Electrochemical Stability of Poly(3,4-Ethylenedioxythiophene) Derivatives Under Cell Culture Conditions

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Abstract. Poly(3,4-ethylenedioxythiophene) (abbreviated as PEDOT) is widely used in nerve electrodes and biosensors. However, only a few investigations on the electrochemical stability of PEDOT derivatives in the biological environment have been reported. Here, the electrochemical performances of two PEDOT derivatives were evaluated during cell culture. The results showed phosphocholine-functionalized PEDOT (PEDOT-PC) to possess good anti-cell adhesion ability with 0.6% impedance change before and after cell culture. By comparison, hydroxyl-functionalized PEDOT (PEDOT-OH) promoted cell adhesion, in which impedance decreased by 17.6% with cell spreading due to strong adsorption of serum proteins. In sum, these findings look promising for the development of novel and facile PEDOT platforms for in vivo bioelectronics.

1. Introduction

Nowadays, organic bioelectronics for neural prostheses[1-2], artificial muscles[3], and bio-integrated devices[4] are rapidly emerging in biosignal detection and recording just like translators between biological and artificial electronic processing signal function systems. Poly(3,4-ethylenedioxythiophene) (abbreviated as PEDOT) possesses good redox stability in biological environments[5], excellent biocompatibility[6], and low-impedance ion/electronic interfaces[7]. Hence, PEDOT is considered a promising material for the preparation of implanted bioelectronics. However, many bioelectronics inevitably adsorb biomolecules in real applications, thereby reducing their electrochemical stability. To solve this issue, some studies have introduced zwitterionic materials containing equal numbers of cationic and anionic moieties. Such structure grants zwitterionic materials with superior hydration capacity, thereby effectively resisting nonspecific absorption from biomolecules and microorganisms.

An example of a zwitterionic conducting polymer is phosphocholine-functionalized PEDOT (abbreviated as PEDOT-PC). This zwitterionic conducting polymer displays high resistance towards nonspecific protein/cell binding[8]. The reason for this has to do with its unique hydrogen-bonded
phosphocholine, allowing it to form ice-like water, leading to strong mechanical strength and good resistance toward potential interferences\cite{9}. By comparison, hydrosyl-functionalized PEDOT (PEDOT-OH) has good biocompatibility and may promote cell adhesion. PEDOT-PC and PEDOT-OH have so far been studied in terms of anti-adhesion ability and electrochemical performance but their electrochemical impedance stability features during real-time cell culture are still lacking.

In this study, PEDOT-OH and PEDOT-PC were deposited on a conductive substrate by electrochemical polymerization. The impedance changes during the protein/cell adhesion process were explored to gain a better understanding of the interaction mechanism between PEDOT materials and biological cells, which might benefit the fabrication of high impedance stability bioelectronic devices.

2. Materials and methods

2.1 Materials

EDOT-OH was purchased from Sigma-Aldrich, and EDOT-PC was synthesized according to previously reported methods\cite{8}. LiClO_4 was obtained from Aladdin biochemical technology, acetonitrile (MeCN) and sodium dodecyl sulfonate (SDS) from Adamas Reagent and dioctyl sulfosuccinate sodium salt (DSS) from Wako Pure Chemical Industries. All materials used for cell culture were purchased from Thermofisher Scientific.

2.2 Preparation of PEDOT-PC and PEDOT-OH films

The electrochemical experiments were performed on an Autolab PGSTA204 equipped with an impedance module.

The electrochemical solution of EDOT-OH contained 10 mM EDOT-OH monomer, 50 mM SDS, and 100 mM LiClO_4 dissolved in distilled water. The electropolymerization of EDOT-OH was carried out by cyclic potential scans in the voltage range of -0.6 V to 1.13 V at the scan rate of 100 mV/s for two scan cycles. A three-electrode electrochemical cell containing a Pt mesh as counter electrode and Ag/AgCl as the reference was used for the studies.

The electrochemical solution of EDOT-PC contained 10 mM EDOT-PC monomer, 50 mM DSS, and 100 mM LiClO_4 dissolved in MeCN. The electropolymerization of EDOT-PC was carried out by applying cyclic potential scans from -0.6 V to 1.025 V at the scan rate 100 mV/s for two scan cycles. A three-electrode cell made of Pt mesh as counter electrode and Ag/Ag^+ (10 mM AgNO_3 and 100 mM LiClO_4 in MeCN) as reference was utilized.

2.3 Quartz crystal microbalance (QCM) measurements

The QCM measurements were performed on a Q-Sense E4 instrument (Biolin Scientific) at 25 °C. The PEDOT-OH and PEDOT-PC were first electrochemically deposited on a QSX 301 sensor (Biolin Scientific) and then placed in the measurement chamber. A baseline signal was established by allowing PBS buffer to flow at the rate of 30 μL/min until the stabilization of the baseline. Solutions containing 10% cell culture medium were delivered to the measurement chamber at the flow rate of 30ul/min. Next, a blank PBS was employed to flush the chamber and remove nonspecific adhesions. The tests were performed at resonance frequencies of 5, 15, 25, 35, 45, 55, and 65 MHz. Changes in the third overtone (15 MHz) were used for analysis.

