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Immobilization and electrochemical behavior of gold nanoparticles modified leukemia K562 cells and application in drug sensitivity test

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Abstract

A new strategy for immobilization of tumor cells on electrode surface and accelerating electron transfer between electrode and the immobilized cells was proposed to study the electrochemical behavior of cells and the effect of antitumor drug on cell viability. The leukemia K562 cells immobilized in a microporous cellulose membrane were firstly modified with colloidal gold nanoparticles to retain efficiently the activity of immobilized living tumor cells and promote electron transfer between electroactive centers of the cells and the electrode, exhibiting a well-defined anodic peak of guanine at +0.830 V at 50 mV s^{-1}. The electrochemical response could be used to describe cell growth and evaluate the effectiveness of antitumor drug methotrexate on tumor cells. The proposed method offered potential advantages for drug sensitivity test with little usage of cells. It could be developed as a convenient means for the study of the tumor cells growth and the cytotoxicity of antitumor drugs.

Keywords: Biosensors; Drug sensitivity test; Electron transfer; Leukemia K562 cells; Colloidal gold nanoparticles; Cellulose acetate membrane

1. Introduction

A series of very important biochemical and physiological processes in a living cell involve electron generation and transfer. That is to say, the activity of life may have very intimate relation with the electron behavior in living cells. Therefore, numerous studies have focused on electric behavior and characters of living cells using electrochemical methods. For example, cyclic voltammetric behavior of cells can be used for probing intracellular redox activity of initiation of apoptosis [1] and investing the sensitivity of tumor cells to antitumor drugs [2]; amperometric method can be applied for real-time monitoring of intracellular redox enzyme activity with help of electron transfer mediators [3]; outer cell potential measurement is applicable to noninvasive extracellular measurement of the electrical activity in insect cells growth [4]; electrochemical impedance methods have been utilized for rapid detection of *Escherichia coli* O157:H7 [5] and the study of the attachment and spreading of fibroblast cells [6]; and scanning electrochemical microscope has been used to probe the redox activity of individual purple bacteria [7] and detect the nitric oxide release from human umbilical vein endothelial cells with a NO-sensors [8]. This work proposed a novel immobilization method of cells for studying electrochemically the voltammetric behavior of leukemia K562 cells and the action of antitumor drug on cell growth.

The rapidly increasing knowledge of the mechanism of cell–cell interaction and cell–substrate interaction provides a strong driving force for advances in biotechnology and biomaterials [9,10]. In particular, microporous cellulose

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carriers with a large specific surface area and good biocompatibility have suitable characteristics for the immobilization of animal cells [11,12], which have also been applied for the immobilization of biomolecules [13] and bacteria [14]. Here, a cellulose acetate (CA) membrane was used to immobilize leukemia K562 cells on a glassy carbon electrode. The membrane could retain efficiently the activity of living tumor cells and prevent cell leakage from electrode interface. In order to study the electrochemical behavior of the immobilized cells the colloidal gold nanoparticles were firstly used to modify the cells, which promoted the electron transfer between electroactive centers of the cells and the electrode.

Colloidal gold nanoparticles can provide an environment similar to native system of the immobilized biomolecules to retain efficiently their biological activity and facilitates the electron transfer between these molecules and electrodes. Recently, this matrix has been used as a mimetic interface for cell proliferation and assembly of human hepatocytes to construct a cellular biosensor [15]. In this work the colloidal gold nanoparticles modified leukemia K562 cells, which were immobilized in the cellulose acetate membrane, showed a well-defined anodic peak of guanine [2], producing a cellular biosensor for cytotoxicity study of antitumor drugs or other exogenous factors. The action of an antitumor drug, methotrexate, to the leukemia K562 cells was examined to verify the potential application of the immobilization method. This proposed method combined the advantages of electrochemical and immobilization techniques with the advantageous features of colloidal gold nanoparticles and microporous CA membrane, and could be conveniently used for accurate monitoring of the viability of living tumor cells and the effectiveness of antitumor drugs on the tumor cells. In comparison with other methods for drug sensitivity test this process needed less amount of cells for the same purpose.

