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Biocompatible conductive architecture with surface-confined probe for non-invasive electrochemical cytosensing

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ABSTRACT

The possibility of using electrochemical methods to investigate cell immobilization and natural physiological states provides great advances in life science research and public health protection. Herein, cytosensors with surface-confined ferrocene as signal indicator were developed to study the immobilization of human cervical carcinoma (HeLa) cells. With layer-by-layer (LBL) technique positively charged ferrocene functionalized poly(allylamine hydrochloride) (Fc-PAH) and negatively charged single-wall carbon nanotubes (SWNTs) were alternately assembled on 3-mercaptopropionic acid (MPA) modified gold substrates. The as-prepared cytosensors presented good biocompatibility and HeLa cells could keep viability for 72 h on the materials according to the proliferation results. With differential pulse voltammetry (DPV) measurements the cytosensors exhibited high sensitivity to the detection of HeLa cells within a wide concentration range from 10 to 10^7 cells/mL.

1. Introduction

Recently, increasing interest has focused on the investigation of immobilizing living cells on biocompatible material surface within such fields as life science research and public health protection [1]. Due to the insulating property of cell membrane the access of electron transfer (ET) through the complex materials is hindered by the immobilized cells. The inhibiting property on ET is directly related to the amount of the immobilized cells. As a result, simple and nondestructive electrochemical methods have attracted considerable attention for developing cytosensor to investigate the viability and natural physiological state of cells [2]. For example, electrochemical impedance spectroscopy (EIS) was used to monitor the immobilization of bacterial cells [3–6]. Ju and co-workers investigated the viability of immobilized K562 cells by monitoring current response with DPV method [7,8]. In previous investigations, the changes of the electrochemical properties were usually studied by monitoring current or resistance change of solution-phase redox probes at electrode/solution interface. However, the accumulation of solution-phase redox probes on the substrate surface might affect the viability of the immobilized cells [1]. Therefore an increasing interest has focused on the search of new culture substrates [9,10]. Keeping that in mind, we developed a "surface-confined probe" technique for the first time to detect cell immobilization with DPV measurements in solution free of redox probes. Based on LBL technique positively charged Fc-PAHs were immobilized on electrode surface as signal indicator. Negatively charged SWNTs were introduced to the surface since it can promote ET of redox probes, which were widely used in the fabrication of electrochemical biosensors [11,12]. With LBL technique large amount of redox probes were immobilized on the cytosensor surface to provide an amplified current signal for improving detection sensitivity. The as-prepared cytosensors presented good biocompatibility and high sensitivity for monitoring immobilization of HeLa cells.

2. Experimental procedure

2.1. Materials

3-mercaptopropionic acid (MPA) was purchased from Alfa Aesar company and PAH was from Sigma-Aldrich company. Ferrocenecarboxaldehyde was purchased from J&K Chemical LTD. SWNT came from Shenzhen Nanotech Port Co., Ltd. Acridine Orange (AO) was from Dingguo Co. China. The Fc-PAH was synthesized according to the previous procedure [13]. Phosphate buffered saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 0.087 M Na_2HPO_4, and 0.014 M KH_2PO_4.

2.2. Fabrication of the sensing interface

The gold electrode (0.11 cm^2) was firstly immersed into 0.1 mM MPA solution for 2 h. After being taken out, the electrode was rinsed

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with large amount of ethanol and Milli-Q water and then alternatively immersed into Fc-PAH (1 mg/mL, pH = 6) and SWNT (0.1 mg/mL) solutions for 30 min. The resultant membrane was washed thoroughly with Milli-Q water. This process was repeated until the desired number of $(Fc\text{-}PAH/SWNT)_n$ was obtained. The modified electrode was named as $Au/MPA/(Fc\text{-}PAH/SWNT)_n$. The positive charged PAH layer was designed as the outermost layer, leading final modified electrode, $Au/MPA/(Fc\text{-}PAH/SWNT)_5/PAH$.

2.3. Cell culture and cell immobilization

The HeLa cell line was purchased from Kunming Institute of Zoology. 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. The cells were trypsinized and subcultured every two days. The cell number was detected using a Petroff Hausser cell counter (USA).

For immobilization on the cytosensor surface, the cells were separated from the medium by centrifugation at 2000 rpm for 3 min and then washed twice with sterile PBS. The sediment was carefully redispersed in PBS (3 mL) to obtain a homogeneous cell suspension at a certain concentration. Then the $Au/MPA/(Fc\text{-}PAH/SWNT)_5/PAH$ electrode was immersed into the cell suspensions for 50 min.

2.4. Electrochemical measurements

Cyclic voltammograms were recorded in a classical three electrode configuration by CHI 832B (Co. Chenhua, China). Platinum foil and Ag/AgCl electrode worked as counter and reference electrode, respectively. 0.9% NaCl solution was used as supporting electrolyte.

