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Detection and distinguishability of leukemia cancer cells based on Au nanoparticles modified electrodes

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ABSTRACT

The Au nanoparticles (Au NPs) modified interface has been fabricated by multi-potential step electrodeposition in this study. Based on the nano-Au interface, we have proposed an electrochemical approach to detect the cancer cell numbers sensitively with a detection limit of about 500 cells. More interestingly, the drug sensitive leukemia K562 cells and drug resistant leukemia K562/adriamycin could be electrochemically distinguished on the interface by the oxidation potential, which did not show any evident differences on the bare electrode. These results indicate the promising application of this nano-interface for constructing the unlabeled potential-discriminative cell biosensors.

1. Introduction

The identification and detection of pathogens are often painstaking in the clinic due to the low abundance of diseased cells in sputa, blood, and other clinical samples [1]. Diagnosis in combination with nanotechnology could overcome the difficulty [2], and it may offer a method to realize the high sensitive identification and detection of the related disease biomarker and some important biological process [3–9]. Especially, the well-known biocompatible Au NPs have been extensively applied in the biological assays [10,11]. There are many methods such as self-assemble, covalent bond modified and electrochemical converge to form Au NPs modified electrode to make high sensitive biosensors [12–15], but all these take a long time. In this study, the Au NPs modified interface fabricated by us only needs less than 12 min to make it, which could be further adopted to detect the different cancer cells.

In this contribution, we mainly focused on the study of the Au NPs modified glassy carbon electrodes (GCE), which has been utilized to distinguish the different leukemia cancer cells. The Au NPs with a series of sizes could be deposited on the GCE by varying the ratio of the reagents and the depositing time. The increase of the cysteine concentration could cause a significant enlargement of the Au particles [16]. The electrochemical behaviors of the probe have been detected on the Au NPs modified GCE, where the change of the respective electrochemical signal for the different cancer cells is able to identify the target cells. These observations indicate that the functionalized Au NPs modified electrodes could provide a new strategy for the rapid identification of the carcinoma cells.

2. Experimental

2.1. Chemicals

Hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$) was purchased from Sino-reagent Company (Shanghai, China). Cysteine, ferricyanide and all other reagents were of analytical grade and purchased from Sigma–Aldrich Corp. (St. Louis, USA). Phosphate buffer saline (PBS, 0.1 M, pH 7.2) was prepared with double distilled water.

2.2. Cell culture

The leukemia K562 cell lines were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 mU mL^{-1}), and streptomycin (100 mU mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. Additionally, the K562 cells (KA) were maintained with 1 μg mL^{-1} adriamycin (Sigma). All aqueous solutions of drugs and target cells were freshly prepared at ambient temperature (20 ± 2 °C).

2.3. Apparatus

The cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectrum (EIS) were performed on a CHI 660b electrochemical workstation. All

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measurements were carried out at 20 ± 2 °C in a three-component electrochemical cell consisting of a GCE as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the counter electrode. Scanning electron microscopy (SEM) was performed by using S-3000N scanning electron microscope (HITACHI).

2.4. Preparation of Au NPs modified electrode

The fabrication of the electrode and the cell coating processes were as follows: Initially, the GCE was polished to a mirror-like plane, then the Au NPs were deposited onto the carbon substrates by the multi-potential step technique. The depositing media were 0.5 M H_2SO_4 aqueous solution, containing 2.0 mM $HAuCl_4$ precursor and 0, 25, 50, 75 or 100 μM cysteine, respectively. In this deposition process, the cysteine was added to the media to control the size of Au NPs and the potentials from 0 to 0.45 V (*vs* SCE) were applied to the system in turn. The running time and the period were optimized to obtain the best conditions. After this process, the surfaces of the electrode were carefully washed. Finally, different kinds of 10 μL cells were dropped onto the modified electrode, and air-dried in the ambient environment for 2 h. The cells covered electrodes were used in the subsequent electrochemical experiments.

3. Results and discussion

3.1. Characterization and optimization of Au NPs modified electrode

The coatings of the Au NPs were performed according to the literature [16] with some modifications. In the multi-potential step deposition process, the applied potential was the key parameter. In the electrolysis process, the potential under the reduction potential of the $AuCl_4^-$ could lead to the deposition of Au on the electrode. Here the standard potential of the following equation was about 0.76 V (*vs* SCE).

$$AuCl_4^- + 3e \rightarrow Au + 4Cl^-$$

Actually, for the CV curve of 2.0 mM $AuCl_4^-$ in the acidic media, the cathodic current increased obviously below 0.6 V and reached a peak at 0.45 V. Thus, the applied potential of 0.45 V (*vs* SCE) was selected in the following experiments.