2. Material and methods

2.1. Chemicals and reagents

Twentyfour-nanometer-diameter colloidal gold nanoparticles solution was prepared according to literature [16] and stored in a brown glass bottle at 4 °C. Cellulose acetate (CA, approximately 40% acetate) was obtained from Shanghai Biochemical Reagent (China). 3-(4,5-dimethylthizade-2,5-diphenyltetrazolium bromide (MMT) was purchased from Sigma (St. Louis, MO). Methotrexate (Jiangsu Hengrui Pharmaceutical Limited Co., China), penicillin (Huabei Pharmaceutical Limited Co., China) and streptomycin (Shangdong Lukang Pharmaceutical Limited Co., China) were used without further purification. Phosphate buffer solution (PBS, pH 7.4) contained NaCl (137 mM), KCl (2.7 mM), $NaHPO_4$ (87 mM) and KH_2PO_4 (14 mM). All other reagents were of analytical grade. Doubly distilled water was used for all experiments.

2.2. Cell culture and collection

Human leukemia K562 cells were obtained from Jiangsu Institute of Cancer Prevention and Cure. The cells were cultured in RPMI 1640 medium (Gibco) supplemented with fetal calf serum (15%), penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) in an incubator (5% CO_2, 37 °C). For studying the cytotoxic effect of antitumor drug on cell viability, methotrexate was added into other sets of cell culture medium. The same culture conditions were provided for both the control and experimental groups. The cells were counted with Petroff–Hausser counter (USA). After cultured for 72 h, the obtained leukemia K562 cells were separated from the culture medium by centrifugation at 1000*g* for 10 min, and then washed twice with a sterile pH 7.4 PBS. The sediments were suspended in PBS to obtain 250 μL cell suspensions for electrochemical measurements and immobilization.

2.3. Electrode preparation and cell immobilization

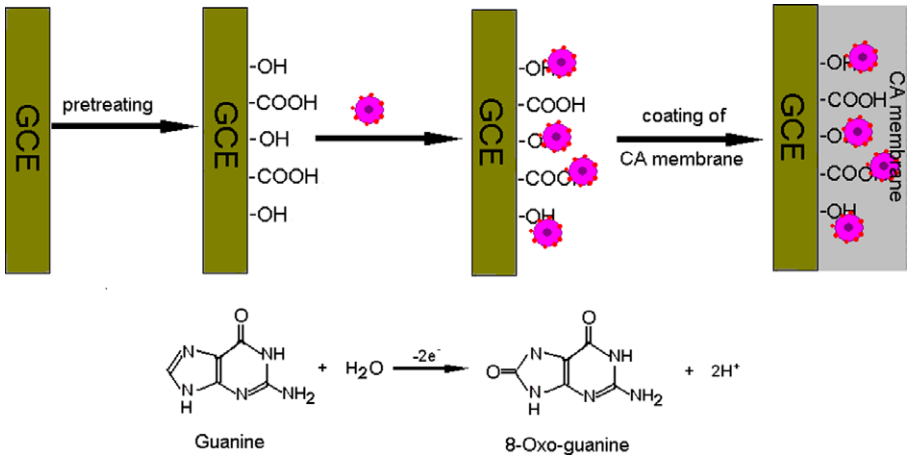
Prior to modification, a glassy carbon electrode (GCE, 3 mm in diameter) was polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively, followed by rinsing thoroughly with doubly distilled water. Then, it was pretreated electrochemically by applying a potential of +1.75 V in 0.1 M pH 5.0 PBS for 300 s, and scanned between +0.3 and +1.25 V and then +0.3 and −1.3 V until a steady-state current–voltage curve was observed. This process led to the formation of –OH and –COOH groups on the GCE surface and increased surface hydrophilicity of the GCE, which made it easy for cell adhesion [17].

About 1% CA solution was prepared by dissolving 1.0 g CA in a mixture of 50 ml acetone and 50 ml of cyclohexanone. After 10 μL leukemia K562 cell suspension and 10 μL colloidal gold solution were mixed thoroughly, the obtained mixture was dropped on the pretreated GCE and dried in a silica gel desiccator. Five microlitre 1% CA solution was then dropped on the electrode surface to form a CA membrane for cell immobilization. The preparation process could be described in Scheme 1. After rinsing with a sterile pH 7.4 PBS, the obtained biosensor (CA/K562 leukemia cells-Au/GCE) was stored in pH 7.0 PBS at 4 °C.

2.4. Electrochemical measurements

Electrochemical experiments were performed on a CHI 730 electrochemical analyzer (USA) with a conventional three-electrode system comprising a platinum wire as auxiliary, saturated calomel electrode as reference and different modified GCEs as working electrodes. The cytotoxicity of drug was calculated as follows:

$$\text{Cytotoxicity } (\%) = 100\% \times (I_{\text{pa, control}} - I_{\text{pa, exp}})/I_{\text{pa, control}} \quad (1)$$



Scheme 1. Preparation process of cellular biosensor (CA/leukemia K562 cells-Au/GCE) and the oxidization of guanine. is colloidal gold nanoparticles modified leukemia K562 cell.

where $I_{pa,\ control}$ and $I_{pa,\ exp}$ are the anodic peak currents of the immobilized cells without and with antitumor drug treatment.

