoresis with a merit that the pore size can be well tuned by changing the concentration of metal ions in the electrolyte. An ultrathin conformal PPy layer around SWNT bundles in a uniform manner within the entire films was subsequently obtained by pulsed electropolymerization. The adhesion of the SWNT coated electrodes was tested by repeatedly inserting the coated electrode into agar gel to demonstrate the better adhesive force of the coating. Electrochemical results showed that the SWNT/PPy coated metal electrodes have much lower impedance and higher charge storage capacity than the bare metal substrates. Further in vitro culture of rat pheochromocytoma (PC12) cells revealed that the porous SWNT/PPy composite film was non-toxic and well supported the growth of neurons. We demonstrate that the prepared composite film has potential applications in chronic implantable neural electrodes for neural stimulation and recording.

1. Introduction

Neural electrodes are multichannel neural microelectrode arrays micromachined with special techniques [1]. They have significantly improved the quality of life for an increasing number of people suffering from neurological disorders and injuries [2–4]. Nowadays, diagnostic, therapeutic, and treatment strategies for problems of central nerve system (CNS) increasingly rely on external electrical stimulation and recording through neural electrodes. Most neural electrodes are made of inert metals, such as platinum, gold, iridium, titanium and stainless steel. The performances of bare metal electrodes, including mechanical modulus, electronic transfer efficiency, signal sensitivity, stability and biocompatibility, are unsatisfactory in the long-term application. Hence, numerous attentions have been paid to the fabrication of diverse functional coatings on electrode surface, including methodology and materials [5–7].

As a new promising material, carbon nanotubes (CNTs) have attracted enormous interest due to their excellent properties, such as unique structure, high tensile strength, high chemical stability and metallic conductivity [8]. Additionally, their structural features and dimensions are similar to many elements of the CNS (ion channels, signaling proteins and elements of the neuronal cytoskeleton) [9]. In recent years, CNTs are employed in neuromedicine, such as scaffolds for neuronal growth [10], electrical interfaces [11] and modifying materials for neural electrodes [12,13]. The high specific surface area of CNTs can provide sufficient effective area for electron transfer, which specifically meets the requirements of miniaturization of neural electrodes. However, the success of the biological application of CNTs in the CNS is closely dependent on their compatibility with the tissue thus the cytotoxicity considerations of CNTs must be taken into account. It was reported that CNT in its pure form tends to induce phagocytosis and cell death when released into biological media [14]. To address the concern, Casan-Pastor's group recently developed nanostructured iridium oxide-CNT hybrids as novel materials for neural electrodes [15]. While the hybrid presented greatly improved mechanical and

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enhanced electrochemical properties, no release of CNTs to the media was observed.

In recent decades, conducting polymers (CP) have been extensively used as electrode materials because of their excellent electrical properties, ease of preparation and good biocompatibility [16,17]. However, the poor electrical stability and weak mechanical strength still hinder their applications in the aspect. In particular, the poor adhesive force of the CP films to the metal electrode substrates remains challenging in the research field and hinders the development of neural electrodes. Recent studies have found that the CP bilayers, with polypyrrole (PPy) deposited on poly(3,4-ethylenedioxythiophene) (PEDOT), showed a good adherence and are highly reproducible [18]. Studies also found that the combination of CNT and CP demonstrated significant advantages, including low impedance, high capacitance, fast charge transfer rate as well as good mechanical stability due to the excellent conductivity of CNT [19–21]. Moreover, the micro- and nanoscale features of the composite coatings can significantly increase the effective surface area, improve the electron transfer efficiency, decrease the impedance at electrode/neuron interface and promote the growth of neurons and their adhesion to the neural electrodes [22,23].

By electrochemical polymerization, the CNT/CP composite can be directly and specifically deposited onto the electrode sites under the electric field to form continuous and uniform films [24,25]. As reported, the CNT/CP composite films have been prepared through either drop casting method or co-deposition. The former is the most readily operated method where CNTs are dropping-deposited directly onto the electrode surface prior to electropolymerization of CPs [26]. The CNTs immobilized in this way are scattered randomly over the entire electrode surface and good adhesion between the composite coating and substrate cannot be guaranteed. In the latter approach, the chemically functionalized CNTs are incorporated in CP films as normal anionic dopants [27,28]. However challenges remain to be addressed: (1) the surface morphology and porosity of the fabricated composite are generally uncontrollable and (2) good electrode/coating adhesion is hard to achieve due to the substantial volume changes during switching between oxidation and reduction states of CP, which has been the prime hindrance for the long-term application of the implanted electrodes. Hence, we herein proposed a new strategy that SWNT/CP coatings were fabricated by electrophoresis of SWNTs onto the metal electrode, followed by pulsed electrochemical polymerization of PPy. Porous SWNT films were obtained and the pore size was well controlled by adjusting the concentration of Al^{3+} ions in the electrolyte during electrophoresis. Pulsed electropolymerization was employed since it allows the formation of an ultrathin conformal PPy layer around SWNT bundles in a uniform manner within the entire films. Electrochemical properties, such as CV and electrochemical impedance were investigated. An equivalent circuit model for the electrochemical impedance spectrum (EIS) data was used to derive the physical and chemical characteristics of the modified electrode surface. The biocompatibility of the SWNT/PPy modified electrodes was studied by growing PC12 cells on the electrode surface and further observation of the neurite differentiation and growth.