2.4 Electrochemical impedance measurements

Faraday impedance of serum adhesion: QCM was used for the measurement of the impedance values by the three-electrode method using 5 mM K_3Fe(CN)_6 / K_4Fe(CN)_6 as a redox probe. The bias voltage was used as an open circuit potential (OCP) at the frequencies of 0.1 Hz to 100000 Hz and voltage amplitude of 10 mV.

Cell culture by in vitro electrochemical impedance measurements: HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO_2. PEDOT-OH and PEDOT-PC were
electrochemically deposited on an Indium Tin Oxide (ITO) coated glass (5 cm*5 cm) separately. Next, the ITO was placed in a chamber (Figure 1), and the two-electrode electrochemical impedance measurements were performed during cell culture. The counter electrode was Au, the bias voltage was an open circuit potential (OCP), the frequency range was 0.1 Hz to 100000 Hz, and the voltage amplitude was 10 mV.

3. Results & Discussion

Figure 1. Schematic diagram of the electrochemical impedance testing device

In this experiment, PEDOT-PC and PEDOT-OH films were deposited on ITO glass by electrochemical polymerization. The resulting samples were then placed in the experimental device for cell culture. As shown in Figure 2, Hela cells started to adhere to PEDOT-OH films after 2 h of culture followed by further spread after 12 h of culture. After 2 h culture, the cells shrunk into spherical shapes deposited on PEDOT-PC surface. After a long period of culture, the cells tended to agglomerate, indicating PEDOT-PC to possess excellent anti-cell adhesion ability.

Figure 2. (a,b) Pictures of Hela cells cultured on PEDOT-OH films for 2 h and 12 h. (c,d) Pictures of Hela cells cultured on PEDOT-PC films for 2 h and 12 h. Conditions: seeding density of 1*10^5 cell/mL. The scale bar is 100 μm.

In situ serum protein adhesion experiments were also carried out on PEDOT-PC and PEDOT-OH using QCM measurements. As shown in Figure 3a, PEDOT-OH illustrated serious serum protein adhesion ability but PEDOT-PC was almost unable of adhering to serum proteins. Some studies have shown that the adsorption of charged serum proteins would contribute to cell adhesion[10]. The latter confirmed that PEDOT-OH promoted cell spreading, but PEDOT-PC was not capable of adhering to cells. Here, the impedance at 1 kHz was used to investigate the deviation in PEDOT profiles. This value is often employed as a typical frequency to predict device performance in bioelectronics[11]. In faraday impedance, the change in impedance is related to the interface charge transfer capability[12]. As shown
in Figure 3b, the impedance of PEDOT increased by 12.3% while that of PEDOT-PC only changed by 1.8%, which may be due to the adhesion of serum proteins hindering the charge transfer.

Figure 3. a) QCM frequency responses taken from the adsorption serum to PEDOT-OH and PEDOT-PC films. b) PEDOT-OH and PEDOT-PC impedance profiles in PBS and serum at 1kHz

The experimental device shown in Figure 1 was utilized for real-time measurements of electrochemical impedance during cell culture. Two-electrode electrochemical impedance without adding redox probes was used as an alternative to other labeled sensor methods\[13\textendash14\]. The DC bias was set to OCP, in which zero net currents will not affect the redox state of the material in the solution. The electrochemical impedance spectroscopy was investigated during cell culture and the data are provided in Figure 4a and 4b. In non-faraday impedance, the impedance change is related to the interface capacitance affected by the relative dielectric constant of the interface\[12\]. As culture time increased, the cells spreading further on PEDOT-OH, thereby changing the dielectric properties of the interface and causing a continuous decline in impedance by 17.6%. By comparison, the good anti-adhesion ability of PEDOT-PC to cells and serum proteins prevented the impedance of PEDOT-PC from varying after an initial slowing a decline by 0.6% (Figure 4c).

Figure 4. a) Bode plot of PEDOT-OH after different cell incubation times, b) Bode plot of PEDOT-PC after different cell incubation times, and c) relationship between the impedance value of PEDOT-OH and PEDOT-PC at 1 KHz and incubation time.

Cells did not spread on PEDOT-PC surface, leading to large area cell apoptosis. Thus, a difference between the log impedance data (\(\log|Z|_{\text{cell}}\)) after 12 h of cell culture and the initial log impedance data in the cell culture medium (\(\log|Z|_{\text{cell culture medium}}\)) was drawn. No obvious differences in PEDOT-PC impedance before and after cell culture were observed when compared to PEDOT-OH, which further demonstrated the impedance stability of PEDOT-PC.
4. Conclusions
The electrochemical impedance of two different PEDOT derivatives was successfully studied during real-time cell culture. PEDOT-OH showed severe serum protein adhesion. After protein adhesion, the impedance changed by 12.3%. Meanwhile, the impedance further changed by 17.6%. PEDOT-OH after the spread of cells on the surface, revealing severe serum protein adhesion that could promote cell adhesion. By comparison, PEDOT-PC displayed excellent anti-adhesion to serum proteins, displaying stable electrochemical impedance before and after serum adhesion and cell culture. In sum, these findings look promising for the future development of new and facile platforms for in vivo bioelectronics.

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