Poly(3,4-ethylenedioxythiophene)/Graphene Oxide Composite Coating for Electrode-Tissue Interface

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Abstract— **Owing to interacting with the living tissue directly, the electrode-tissue interface largely determines the performance of the whole bioelectronics devices. The miniaturization of biomedical electronic components requires interface materials to possess properties including excellent electrical performance, good biocompatibility and compatibility with microelectronic fabrication process. Considering the unique characteristics and wide applications in biomedical domain of conducting polymer and graphene, composite film consists of poly(3,4-ethylenedioxythiophene) (PEDOT) and graphene oxide (GO) is proposed as electrode-tissue interface in this work. The facilely electrochemically synthesized PEDOT/GO coating on microelectrodes shows low impedance, high charge storage capacity and good biocompatibility to act as electrode-tissue interface. As a result, the composite film is a potential biomaterial as electrode-tissue interface for tissue engineering and further implantable electrophysiological devices.**

I. Introduction

Microelectrodes play an important role as electrode-tissue interface in diagnosing and treating diseases by electrophysiological recording or electrical stimulation [1]. More and more minimized electrodes are developed to perform sophisticated electrophysiological research by providing excellent spatial selectivity, low power consumption and low response from tissue [2]. However, the shrinkage of electrode dimension will result in the degradation of electrode performance. For instance, when the electrode area decreases, the impedance goes up, leading to a declination in recorded signal quality and tissue damage during electrical stimulation. Therefore, it is necessary to develop electrode-tissue interface materials for electrode site modification.

As performing neural recoding or stimulation in living tissue for a period of time, the electrode-tissue interface materials ought to possess properties including: compatibility with micro electrode fabrication process, excellent electrochemical performance and biocompatibility. Herein, we proposed electrochemically synthesized composite film consisted of graphene oxide (GO) and poly(3,4-ethylenedioxythiophene) (PEDOT) for electrode-tissue interface. Owning excellent mechanical property, GO doped in PEDOT acted as the structural material to enlarge the effective surface area of electrode site and enhanced the stability of the composite. PEDOT dispersed in the interspaces of GO nets served as stable charge transfer medium. Moreover, electrical characterizations including electrochemical impedance spectroscopy (EIS), cyclic voltammogram (CV) and charge injection limit demonstrated excellent electrical properties of PEDOT/GO composite film. Furthermore, biocompatibility and cell adherence of PEDOT/GO were verified by the cultivation of highly differentiated rat pheochromocytoma PC-12 cells (hdPC-12 cells) and mouse embryonic fibroblasts NIH/3T3 (NIH/3T3 cells). Finally, we evaluated the PEDOT/GO modified wire electrode by electrically stimulating rabbit orbicularis oculi muscle for paralysis recovery.

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II. Materials and Methods

A. Electrochemical Synthesis

The electrolyte for PEDOT/GO or PEDOT/PSS synthesis consisted of 0.01 M EDOT (Sigma-Aldrich, USA) and 1 mg/ml graphene oxide aqueous (XFNano, China) or 5 mg/ml poly(sodium-p-styrenesulfonate) (PSS, Sigma-Aldrich, USA). The solution was then stirred for over 2 hours to dissolve EDOT and purged with pure nitrogen gas for 10 minutes to prevent pre-oxidation of EDOT. The PEDOT/GO film was electrochemically deposited on Au plane and wire electrode in galvanostatic mode with deposition current density of 0.2 mA/cm^2, respectively. The electrode site diameter of the plane electrode is 100 μm which is equal to the diameter of wire electrode.

B. Electrochemical Characterization

The measurement of electrochemical properties including cyclic voltammogram (CV) and electrochemical impedance spectrum (EIS) were performed with electrochemical station 660c (CH Instrument) in phosphate buffered saline (PBS, pH 7.2-7.4) versus saturated calomel electrode (SCE, CH Instrument). CV was scanned over the potential between -0.6 V and 0.8 V at scan rate of 50 mV/s. EIS was measured at frequency ranging from 0.1 Hz to 100,000 Hz.

C. Cell Culture and Viability Test

Highly differentiated rat pheochromocytoma PC-12 cells (PC-12 cells), and mouse embryonic fibroblasts NIH/3T3

(NIH/3T3 cells) were employed in this study, purchased from Chinese Academy of Sciences. PC-12 cells and NIH/3T3 cells cultivated with DMEM (Gibco, USA) supplied with 5% CO_2 at 37°C.