2.5. MTT assay

1×10^5 leukemia K562 cells in 200 μL medium alone or medium containing an antitumor drug at various concentrations were added to each well of 96-well plate. The plate was incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 3 days. MTT (20 μL, 5 mg mL^{-1}) was then added to each well. After the plate was further incubated for 4 hours, sodium dodecyl sulfate (150 μL, 0.520 M) was added to each well to solubilize formazan dye. After 1 hour the incubation absorbance in control and drug treated wells was measured on a Victor2 1420 Multilable Counter (Wallac, Finland) at 490 nm. The cytotoxicity of the antitumor drug was calculated as follows:

$$\text{Cytotoxicity}\ (\%) = 100\% \times (1 - \text{absorbance of drug treated well}/\text{absorbance of control well}) \quad (2)$$

3. Results and discussion

3.1. Voltammetric behavior of immobilized leukemia K562 cells

During the mixing process the colloidal gold nanoparticles could adsorbed on leukemia K562 cells through the weak interaction between the nanoparticles and mercapto or primary amine groups on cell membrane to obtain gold nanoparticles modified leukemia K562 cells. CA membrane could efficiently prevented cell leakage from the electrode interface, as shown in Scheme 1. The immobilized gold nanoparticles modified cells showed a well-defined anodic peak at +0.830 V at 50 mV s^{-1} (Fig. 1), while the CA modified GCE and the CA and colloidal gold nanoparticles CA co-modified GCE showed acceptable background currents in pH 7.4 PBS and no redox peak was observed (curve a in Fig. 1). The leukemia K562 cells immobilized in the CA membrane did not also show any detectable voltammetric response in the potential range from 0 to +1.0 V (curve b in Fig. 1). Obviously, the presence of colloidal gold nanoparticles on the cell surface promoted the electron transfer between electroactive centers of the cells and the electrode and reduced the overpotential for the oxidation of the electroactive centers.

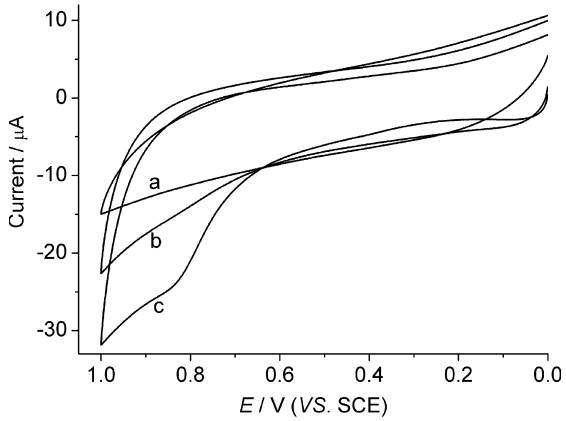


Fig. 1. Cyclic voltammograms of (a) CA/Au/GCE, (b) CA/leukemia K562 cells/GCE, and (c) CA/leukemia K562 cells-Au/GCE in pH 7.4 PBS at 50 mV s^{-1}. Leukemia K562 cells were firstly cultured for 72 h.

Our previous works demonstrated that the electroactive centers of the cells was the guanine presented in the cytoplasm of the living cells, and the conversion of guanine to 8-oxo-guanine [18] (Scheme 1) led to the irreversible electrochemical response of K562 tumor cells [2,19]. During the electrochemical process, the guanine molecules within the cytoplasm of the living cells were able to cross the cell membrane rapidly. Colloidal gold nanoparticles immobilized on leukemia K562 tumor cells facilitated the electron transfer between guanine and the electrode through the conducting tunnels [20], thus enhancing the

electrochemical response of the cells. In additional, the colloidal gold nanoparticles were efficient for preserving the activity of immobilized cells [19] and amplifying the electrochemical signal, which was suitable for studying the viability of tumor cells and the effect of antitumor drug on tumor cells.

The peak current dropped dramatically after several cyclic voltammetric scans (not shown), and no corresponding reduction peak appeared in the inverse scan, which was characteristic of an irreversible electrode process. Considering the irreversibility of the electrode process of K562 cells and the decrease of peak current upon continuous cyclic sweep, the peak current on the first scan could be used for the electrochemical study of tumor cells and the voltammetric measurement of the cytotoxicity of antitumor drugs. This response was reproducible for similar cell coatings. For example, the variation coefficient of the peak currents on the first scan of five coatings of the same amounts of K562 cells cultured in absence or presence of 15 μg mL^{-1} methotrexate for 72 h was 5.3% and 6.7%, respectively.