In order to improve the sensitivity of the electrodes, the factors for the electrolysis should be optimized. In this research, the DPV was explored for the Au NPs modified GCEs to evaluate the performances of the relevant electrodes. During the deposition process, the bare GCEs were electrodeposited in the media containing the series concentrations of cysteine for 700 s. It has been observed that the increase of the cysteine concentration caused a significant enlargement in the size of the Au NPs, probably due to the self-assembly of cysteine on the surface of the Au NPs, which then affected the growth of their lattices [16]. Fig. 1A shows the DPV study of 10 mM $[Fe(CN)_6]^{3-/4-}$ on these modified electrodes. It could be found that the concentration of cysteine significantly affected the peak current which increased with it and then decreased. When the concentration of cysteine was 25 μM, the Au NPs on the electrode displayed the best electrochemical behavior, which was about 81% higher than that of the bare electrode. This concentration was changeless in the following optimization.

The running time for the deposition process was considered. This parameter was chosen from 300 s to 800 s with a fixed period of 1 s. From the DPV plot shown in Fig. 1B, the peak current of $[Fe(CN)_6]^{3-/4-}$ increased with the running time. However, when the time surpassed 700 s, the current decreased. So 700 s was the best electrodeposition time. Meanwhile, the relevant scanning electron microscopic (SEM) images (see Fig. 2) were explored to show the sizes of the NPs deposited on the electrodes. The mean size of the Au NP was about 125 ± 10 nm for the running time of 700 s and about 250 ± 10 nm for 300 s. Obviously, the size was smaller for the longer running time. The results were coincident with that reported for hydroxyapatite crystal [17].

Finally, on the basis of the above studies, the best conditions for electrodeposition were 25 μM cysteine as additives, the running time of 700 s and the cyclic period of 1 s.

3.2. Electrochemical behaviors of cancer cells on Au NPs modified GCE

Based on the Au NPs modified surface, we have investigated the feasibility for monitoring the cell adhesive behaviors. As shown in Fig. 3, a serial concentration of the K562 cells was placed on the surface of the electrode and dried. The EIS technique was used to detect the changes in the electronic parameters. The EIS values of ferricyanide enlarged with the increase of the concentration of the cells (Fig. 3), indicating that the cell adhesion could significantly block the electron transfer of the electrochemical probe. The charge transfer resistance, R_{ct} value, increased obviously from 12.1 Ω to 135 Ω with the increase in the amount of cells due to the electronic exchange blockade. While the R_{ct} value of the Au/GCE

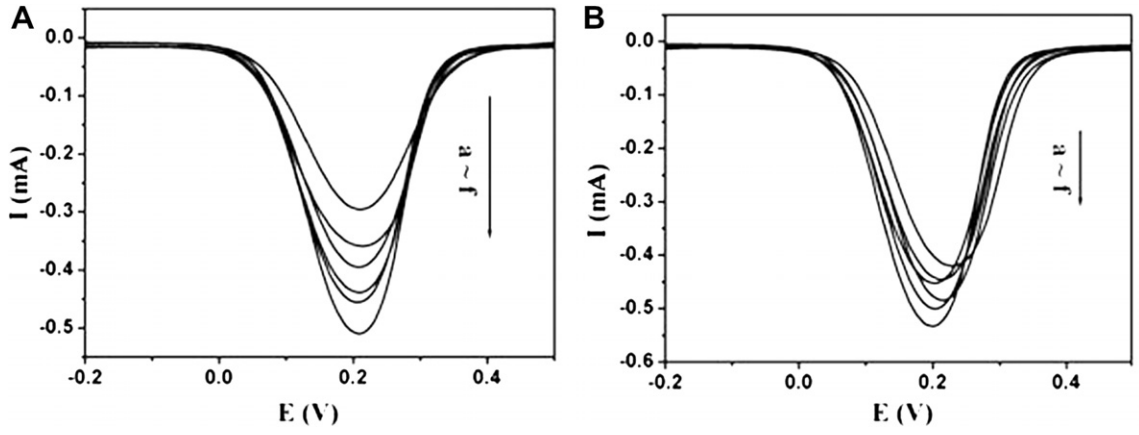

Fig. 1. A. DPV behaviors of 10 mM $[Fe(CN)_6]^{3-/4-}$ (0.1 M KCl) on the bare GCE (a) and Au NPs modified GCE (b~f). The depositing media are 0.5 M H_2SO_4, 2.0 mM $HAuCl_4$ and 100 μM (b), 0 μM (c), 75 μM (d), 50 μM (e), or 25 μM (f) cysteine. B. DPV behaviors of 10 mM $[Fe(CN)_6]^{3-/4-}$ (0.1 M KCl) on the Au NPs modified electrodes with a series of deposition time: 300 s (a), 400 s (b), 800 s (c), 500 s (d), 600 s (e), 700 s (f).