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A simple and rapid electrochemical strategy for non-invasive, sensitive and specific detection of cancerous cell

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ABSTRACT

Developing non-invasive, sensitive and specific sensing strategies for cancerous cell detection with simple and low cost instrumentations provide great advantages in cancer research and early diagnosis of diseases. In the present work, gold nanoparticles (Au NPs) functionalized with recognition components (folic acid) and signal indicator (ferrocene) was designed to fabricate electrochemical cytosensor. The Au NPs can not only accelerate electron transfer between signal indicator and the underlying electrode but also accumulate more ferrocene on the cytosensor surface to magnify signal for improving detection sensitivity. The surface-tethered folic acid plays a key role in specific binding folate receptor-riched HeLa cells on the cytosensor surface, resulting in corresponding current signal change measured by differential pulse voltammetry method. A wide detection range from 10 to 10^6 cells/mL with a detection limit as low as 10 cells/mL for cancerous cells was reached in the presence of a large amount of normal ones with fast differential pulse voltammetry measurement. Detection of the captured cells can be finished within 1 min. The developed strategy provides a new way for operationally simple, rapid, sensitive and specific detection of cancerous cells.

1. Introduction

Cancer has become one of the most likely causes of death worldwide. The chance of being cured increases with early diagnosis and treatment of the cancer disease [1]. Most of conventional approaches including fluorescent, radioactive and spectrophotometric procedures need for stringent laboratory conditions and expensive instrumentation and are usually high-cost and time-consuming in preparation steps. Moreover, some approaches may couple with radioactive risk. Therefore, it is highly desirable to develop simple, rapid and non-destructive methods for early detection of cancer with low cost [2]. To meet these specific requirements, electrochemical techniques have attracted increasing attention due to the high sensitivity, rapidity, simple operation, broad dynamic range, minimal power requirement and excellent reproducibility [3–6]. Electrochemical cytosensors are generally fabricated based on measuring the changes in current or resistance at the cytosensor interface that are related to the biological status of the cells, including cellular viability, proliferation, apoptosis and immobilized cell number [3]. Some cytosensors are developed based on electrostatic attraction between the cells and the cytosensor surface, which are used to investigate cell adhesion, proliferation and apoptosis [7–9]. While, the normal cells are also negatively charged, therefore, such cytosensors lack detection specificity and could not distinguish cancerous cells from nonconcerous ones. To meet the requirements of clinical diagnosis and therapy, an increasing interest has focused on "target-binding" technology to develop cytosensors with selectivity [10]. Owing to the highly specific recognition between antibody and antigen, electrochemical immunoassay has been developed to evaluate cell surface carbohydrates and glycoprotein [11,12]. Based on high affinity of folic acid for folate receptor, several electrochemical cytosensors were developed to selectively detect cancerous cells from normal ones [13,14]. To accurately determine cancerous cells at ultralow level for the early stage detection of diseases, various signal amplification approaches have been developed to enhance the electrochemical current signal of recognition events [15–18]. Gold nanoparticles functioned with both

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recognition component and signal amplification component were used to fabricate cytosensor for cytosensing and evaluating cell surface carbohydrate and glycans [19,20]. By binding aptamer on graphene modified electrode surface, Qu's group developed a cytosensor to distinguish cancerous cells from normal ones [21].

From a clinical point of view, one key goal of developing reliable cytosensing technology is to treat sample without introducing of extraneous reagents and reduce time consumption [22]. To decrease the risk of false-positive results, any possible contamination on the cells should be decreased as low as possible. Though the developed electrochemical cytosensors present advantages on cytosensing, these studies mainly suffer from the following drawbacks. (1) Electrochemical impedance spectroscopy (EIS) is a commonly used method to monitor live cells. Recently; however, Cheng's group found that a larger resistance change occurred during EIS measurement [23]. They speculated the possible reason might be detachment of some cells from the cytosensor film due to deterioration of cell vitality under a longer time of electrical field effect or accumulation of external redox probe within the film. Thus a fast response electrochemcial technique, differential pulse voltammetry (DPV), was used as an alternative method. (2) To produce detection signal, enzymatic substrates, for example H_2O_2, or external redox probe, for example $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$, are often required to be added in detection solution. While, it was reported that H_2O_2 might induce cell apoptosis [24]. $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ can be easily metamorphic in biological condition, producing unexpected effects on viability of tissue [25,26]. So the detection results might be influenced by the added substrates in detection solution. It remains a challenge to develop approaches for non-invasive cell detection.

