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Highly sensitive and selective detection of cancer cell with a label-free electrochemical cytosensor

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ABSTRACT

Electrochemical methods have attracted considerable attention for developing cytosensing system since they can decrease the cost and time requirement for cell detection with simple instrumentation. Herein, a label-free electrochemical cytosensor with surface-confined ferrocene as signal indicator was developed for highly sensitive and selective detection of cancer cell. With layer-by-layer (LBL) self-assembly technique, positively charged poly(ethylene imine) functionalized with ferrocene (Fc-PEI) and negatively charged single-wall carbon nanotubes (SWNTs) were alternately assembled on 3-mercaptopropionic acid (MPA) modified gold substrate. Folic acid (FA) was covalently bonded onto SWNTs surface to specifically recognize cancer cells according to the high affinity of FA for folate receptor (FR) on cellular surface. The developed cytosensor presented high sensitivity and selectivity for the detection of human cervical carcinoma (HeLa) cell. By using fast-response differential pulse voltammetry (DPV) method, a wide detection range from 10 to 10^6 cells/mL with a detection limit as low as 10 cells/mL was reached even in the presence of a large amount of non-cancerous cells.

1. Introduction

Cancer is considered as the number one killer in many countries and has become a major public concern nowadays. Various techniques have been developed for cancer cell detection, including cytologic testing, fluorescent imaging, magnetic resonance imaging, computerized tomography, X-ray radiography and ultrasound (Brindle, 2008; Fass, 2008; Mosmann, 1983; Zhang et al., 2002). However, most of these modalities are high cost and time consuming in either experimental process or instrumentation. Moreover, these approaches may couple with radioactive risk. Therefore, it is highly desirable to develop rapid, simple and non-destructive methods for early detection of cancer cells, which is important for preclinical diagnosis and reduction in mortality for certain cancers (De la Rica et al., 2009). To address these specific requirements, electrochemical methods have attracted considerable attention for developing cytosensing platforms since they are usually performed with simple instrumentation and also can reduce the cost and time required for analysis (Giaever and Keese, 1993; Hong et al., 2011; Liu et al., 2011; Seriburi et al., 2008; Weng et al., 2011).

Typically, electrochemical cytosensors are fabricated based on measuring the changes in current or resistance at the cell/sensor interface (Andreescu and Sadik, 2005; Privett et al., 2008; Spegel et al., 2008; Ding et al., 2008). Cheng et al. developed a electrochemical cytosensor array for dynamic analysis of a cell-surface glycome according to the special binding between horseradish peroxidase and the carbohydrate on the cell surface. (Cheng et al., 2009). Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry have been used to monitor the adhesion, viability, proliferation and apoptosis of cancer cells (Ding et al., 2007a,b; Hao et al., 2007a,b; Ruan et al., 2002; Yang et al., 2004). In some investigations, cancer cells were immobilized on the sensor interface based on electrostatic adsorption. Such cytosensors could not distinguish cancer cells from normal ones due to the low specificity, which could not meet the requirements of clinical diagnosis and therapy. An increasing interest has focused on "target-binding" technology for developing cytosensors with specific recognition function. Since the abnormal alterations of tumor markers, such as telomerase, glycan and folic receptor (FR), are associated with a variety of diseases especially cancers, tumor markers have been selected as a target molecule for developing

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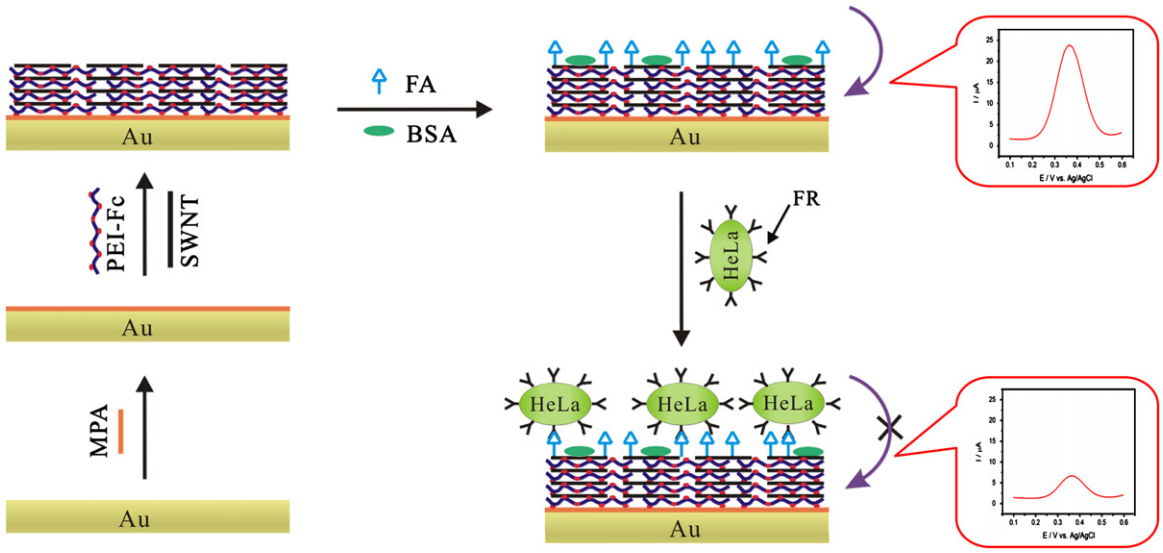