In acute cytotoxicity test, the cells of 5×10^4 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 (Corning, USA), for incubating with DMEM supplied with 5% CO_2 at 37°C for 24 hours. To estimate the cytotoxicity, medium was removed and cells on samples were treated with 150 µl fresh DMEM with CCK-8 for 3 hours. 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. And the formula was used to calculate the viability of cell growth:

Cell viability (%) = 100% × OD value of sample / OD value of control

Where the OD value of sample is cell cultivated on sample, and OD value of control is the OD value of cell cultivated on the cell plate. The high cell viability value indicates low cytotoxicity.

D. Immunofluorescent Staining

For observation of cell morphology, cells were labeled after 24 hours of cultivation. After the removal of the medium, the cells on samples were washed with PBS, and then fixed for 5 minutes in 3.5% formaldehyde in PBS. Then they were immersed in 0.1% Triton X-100 for 5 minutes, followed by washing in PBS for 3 times. The cells on samples were then stained with phalloidin-TRITC (Sigma, USA) for 1 hour at room temperature without illumination. Subsequently, the nucleuses of these samples were quickly stained by DAPI (4,6-diamidino-2-phenylindole dihydrochloride, Sigma, USA) of 5 µg/ml for 5 minutes 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).

III. Results and Discussion

As illustrated in Figure 1, when constant current was applied through the electrolyte, the EDOT monomer was firstly oxidized to form PEDOT polymer chain at anode. Then the positively charged PEDOT molecule combined with the negatively charged GO, which was named counterion, by ionic bond. The PEDOT/GO composite was deposited onto the electrode site to serve as electrode-tissue interface.

The PEDOT/GO composite was electrochemically deposited on both planar and cylindrical electrode sites for morphology investigation, respectively. As shown in Figure 2, PEDOT uniformly dispersed among the stable framework composed of GO layers with excellent mechanical property. Also, the incorporation of GO caused rough morphology on the surface of film, which contributed to increase the effective electrode-tissue interface area. The GO doped in PEDOT randomly distributed in the composite film on the planar substrate (Figure 2a and 2b), while the GO on the cylindrical substrate orderly aligned with the direction of gold wire electrode. This aligned surface morphology may be applied as extracellular matrix guiding cells directional growth in tissue engineering.

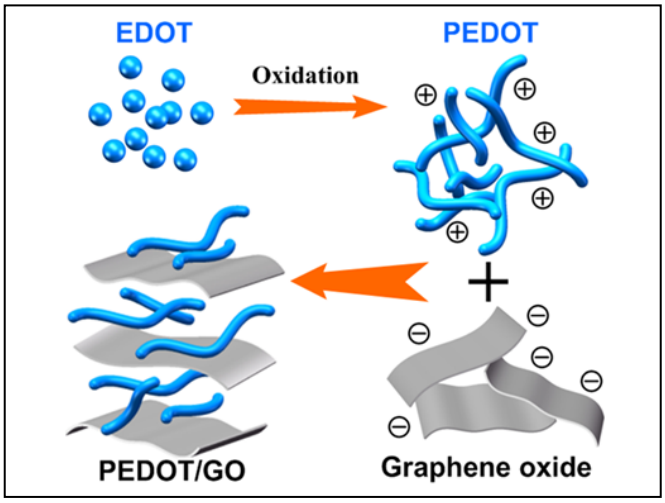

Figure 1. Illustration of the synthesis procedure and structure of the PEDOT/GO composite film

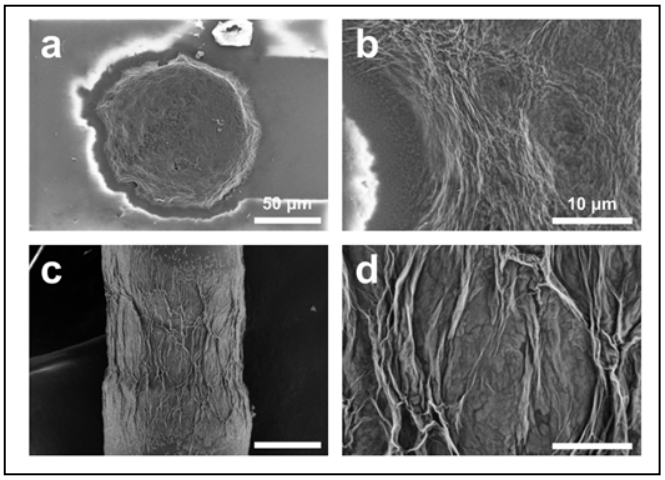

Figure 2. Scanning electronic microscopy (SEM) of PEDOT/GO film deposited on planar (a, b) and cylindrical electrode sites (c, d), respectively..