Keeping that in mind, we have developed a novel electrochemical cytosensor which can sensitively differentiate cancerous cells from control ones by making use of the advantages of tumor marker and gold nanoparticles. To avoid the potential contamination, the current signal indicator did not dissolve in detection solution while it was immobilized on the electrode surface and did not directly contact with cancerous cells. With fast response DPV approach, the immobilized cells can be quickly detected within 1 min with simple instrumentation. The rapid detection can further avoid possible contamination and reduce the time of electrical field effect on the cells and thus reduce the loss of cell viability.

2. Experimental

2.1. Materials

1,6-Hexanedithiol and N-hydroxysuccinimide (NHS) were purchased from Acros Organics. 16-Mercaptohexadecanoic acid, 6-(Ferrocenyl) hexanethiol and N-(3-Dimethylaminoprophyl)-N′-ethylcarbodimide hydrochloride (EDC) were purchased from Sigma-Aldrich Company. Folic acid was obtained from Beijing Dingguo Changsheng Biotech Co. Ltd. Chloroauric acid was obtained from Shanghai Chemical Reagent Co. Ltd. Sodium citrate was obtained from Beijing Chemical Works. Physiological saline (0.9%) was used as the buffer solution. The water used was purified through a Millipore system. All chemicals were used as received.

2.2. Preparation and characterization of gold nanoparticles

Au NPs were prepared by a conventional citrate-reduction method [27,28]. After sodium citrate solution (1% by weight, 0.5 ml) was added to a boiling chloroauric acid solution (0.01% by weight, 50 ml), the mixture was kept boiling and stirring for another 20 min. After being cooled to room temperature, the resultant Au NPs solution was stored at 4 °C.

The gold hydrosol was characterized from 350 to 800 nm by a VARIAN-50 Conc UV–Visible spectrophotometer (Fig. S1 in Supporting Information (SI)) and Transmission electron microscopy (Fig. S2 in SI). The diameter was evaluated as 15 nm.

2.3. Fabrication of the sensing interface

A fresh gold bead electrode (0.11 cm^2) was firstly immersed into 1 mM 1,6-Hexanedithiol ethanol solution (1.0 mM) for 2 h. After being taken out, the electrode was rinsed with large amount of ethanol and Milli-Q water and then immersed into the Au NPs solution for 1 h. The resultant modified electrode was washed thoroughly with Milli-Q water and then immersed in a mixture solution of 16-mercaptohexadecanoic acid (1 mM) and 6-(ferrocenyl) hexanethiol (1 mM) with volume ratio of 1:5 for 8 h. To active the carboxylate terminal group of 16-mercaptohexadecanoic acid the obtained electrode was then immersed in the mixture of EDC (75 mM)/ NHS (15 mM) for 1 h. At last, the activated modified electrode was immersed into folic acid solution (0.5 M) for 2 h. Thus a cytosensor was prepared for selective detection of cancerous cells.

2.4. Cell culture and cell immobilization

The cell lines, HeLa cells (human cervical carcinoma cell) and normal cells (HEK 293), were obtained from the Kunming Institute of Zoology, Chinese Academy of Sciences. The cell lines were cultured cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin (100 mg mL^{-1}) in a 5% CO_2 humidified chamber at 37 °C. After the concentration of cells reached about 1×10^6 cells mL^{-1}, HeLa and HEK 293 cells were trypsinized in the presence of 0.25% trypsin solution and collected from the medium by centrifugation at 3000 rpm for 5 min. Then the collected cells were washed twice with a sterile PBS (pH 7.2). The cell sediment was re-suspended in the physiological saline (0.9% NaCl) solution to obtain a homogeneous cell suspension. Then the as-prepared cytosensor was incubated in the cell suspensions at different concentrations at 37 °C for 10 min.

2.5. Electrochemical measurements

Electrochemical measurements were conducted with a conventional three-electrode system by CHI 832B electrochemistry workstation (Co. Chenhua, China). Platinum foil and Ag/AgCl electrode worked as counter and reference electrode, respectively. $NaClO_4$ solution (0.1 M) was used as supporting electrolyte.

3. Results and discussion

3.1. Fabrication and characterization of the cytosensor

The excellent biocompatibility and the ability to facilitate electron transfer enable gold nanoparticles (Au NPs) to be a promising platform for construction of biosensors and improving the sensing performance [29]. As shown in Scheme 1, the electrochemical cytosensor was prepared by first assembling dithiol molecules, 1,6-hexanedithiol, on a gold electrode surface to produce a monolayer with –SH as terminal group. Thus, Au NPs can be easily assembled on the 1,6-hexanedithiol layer through Au-S bond. After that, the Au NPs was functionalized with a mixed monolayer of 16-mercaptohexadecanoic acid and 6-(ferrocenyl) hexanethiol. The 16-mercaptohexadecanoic acid was further used to covalently bond folic acid onto the electrode surface to