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An electrochemical method to detect folate receptor positive tumor cells

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Abstract

This paper reports an electrochemical method to detect folate receptor positive tumor cells by making use of the interaction between folic acid immobilized on gold nanoparticles and its receptor over-expressed on tumor cell membrane. Experimental results have shown that a gold electrode modified with folic acid functionalized gold nanoparticles can clearly denote folate receptor positive tumor cells, such as ovarian tumor cells and human cervical cancer cells. So, electrochemical technique has been introduced for cancer cells detection and a simple method to detect folate receptor positive tumor cells has been developed.
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Keywords: Electrochemistry; Tumor cell; Gold nanoparticles; Folic acid; Folate receptor

1. Introduction

As is well known, to diagnose cancers in early stage is of great importance. Therefore, more detection methods are required to be developed on the one hand, how to detect tumor cells simply, sensitively and rapidly is more and more important on the other. Tumor-associated antigens are those tumor-specific or quantitatively more abundant materials on tumor cells. They can be used as markers of cancer cell, as well as the target of medicine. Folate receptor (FR) is a kind of tumor-associated antigen, which will be over-expressed in many human tumors, including ovarian carcinomas, choroid plexus carcinomas, and ependymomas [1–3]. The high affinity between FR and folic acid (FA) makes them work as a bridge connecting medicine with cancer cell, and an attractive property for tumor therapy.

Electrochemical method has been known to be simple, rapid, sensitive and convenient. It has been used for the detection of organic species and small medicine molecules for many years. In recent years, due to the rapid development of the functionalization of the electrode surface, electrochemical technique can be also used to detect biological macromolecules, such as proteins and DNA. In this paper, we report that tumor cells can be detected very simply and conveniently through electrochemical method.

2. Experimental

2.1. Chemicals and reagents

Chloroauric acid ($HAuCl_4$) was obtained from Shanghai Jiushan Chemicals Co. Limited. *N*-(3-Dimethylaminopropy)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), FA and cysteamine were purchased from Sigma Corporation. 3-Mercaptopropinic acid (3-MPA) was obtained from Fluka (Germany). Other chemicals were of analytical grade. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >18 MΩ cm, and stored in refrigerator at 4 °C.

2.2. Preparation of FA functionalized gold NPs

Gold NPs were prepared basically as we did before [4]. All the used glasswares in the following procedures were

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cleaned in a bath of freshly prepared 3:1 HNO_3–HCl, rinsed thoroughly in double-distilled water and dried in air. $HAuCl_4$ and trisodium citrate solutions need to be filtered through a 22 mm microporous membrane filter prior to use. Gold NPs were prepared by adding trisodium citrate solution to a boiling $HAuCl_4$ aqueous solution at reflux. The molar ratio of $HAuCl_4/Na_3$ citrate was ca. 0.026. The gold NPs solution was stored in brown glass bottles at 4 °C.

A 5 mL gold NPs solution was deaerated by pure nitrogen (>99%). Then, 15 μL of 0.1 mol L^{-1} 3-MPA solution was added into the above solution. The resulting solution was stirred for 3 h for reaction, and then was dialyzed for another 3 h to remove the extra free small molecules. A 10 μL of EDC solution (0.05 mol L^{-1} in dimethyl sulfoxide (DMSO)) and the same amount of NHS solution (0.05 mol L^{-1} in DMSO) were added into the above dialyzed solution, the mixture of which was stirred for 30 min for reaction and then dialyzed again. After dialysis, FA was added into this solution, which was stirred overnight and then also dialyzed. The FA functionalized gold NPs were thus obtained in this final solution.

2.3. Preparation of the modified electrode

The surface of the substrate gold electrode was first polished with 0.5 μm Al_2O_3 powder, and washed ultrasonically in absolute alcohol and deionized water for 3 min, respectively. Then, the gold electrode was dipped into a 1:1 H_2SO_4–H_2O_2 mixture for 30 s, rinsed with distilled water, and dried. The cleaned substrate gold electrode was immediately immersed in a 50 mM cysteamine solution for 1 h at room temperature, avoiding light. The resulting self-assembled monolayer (SAM) modified electrode was thoroughly rinsed with absolute ethanol and deionized water to remove physically absorbed thiols. Finally, the SAM modified gold electrode was immersed in the above prepared solution containing FA functionalized gold NPs for 7 h for immobilization of gold NPs with the SAM on the electrode surface, followed by thoroughly rinsing with water for 20 s. The FA-gold NPs modified electrode was then prepared.

2.4. Preparation of cell suspensions

The cell lines, human cervical tumor cells (Hela cells), Chinese hamster ovarian cancer cells (named as CHO-1 cells) and Chinese hamster ovarian cells (named as CHO-2 cells), were obtained from Institute of Biochemistry and Cell Biology in Shanghai, Chinese Academy of Sciences. Hela cells, a human cervical cancer cell line, and CHO-1 cells, a Chinese hamster ovarian cancer cell line, are known to overexpress FR. CHO-2 cells are Chinese hamster ovarian cell line, which used as a FR negative control. These three kinds of cells were cultured in 1640 medium supplemented by fetal calf serum and L-glutamine, and then subcultured in flasks at 37 °C in a 5% CO_2 atmosphere. Cells were used during the end of log phase of growth and were digested by 0.25% trypsin to prepare the cell suspensions. The magnitude of cell suspensions used in this work was around 10^5.

2.5. Apparatus

Electrochemical experiments were carried out with a 263A Potentiostat/Galvanostat (EG&G, PARC, USA) and a three-electrode system. The modified electrode was used as the working electrode. A saturated calomel electrode served as the reference electrode, and a platinum wire auxiliary electrode as the counter electrode. M270 electrochemical software was used for collecting and calculating data.

3. Results and discussion

Fig. 1a shows the cyclic voltammograms (CVs) of the very commonly used electrochemical species, $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, obtained at a gold electrode

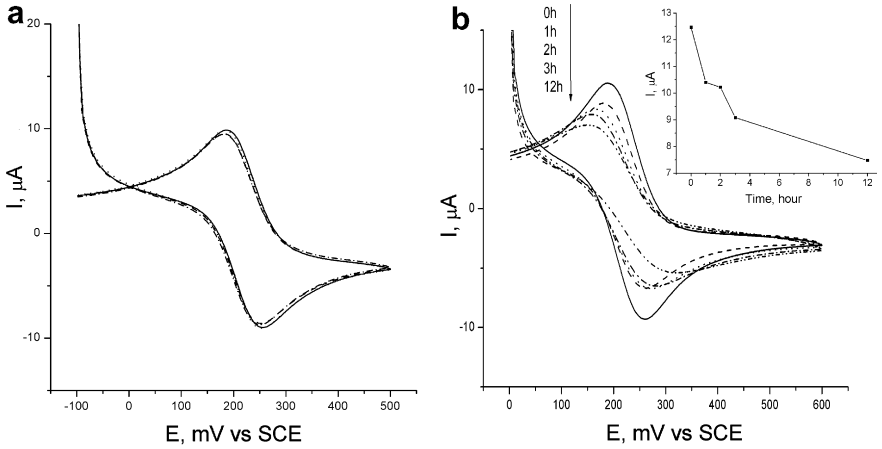

Fig. 1. CVs obtained at a FA-gold NPs modified electrode for 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution before (the solid line) and after (the dash line) the electrode has been previously immersed in 1.3×10^5 (a) CHO-2 cell suspension for 1, 2 and 3 h, or (b) CHO-1 cell suspension for 1, 2, 3 and 12 h. Scan rate: 100 mV s^{-1}. Inset shows the relationship between the anodic peak current and immersing time.

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