3.2. Effect of the amount of colloidal gold nanoparticles on electrochemical response

The anodic peak current of the immobilized leukemia K562 cells depended on the amount of colloidal gold nanoparticles used for cell modification. The modification led to the voltammetric response of the immobilized leukemia K562 cells. With the increasing volume of colloidal gold solution the peak current increased gradually and reached a maximum value at the volume of 10 μL colloidal gold solution (Fig. 2). Further increase of the volume led to decrease of the response, which was attributed to the increase of resistance and double layer capacitance of the modified electrode. Therefore, 10 μL colloidal gold solution was selected for modifying 10 μL leukemia K562 cells to construct the cellular biosensor for cytotoxicity study of antitumor drugs and other exogenous factors.

3.3. Voltammetric behavior of leukemia K562 cells in the whole culture process

The immobilization method could be used to monitor the growth of leukemia K562 cells. With the same volume used for immobilization step the anodic peak current depended on the concentration of tumor cells in the suspension and the viability of the cells, which were decided by the culture time and components of the culture medium. The dependence of the anodic peak current on culture time without presence of any exogenous factor was shown in Fig. 3. The curve was found to be similar to the curve of the cells, which has lag growth period, exponential growth period, stationary growth period and dead period. When the tumor cells were cultured for less than 10 h, the peak current was very small. After a culture time of 10 h the peak current increased gradually with the increasing culture time. In the period of 20–34 h the response increased quickly (an early exponential growth period). From about 34–70 h, the peak current increased slowly again (an exponential growth period and a stationary growth period) and reached the maximum. Then the peak current began to drop down. After a culture time of 94 h (a dead period), the peak current decreased greatly.

The electron generation and transfer system in the living cells, such as the oxidation of guanine in the cytoplasm, depends on the surface charge established on the cell membrane system [21]. During the whole culture process, the state of the cell membrane and the surface charge were changed and related to the culture conditions at the same culture time, which could interpret the change of the peak current of the immobilized leukemia K562 cells. These results showed that this method could be used to monitor cell growth in cell culture process and describe cell growth curve, which was parallel to that described by cell counting.

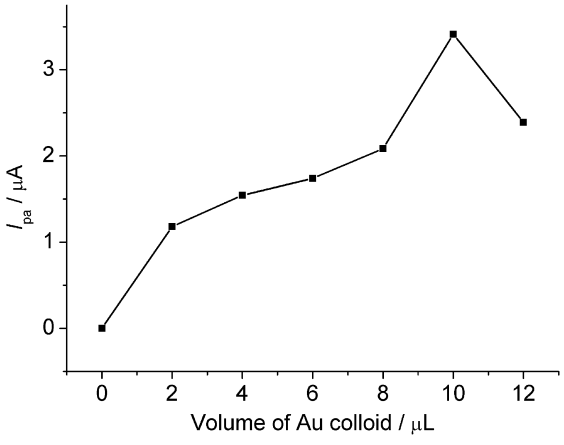


Fig. 2. Dependence of the peak current of colloidal gold nanoparticles modified leukemia K562 cells (10 μL) immobilized on GCE surface in pH 7.4 PBS at 50 mV s^{-1} on the volume of colloidal gold solution used for modification.

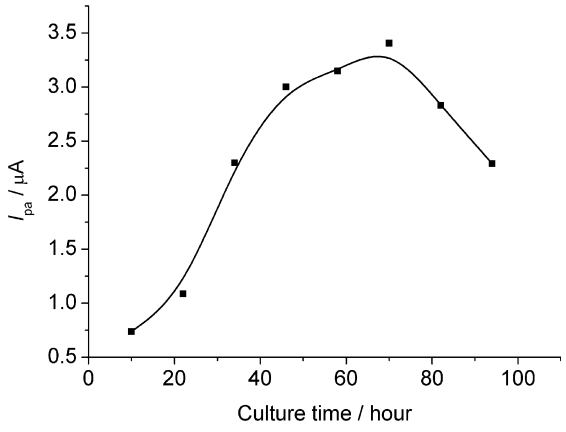


Fig. 3. Dependence of the peak current of colloidal gold nanoparticles modified leukemia K562 cells immobilized on GCE surface in pH 7.4 PBS at 50 mV s^{-1} on the culture time of K562 cells.