The chemical structure of the PEDOT/GO composite film was confirmed by and UV-Vis spectra (UVI). As shown in Figure 3, the UVI spectra of PEDOT, GO and PEDOT/GO composite aqueous dispersion was analyzed to confirm the composition of electrochemical deposited PEDOT/GO. The peaks at 228 nm and 300 nm corresponded to π-π* and n-π* transitions of C=C and C=O bonds, respectively [3]. The UVI curve of PEDOT/GO consists not only peak at 255 nm of PEDOT but also peak at 300 nm of GO, which proves the coexistence of PEDOT and GO.

The electrochemical impedance spectroscopy of PEDOT/GO coated electrodes was measured at the frequency range from 0.1 to 100,000 Hz. As shown in Figure 4a, the impedance of electrode coated with PEDOT/GO film was obviously smaller than one coated with PEDOT/PSS film. It was mentioned that the impedance at 1 kHz was related to the neuronal recording and power consumption during electrical stimulation [4]. The impedance of electrode with PEDOT/GO

film decreased almost two orders of magnitude than that of bare gold electrode. It exhibited an outstanding characteristic that could be compared with some special structures of electrode materials [5].The phase plot of the impedance spectroscopy (Figure 4a) demonstrates that PEDOT/GO composite film acts as resistive material at high frequency and as capacitive material at low frequency, because their phase angles of the corresponding frequency range are approximately 0° and 90°, respectively.

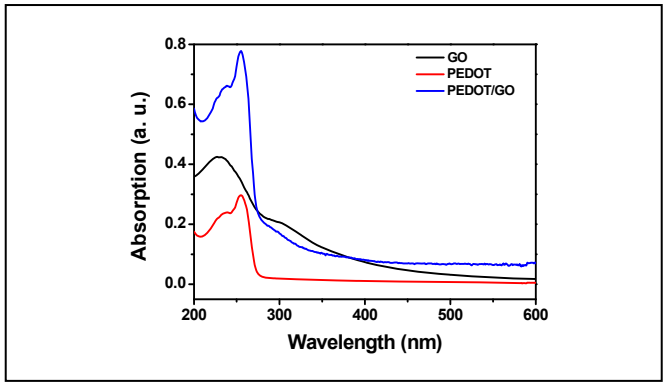

Figure 3. UV-Vis spectra of GO, PEDOT and PEDOT/GO.

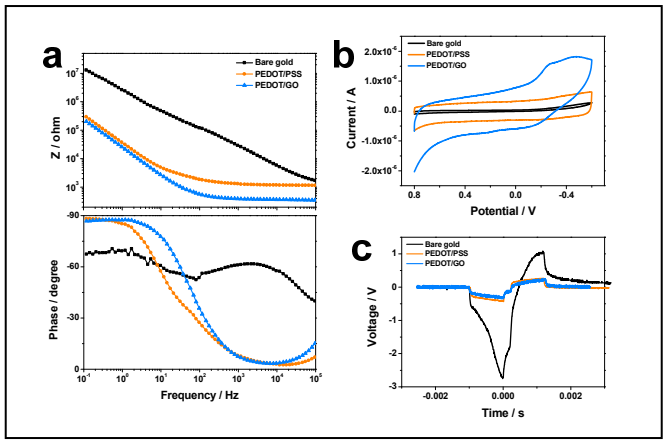

Figure 4. (a) Electrochemical impedance spectroscopy (EIS) of bare gold electrode (black), PEDOT/PSS (orange) and PEDOT/GO (blue) coated electrode. (b) CV measurement curves of three materials corresponding to the EIS curves.

Cyclic voltammogram (shown in Figure 4b) was measured to evaluate the redox characteristics and the charge storage capacity of the electrodes. Widely used poly(sodium-p-styrenesulfonate) (PSS) was doped as counterion to form PEDOT/PSS composite film for comparison. Both PEDOT/GO film and PEDOT/PSS film were deposited on gold electrode sites with a charge density of 0.36 C/cm^2 and scanned from -0.6V to 0.8V (vs. SCE) at a scan rate of 50 mV/s. The charge storage capacity (CSC) of electrode coated with PEDOT/GO film was over 10 times of that of bare gold. And it was obviously greater than one of gold electrode covered by PEDOT/PSS film, which was evaluated based on the enclosed area of CV curve. As expected from the morphology, the increased effective surface area and excellent electrical property of the PEDOT/GO film contribute to the improvement of capacitive property.

