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Graphene oxide doped conducting polymer nanocomposite film for electrode-tissue interface

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ABSTRACT

One of the most significant components for implantable bioelectronic devices is the interface between the microelectrodes and the tissue or cells for disease diagnosis or treatment. To make the devices work efficiently and safely in vivo, the electrode-tissue interface should not only be confined in micro scale, but also possesses excellent electrochemical characteristic, stability and biocompatibility. Considering the enhancement of many composite materials by combining graphene oxide (GO) for its multiple advantages, we dope graphene oxide into poly(3,4-ethylenedioxythiophene) (PEDOT) forming a composite film by electrochemical deposition for electrode site modification. As a consequence, not only the enlargement of efficient surface area, but also the development of impedance, charge storage capacity and charge injection limit contribute to the excellent electrochemical performance. Furthermore, the stability and biocompatibility are confirmed by numerously repeated usage test and cell proliferation and attachment examination, respectively. As electrode-tissue interface, this biomaterial opens a new gate for tissue engineering and implantable electrophysiological devices.

1. Introduction

With the rapid development of micro fabrication technology, biomedical devices can be manufactured considerably tiny and structurally diverse, which minimize the damage during and after implantation for both short term and long term requirement [1]. The most important factors to decide the performance of implanted micro device are the efficiency and reliability of microelectrodes, which can be used to diagnose and treat many diseases by electrophysiological recording and functional electrical stimulation respectively [2]. With the help of microelectrodes, researchers have already achieved clinical effectiveness, such as relieving Parkinson's disease symptoms by deep brain stimulation [3], restoring movement of paralyzed limb through electrical stimulation based on neural recording [4], recovering visual or auditory capability by visual prosthesis and cochlear implants, respectively [5,6].

Nowadays, some dense electrode arrays and tenuous electrodes are developed to undertake complex and precise electrophysiological research with providing excellent spatial selectivity and low power consumption [7–9].The problem is that smaller size, certainly lowers the damage to the tissue, would inevitably damage the performance and safety of the electrodes [10]. Because decrease in the size of electrode will lead to an increase in impedance and a drop in charge storage capacity (CSC), which, as a result, means poor recording signal quality and high stimulating current that may damage tissue. Considering this fact, the interface material plays a significant role to improve the electrode performance. Many efforts are made to develop more ideal electrode-tissue interface materials with properties including: electrical property containing low impedance, high CSC and high charge injection limit; stability for long term work or implantation without significant property variation; biocompatibility ensuring direct contact with tissue without inducing severe tissue response, toxicity or even necrosis.

The most widely applied electrode-tissue interface material is noble metals including platinum, gold, iridium, titanium and their alloys due to long-term stability during implantation process without serious chemical corrosion [11]. Nevertheless, the stimulation and recording performance of bioelectronic devices is restricted mostly by the low charge injection limit and CSC of bare metallic bio-electrodes [12]. Some porous structure and peculiar

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profile is fabricated to form a rough surface of the material, which would consequently improve the electrical properties and biocompatibility by providing a larger effective surface area of electrode sites [13]. Iridium oxide (IrO_x) is another electrode coating material regularly reported which has relatively low impedance and high charge transfer efficiency [14]. However, its oxidation-reduction reaction and poor bonding effect to substrate was observed during electrical stimulation. These defects in some degree restrict IrO_x from becoming an ideal electrode-tissue interface as the possibility of causing tissue damage [15].

Widely explored conducting polymers, especially poly (3,4-ethylenedioxythiophene) (PEDOT), satisfy these requirements of electrode-tissue interface [16]. Besides, the structure and property of conducting polymers can be changed by doping counterions like polymers, biomolecules and carbon nano material, which provides enormous possibilities for the use of conducting polymer in implantable prostheses [17–19]. However, fragmentation and exfoliation of conducting polymer on the substrate may be induced as expansion and contraction of volumes would take place during electrochemical oxidation-reduction procedures [20,21]. This, as a result, arouses the problem of stability and safety for common conducting polymer modified electrode.

As single atom layer structural graphene has excellent electrical, mechanical, thermal and optical characteristics [22,23], it has been applied in various areas, especially in biomedical research, such as acting as the cellular interface of stem cell differential [24], sensing biomolecules [25], promoting cell growth [26], and applying in electrical stimulation [27]. It is reported that mixing graphene, especially water soluble graphene oxide (GO), as counterions into polymers forming new composite materials will improve their performance further [28,29]. For example, multifunctional composite hydrogels consisting of GO and DNA possess high mechanical strength, environmental stability, and dye-loading capacity [30]. Also, hybrid papers fabricated by depositing polyaniline on GO exhibit excellent electrochemical performance and biocompatibility [31].

For these reasons mentioned above, GO was doped into PEDOT to enhance the stability of the composite for its excellent mechanical property, and to promote electrochemical performance by increasing effective surface area for its stacking induced by $\pi-\pi$ bonds interaction. In this paper, we deposited GO as counterion with PEDOT onto surface of microelectrodes. The original GO doped morphology and structure of electrochemically deposited film were observed and analyzed by scanning electron microscope (SEM), transmission electron microscope (TEM) and atom force microscope (AFM). Electrical characterizations containing electrochemical impedance spectroscopy (EIS), cyclic voltammogram (CV) and charge injection limit were performed to investigate electrical property of PEDOT/GO nanocomposite film. Moreover, the stability of PEDOT/GO was evaluated by enduring repetitive CV scanning. In addition, in order to test biocompatibility and cell adherence of PEDOT/GO, we cultured highly differentiated rat pheochromocytoma PC-12 cells (hdPC-12 cells) and mouse embryonic fibroblasts NIH/3T3 (NIH/3T3 cells) for cell viability, proliferation and attachment experiments.