2.5. Confocal imaging

Firstly, three indium tin oxide (ITO) slices coated with $(Fc\text{-}PAH/SWNT)_5/PAH$ film were placed in a 10 cm cell culture plate. Then 1.0×10^6 HeLa cells were introduced into the plate and cultured in DMEM medium supplemented with 10% FBS at 37 °C. At the time of 24, 48 and 72 h, one of the ITO slices was taken out and the cells on which were stained with AO. The fluorescent images of HeLa cells on $(Fc\text{-}PAH/SWNT)_5/PAH$ film were taken by the TCS SP2 confocal laser scanning microscope.

3. Results and discussion

3.1. Electrochemical characterization of the as-prepared biosensor

With LBL self-assembly technique a three dimensional structure on the substrates could be constructed, introducing more ferrocene to produce an amplified signal for improving detection sensitivity. One pair of redox peaks were observed at 0.36 V from Fig. 1A. Current responses increased with increasing number (n) of the bilayer (Fc-PAH/SWNT). The peak-to-peak potential difference was 20 mV and did not obviously change with the increasing of bilayer number. The possible reason might be that SWNT could facilitate ET of redox probes. Within the investigated range, the peak current presented a linear dependence on the bilayer number (inset of Fig. 1A), suggesting the assembling process was well controlled. As known, the current signal should provide appropriate intensity and high sensitivity for the sensor. Moreover, once the detection sensitivity was guaranteed the preparation of biosensors should be as simple as possible. According to our investigations, the bilayer number was fixed at five, which met the above requirements for the further study of cell immobilization. Since cancerous cells are usually negatively charged, positively charged PAH film was designed as outmost layer, which could enhance cell immobilization and favor cell viability [14]. Herein, PAH rather than Fc-PAH was used as outmost layer because we tried

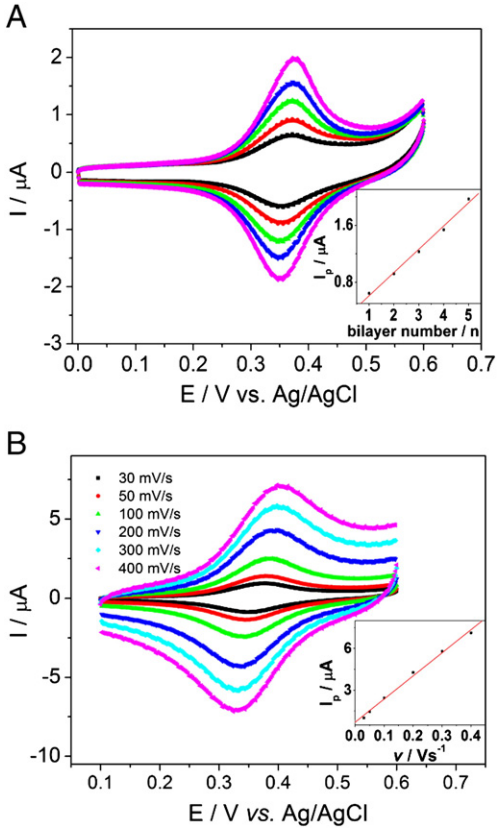

Fig. 1. (A) Current responses of the $Au/MPA/(Fc\text{-}PAH/SWNT)_n$ electrode in 0.9% NaCl supporting electrolyte. n = 1, 2, 3, 4, 5 from inner to outer. Scan rate: 50 mV/s. Inset shows linear dependence of peak current on increasing number of bilayer (Fc-PAH/SWNT). (B) Current responses of the $Au/MPA/(Fc\text{-}PAH/SWNT)_5/PAH$ electrode with different scan rates in 0.9% NaCl supporting electrolyte. Inset shows linear dependence of the peak current on scan rate.

to avoid the effect of ferrocene on the immobilized cells during potential scan. Thus the $Au/MPA/(Fc\text{-}PAH/SWNT)_5/PAH$ modified electrode was used for cells detection, which presented a lower current response than that of $Au/MPA/(Fc\text{-}PAH/SWNT)_5$ due to the assembling of PAH layer, Fig. 1B. The peak current linearly increased with scan rate, indicating that the electron transfer of surface-confined ferrocene was a surface controlled process. The stability of the as-prepared sensors was investigated by keeping $Au/MPA/(Fc\text{-}PAH/SWNT)_5/PAH$ electrodes in desiccator filled with Argon at room temperature for twenty days. The peak current decreased 8.2% only, suggesting that the functionalized electrode was comparatively stable and available for label-free cell detection.

3.2. Biocompatible property of the as-prepared biosensor

The physical and chemical properties of a modified electrode affect the immobilization and growth of cells on it [15]. To guarantee the viability and nature property of cells researchers have paid more attention to construct biomaterials in favor of cells adhering [1]. Herein, the biocompatible property of the as-prepared film was investigated by monitoring cell proliferation on $(Fc\text{-}PAH/SWNT)_5/PAH$ functionalized ITO. Learned from Fig. 2, the density of cultured HeLa cells on the film increased with increasing incubation time. From morphology of the distinguishable filopodia the proliferated cells were alive after incubation for 72 h, indicating good viability of the HeLa cells [16]. Thus, the cells were capable of immobilizing and proliferating on the $(Fc\text{-}PAH/SWNT)_5/PAH$ films during extended culture. The results confirmed that the as-prepared films exhibited