Voltage response to current stimulation was involved to evaluate electrical stimulation performance of PEDOT/GO modified electrode. Here, amplitude of 1 mA charge balanced, cathodic first, biphasic pulse current pulse train at 50 Hz was applied through phosphate buffered saline (PBS, pH 7.4), and an oscilloscope (TDS-2000, Tektronix, USA) was arranged for voltage excursions observation versus saturated calomel electrode (SCE, CH Instrument). As presented in Figure 4c, the voltage amplitude of PEDOT/GO coated electrode was obviously lower than that of unmodified electrode. The charge injection limit was obtained from the maximum charge density per unit area of electrode site when the maximum residual potential reached the potential of water reduction (E_{mc}=—0.6 V) [6]. The charge injection limit was increased from 0.16±0.05 mC/cm^2 of bare gold electrode to 5.60±1.09 mC/cm^2 and 4.71±0.18 mC/cm^2 of PEDOT/PSS and PEDOT/GO, respectively. As a consequence, the high charge injection limit of PEDOT/GO enabled efficient electrical stimulation of microelectrodes with safely low voltage amplitude.

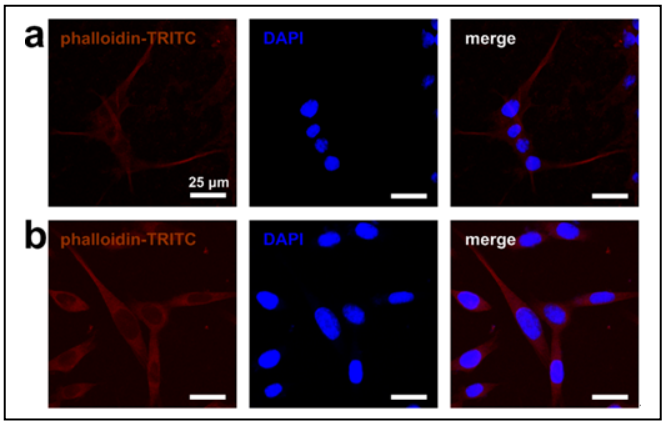

Figure 5. Morphology of cells cultured on PEDOT/GO substrate for 1 day observed by laser scanning confocal microscope (LSCM). (a) and (b) for PC-12 cells and NIH/3T3 cells, respectively.

In order to evaluate biocompatibility of PEDOT/GO for cell in vitro, highly differentiated rat pheochromocytoma PC-12 cells (PC-12 cells) and mouse embryonic fibroblasts NIH/3T3 (NIH/3T3 cells) were cultured on PEDOT/GO film. Morphology of cells cultured for one day was shown in Figure 5 that PC-12 cells grew long neurites interconnected with others, and NIH/3T3 cells grew healthily to form exactly fusiform shape. It can be clearly seen by the SEM cells contrast morphology (Figure 6a and 6b) on the two kinds of material that PC-12 cells grown on PEDOT/GO film generally covering a larger area than on the sputtered gold substrate. Also, no obvious difference is observed for growth of NIH/3T3 cells on two kinds of substrates. That was most likely due to the promotion for neural cells of conducting polymer. As exhibited in (Figure 6c and 6d) in detail, pseudopodia extended out from both kinds of cells as pointed by white arrows, while their extending ends and PEDOT/GO substrate almost fused together. It proves good cell attachment of PEDOT/GO film. Furthermore, the quantitative measurement results of cell viability of PEDOT/GO film were 92.25%±5.82% and 90.61%±9.84% for PC-12 cells and NIH/3T3 cells compared with 94.90%±2.94% and

93.05%±7.90% of gold sputtered substrate (Figure 7), respectively. The approximation for cell viability of PEDOT/GO film to sputtered gold demonstrated that the acute cytotoxicity response caused by PEDOT/GO was nearly same to gold.

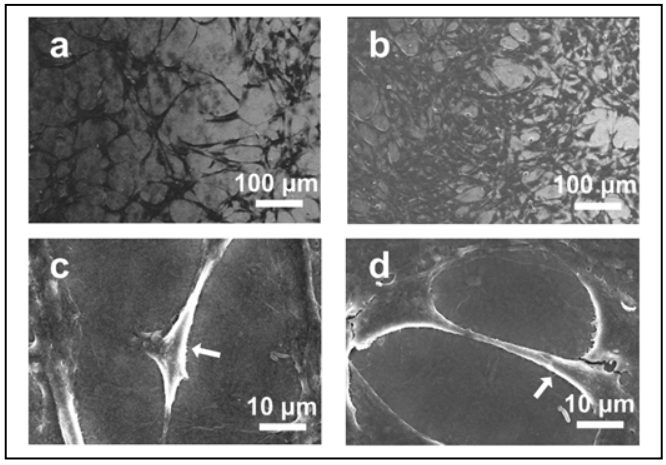

Figure 6. SEM contrast morphology of PC-12 cells (a) and NIH/3T3 cells (b) cultured on PEDOT/GO substrate (left dark side) and sputtered gold substrate (right bright side) for 1 day. Detailed morphology of PC-12 cells (c) and NIH/3T3 cells (d) cultured on PEDOT/GO substrate.