2. Materials and methods

2.1. Electrochemical deposition

Electrolyte for PEDOT/GO polymerization consisted of 2 mg/ml graphene oxide aqueous solution (XFNano, China). EDOT (0.01 M) (Sigma–Aldrich) was added into GO solutions, followed by stirred for 2 h to dissolve EDOT. The solution was subsequently purged with nitrogen gas for 10 min to eliminate the oxygen in the electrolyte. PEDOT/GO film was deposited on glass slices sputtered with 150 nm gold and gold wire electrode (diameter of 100 μm) by applying constant current at density of 0.2 mA/cm^2 through the electrolyte by CHI 660c (CH Instrument) electrochemistry work station.

2.2. Material morphology and characterization

Morphology of PEDOT/GO film deposited on gold wire electrodes and gold sputtered slices was observed by high vacuum scanning electron microscopy (ULTRA 55, Zeiss, Germany). The inner structure of PEDOT/GO fragment was investigated by transmission electron microscopy (JEM-2100, JEOL Ltd., Japan). Detailed morphology and roughness of PEDOT/GO film were obtained with Multimode Nanoscope V Scanning Probe Microscopy System (Bruker, USA). Morphology of cells cultured on PEDOT/GO film was observed by low vacuum scanning electron microscopy (S-4800, Hitachi, Japan). X-ray photoelectron spectroscopy (XPS) analysis was performed by X-ray photoelectron spectrometer (AXIS ULTRA DLD, Kratos) with an excitation source of Al Kα radiation ($\lambda = 1486.6$ eV). Fourier transform infrared spectroscopy (FTIR) of GO and PEDOT/GO was obtained by Fourier transform infrared spectrometer (Nicolet iN10 MX, ThermoFisher).

2.3. Electrochemical characterization

To investigate the electrochemical property, the PEDOT/GO film was deposited on gold wire electrodes with a site area of approximately 0.03 mm^2. Both electrochemical impedance spectrum (EIS) and cyclic voltammogram (CV) were measured by CHI 660c in phosphate buffered saline (PBS, pH 7.4) versus saturated calomel electrode (SCE, CH Instrument). CV was scanned in potentials between −0.6 V and 0.8 V at a scan rate of 50 mV/s. EIS was measured over frequency range from 0.1 Hz to 100,000 Hz.

2.4. Stimulation

To study the electrical performance during stimulation, bare gold wire electrode and PEDOT/GO coated electrode were immersed in phosphate buffered saline (PBS, pH 7.4) and connected with anode of electrical stimulator separately. A platinum foil connected with cathode of electrical stimulator and saturated calomel electrode were also immersed in PBS as counter electrode and reference electrode, respectively. A series of amplitude of 1 mA charge balanced, cathodic first, biphasic pulse current at 50 Hz was generated by an electrical stimulator (Master 8, A. M. P. I., Israel). An oscilloscope (TDS-2000, Tektronix, USA) was used for voltage excursions recording.

2.5. Stability test

Solid plane electrodes based on silicon substrate with site diameter of 100 μm were fabricated for stability assessment. The PEDOT/GO film deposited on the plane electrode at deposition charge density of 0.36 C/cm^2 was sustained 1000 cycles CV scanning ranging from −0.6 V to 0.8 V at rate of 100 mV/s in phosphate buffered saline (PBS, pH 7.4). The electrochemical property of CV and EIS was measured before and after repeated CV scanning for comparison. The mechanical stability was examined by comparison of SEM morphology observed before and after scanning.

2.6. Cell culture

To investigate the cell viability of PEDOT/GO film, highly differentiated rat pheochromocytoma PC-12 cells (hdPC-12 cells), and mouse embryonic fibroblasts NIH/3T3 (NIH/3T3 cells) were employed in this study, purchased from Chinese Academy of Sciences. HdPC-12 cells and NIH/3T3 cells cultivated with DMEM (Gibco, USA) supplied with 5% CO_2 at 37 °C.

2.7. Cell proliferation

The hdPC-12 cells and NIH/3T3 cells of 5×10^3 were seeded onto samples of glass slices sputtered with gold and electrochemically deposited PEDOT/GO film on sputtered gold slices in 48-well flat-bottomed cell plates, respectively, for incubating with DMEM (Gibco, USA) supplied with 5% CO_2 at 37 °C for 1 day, 4 days and 7 days, respectively. For estimating the cell viability, medium was removed and cells on samples were treated with 150 μl fresh DMEM with CCK-8 for 3 h. Then, OD value (optical density) was measured at 450 nm by microplate reader (Multiskan MK3, Thermo Labsystems, Finland). Six parallel replicates were read for each sample.

2.8. Immunofluorescent staining for cell proliferation

For observation of cell proliferation, cells were labeled after 1 day, 4 days and 7 days of cultivation. After the removal of the medium, the cells on samples were washed with PBS, and then fixed for 5 min in 3.5% formaldehyde in PBS. Then they were immersed in 0.1% Triton X-100 for 5 min, followed by washing in PBS for 3 times. After aforesaid processing, the cells on samples were stained with phalloidin-TRITC (Sigma, USA) for 1 h at room temperature without illumination. Subsequently, the nucleuses of these samples were quickly stained by 5 μg/ml DAPI (4,6-diamidino-2-phenylindole dihydrochloride, Sigma, USA) for 5 min at room temperature. Finally, the cells on samples were washed in vast PBS for removing residual stain, and viewed by a laser scanning confocal microscope (Leica TCS SP5, Leica, Germany).