Scheme 1. Schematic diagram of the electrochemical cytosensor with surface-confined probe for detection of HeLa cell.

cytosensor (Weng et al., 2011; Morelli et al., 2011). For example, an electrochemical cytosensor was developed according to the special binding affinity between aptamer AS1411 and cancerous cells. The electron transfer resistance of the cytosensor was increased corresponding to the amount of the attached cells (Feng et al., 2011). Folic receptor can be selected as a target molecule because it is overexpressed on the membrane of epithelial cancer cell while limitedly expressed on the membrane of a normal cell, which was confirmed by flow cytometry experiments (Morelli et al., 2011). Folic acid (FA) is a vital nutrient required by all living cells for nucleotide biosynthesis and for the proper metabolic maintenance of 1-carbon pathways. Aside from its cofactor role for intracellular enzymes, FA also exhibits high affinity for FR. Based on that FA functionalized cytosensors were developed for selective detection of FR-riched cancer cells (Li et al., 2012; Weng et al., 2011).

The investigation on electrochemical cytosensor with specific recognition function is at an early stage and deserves further development. The use of such technology should prevent any possible contamination of the cells and hence decrease the risk of false-positive results (Andreescu and Sadik, 2005). In previous reports, external chemicals were usually introduced into the system to generate signals, which might affect the viability of the immobilized cells. To address the problems, a label-free cytosensor with surface-confined ferrocene as signal indicator was developed to selectively detect human cervical carcinoma (HeLa) cells without external chemicals effect. In our investigations, poly(ethyly imine) functionalized with ferrocene (Fc-PEI) was used as the signal indicator and assembled on the electrode surface with LBL self-assembly technology, which provided an amplified signal to improve the detection sensitivity. FA was immobilized on the electrode surface as the outmost layer to selectively recognize FR-riched HeLa cells. The as-prepared cytosensors presented good biocompatibility and exhibited high sensitivity and selectivity for the detection of HeLa cells. Such sensing strategies provide a new way for importing LBL self-assembly techniques into the non-invasion cytosensing systems.

2. Material and methods

2.1. Chemicals and materials

N-(3-Dimethylaminopropy)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), poly(ethylene imine) (PEI) and folic acid (FA) were purchased from Aldrich. 3-Mercaptopropionic acid (MPA) was purchased from Alfa Aesar company. Ferrocenecarboxaldehyde (98%) was purchased from J&K Chemical Ltd. Single-wall carbon nanotubes (SWNTs) were obtained from Shenzhen Nanotech Port Co. Ltd. The SWNTs were purified with 2.6 M HNO_3 at 120 °C for 24 h and then treated with a 1:3 v/v mixture of HNO_3 (65%) and H_2SO_4 (98%) at room temperature for 8 h with continuous ultrasonication for imparting carboxyl groups onto the surface of SWNTs. Bovine serum albumin (BSA) was bought from Sigma (Missouri, America). Acridine Orange (AO) was from Dingguo Co. China. The Fc-PEI was synthesized according to the previous procedure (Hodak et al., 1997). 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 cytosensor was fabricated with LBL self-assembly technique as shown in Scheme 1. The gold electrode (0.11 cm^2) was firstly immersed into 0.1 mM MPA in absolute ethanol for 2 h. After being taken out, the electrode was rinsed with large amount of ethanol and Milli-Q water and then alternatively immersed into Fc-PEI (pH=6) and SWNT (0.1 mg/mL) solutions for 30 min. The resultant film was washed thoroughly with Milli-Q water. This process was repeated until the desired layers of $(Fc\text{-}PEI/SWNT)_n$ (n=1, 2, 3, 4, 5) were obtained. The modified electrode was named as Au/MPA/$(Fc\text{-}PEI/SWNT)_n$ (n=1, 2, 3, 4, 5). For selective cell detection, the formed Au/MPA/$(Fc\text{-}PEI/SWNT)_5$ electrode was immersed into the mixture of 10 mM NHS and 40 mM EDC in water for 2 h at room temperature to activate the terminal carboxylate group and rinsed with water. Then the activated electrode was immersed into folic acid (25 mM, pH=8) solution for 30 min and rinsed with water. At last, the obtained electrode was dipped into BSA (1%) solution for 1 h to reduce nonspecific adsorption. The cytosensor, Au/MPA/$(Fc\text{-}PEI/SWNT)_5$/FA, was thus fabricated and used for the cell detection, which was characterized by atomic force microscopy experiments (Fig. S1 Supporting Information).

2.3. Cell culture and 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.