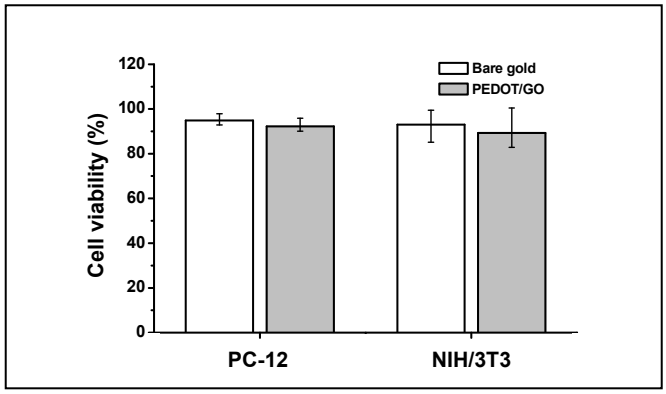

Figure 7. Diagram of cell viability test on substrates of sputtered gold and PEDOT/GO film.

In addition, we evaluated the PEDOT/GO modified wire electrode by electrically stimulating rabbit orbicularis oculi muscle for paralysis recovery (shown in Figure 8). New Zealand rabbits of specific pathogen-free grade, aged 6 months and weighing 2.8 kg were provided by the Laboratory Animal Center, First People's Hospital, Shanghai Jiao Tong University (permission No. SYXK (Hu) 2013–0086). Firstly, unilateral facial nerve of rabbit was cut to disable the rabbit from eye closure. Secondly, a pair of PEDOT/GO modified wire electrodes were easily placed to orbicularis oculi muscle by a hollow injection needle. A series of biphasic stimulation rectangular waves at frequency of 50Hz was applied subsequently. As a result, the cathodic and anodic pulse of rather low current amplitude of 0.15 mA and 0.1 mA, respectively, was able to recover the eye closure.

IV. Conclusion

In summary, we synthesized facilely fabricated PEDOT/GO composite film by electrochemical deposition for electrode-tissue interface. Both the low electrochemical impedance and high CSC indicated that the electrochemical performance of microelectrode was remarkably promoted by PEDOT/GO coating. Also, the voltage response to current pulses and charge injection limit measurement results demonstrated excellent property of PEDOT/GO for electrical stimulation. Moreover, the biocompatibility was verified by cells morphology observation and viability test of cultivated PC-12 cells and NIH/3T3 cells. Furthermore, functional electrical stimulation for recovery of paralyzed rabbit orbicularis oculi muscle was operated to confirm the PEDOT/GO modified electrode performance for practical application. As a consequence, the PEDOT/GO film is promising material as electrode-tissue interface for electrophysiological applications and tissue engineering.

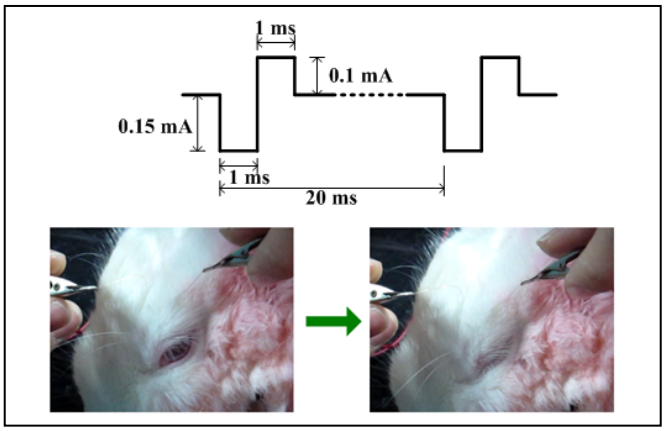

Figure 8. The current stimulation waveforms (upper) and experiment result (under) of electrical stimulation of orbicularis oculi muscle of unilateral facial paralyzed rabbit.

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