2. Materials and methods

2.1. Materials

SWNTs synthesized by electric arc discharge were purchased from Carbon Solutions Inc. (CSI, Riverside, CA), with metal content of <30% (wt% from TGA in air). The nanotubes have an average diameter of 1.4 nm and individual tube lengths ranging from 0.5 to 3 μm. Pyrrole monomer (Py, 98%) was purchased from Sigma–Aldrich (St. Louis, MO), vacuum-distilled until becoming colorless, stored at 4 °C and purged with N_2 for 5 min before each deposition. The reagents used in cell culture including Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, horse serum, L-glutamine, streptomycin, and penicillin, were purchased from Nanjing Tengchun Biological Technology Co. Ltd. (China).

All other reagents (analytical grade) were obtained from Shanghai Chem. Co. (China) and used as received without further purification.

2.2. Construction of SWNT/PPy composite film

2.2.1. Electrophoresis of SWNTs

The 316L stainless steel (SS) was cut into small square electrodes with size of 2.0 cm × 0.5 cm. Prior to use, the SS was bathed in 1 M NaOH at 100 °C for 1 h and washed subsequently with acetone, deionized (DI) water and ethanol for 20 min, respectively.

Before electrophoresis, SWNTs were purified and carboxylated under sonication with sulfuric–nitric acid (3:1, v/v) in a water bath at 60 °C for 4 h. The mixture was diluted with water, extracted through a 0.22 μm filter and washed thoroughly with DI water until the pH value reached ca 6.0. The functionalized SWNTs were then recovered from the filter and re-dispersed in ethanol under ultrasonication to make a stable and homogeneous dispersion with SWNT concentration of 0.5 mg mL^{-1}. Secondly, 0.1 M $Al(NO_3)_3{\cdot}9H_2O$ ethanol solution was diluted with ethanol into different concentrations. The stable SWNT dispersion was slowly added into the above $Al(NO_3)_3{\cdot}9H_2O$ solutions on oscillator. The volume ratio of the SWNTs suspension to Al^{3+} solution was 100:5, 100:10, 100:15, and 100:20, respectively. The treated SS electrodes were used in the electrophoresis cell with an electrode gap of 1 cm. Direct current (DC) voltage of 200 V was applied to deposit a SWNT film on the cathode for 15 min. The nominal area of the SWNT film was 5 mm × 10 mm. After electrophoresis, the electrodes were dried in a desiccator at room temperature for 30 min before soaking in 0.6 M H_3PO_4 for 30 min. Finally, the electrodes were washed with DI water and ethanol to remove any ions adsorbed on SWNT surfaces and dried in a desiccator for 30 min at room temperature.

2.2.2. Electropolymerization of PPy

Pulsed electropolymerization was performed in 0.1 M Py aqueous solution containing 0.1 M *p*-toluenesulfonic acid sodium (TsONa) as dopant to fabricate the SWNT/PPy composite film. The electrochemical polymerization was carried out in a three-electrode system (Autolab PGSTAT302N, Ecochemie, Netherlands), using the above SWNT coated SS electrode as the working electrode (with working area of 5 mm× 10 mm), a platinum wire as the counter electrode and a standard Ag/AgCl electrode as the reference electrode. The working potential was programmed with a waveform consisting of a potential step to 0.75 V for the deposition time (T_d) of 5 s followed by switching off for a rest time (T_r) of 600 s. The waveform was repeated for 12 cycles for a total polymerization time of 60 s. After polymerization, the SWNT/PPy composite film coated electrode was washed repeatedly with DI water to remove any remaining electrolyte and monomer and dried in air for further use.

2.3. Assays and characterizations

2.3.1. Testing of adhesion

Adhesion of SWNT films was tested in agar gel. In particular, 1 g agar powders were soaked in 100 mL DI water for 0.5 h. The obtained suspension was bathed in water at 100 °C until the agar powders were melted completely. The melt was cooled at room temperature and turned to jelly-like transparent agar gel. The SWNT coated electrodes were vertically inserted into the agar gel