3.4. Effect of methotrexate on the viability of leukemia K562 cells

The injection of a clinical antitumor drug methotrexate was selected to evaluate the effect of antitumor drug on the viability of leukemia K562 cells. Methotrexate is one of the antimetabolites, which affects the viability and growth of tumor cells by disturbing DNA synthesis and protein synthesis in cells [22]. Inset in Fig. 4 shows a notable change in the voltammetric signal of the immobilized gold nanoparticles modified leukemia K562 cells obtained after cultured in absence and presence of 15 μg mL^{-1} methotrexate for 72 h. The presence of methotrexate caused a decrease in the peak current recorded for the immobilized leukemia K562 cells, indicating a reduction in the viability and concentration of these cells in the suspension. Methotrexate could restrict the growth of leukemia K562 cells and decrease the expression of guanine in the cytoplasm. Therefore, the change in the peak current of the immobilized gold nanoparticles modified tumor cells could be used for evaluating the effectiveness of antitumor drug on tumor cells.

The effect of methotrexate concentration on the peak current was examined by use of the proposed method. Curve a in Fig. 4 shows the obtained cytotoxicity curve for the 72 h exposure of the leukemia K562 cells in culture medium to methotrexate. When the concentration of methotrexate in the culture medium increased, the cytotoxicity increased. The result was in accordance with that obtained by using the conventional in vitro MTT drug sensitivity test (curve b in Fig. 4). The slight difference between two methods might be attributed to the different culture conditions and assay procedures used. Thus, the proposed electrochemical method could be supported as a credible means for studying the cytotoxicity of antitumor drugs.

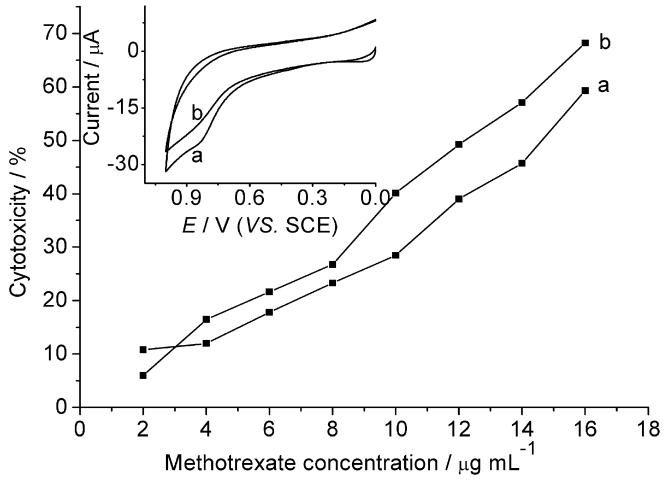


Fig. 4. Cytotoxicity curves for the 72 h exposure of the leukemia K562 cells to methotrexate obtained with (a) the proposed electrochemical method and (b) MTT assay. Inset: cyclic voltammograms of the gold nanoparticles modified leukemia K562 cells in pH 7.4 PBS at 50 mV s^{-1}. The cells were cultured for 72 h in (a) absence and (b) presence of 15 μg mL^{-1} methotrexate.

Although MTT sensitivity test is a conventional assay for evaluating the cytotoxicity, low sensitivity and complex test procedures restrict its development [23]. In comparison, this proposed method had good accuracy attributing to the electrochemical technology and the signal amplification of colloidal gold nanoparticles. In additional, this method of immobilizing tumor cells decreased the usage of cells and made the test procedure simpler in comparison with that of MTT sensitivity test.

4. Conclusions

This work constructs a mimetic interface and develops a novel method for immobilization of tumor cells. Colloidal gold nanoparticles with excellent biocompatibility can be used to modify leukemia K562 cells, which efficiently preserve the activity of tumor cells, accelerate the electron transfer between electrode and the immobilized cells and amplify the electrochemical signal of cells. Microporous cellulose acetate membrane with a large specific surface area can be employed for immobilizing the K562 cells, which prevents cell leakage from electrode interface. The immobilized colloidal gold nanoparticles modified K562 cells exhibit an irreversible voltammetric response relative to the oxidation of guanine in pH 7.4 PBS. After leukemia K562 cells are exposed to an antitumor drug methotrexate, the response of the immobilized K562 cells shows obvious change, which can be used for monitoring the growth of tumor cells and evaluating the cytotoxicity of antitumor drugs. This proposed method has more advantages than conventional MTT sensitivity test, demonstrating the potential to study the antitumor drug sensitivity of tumor cells and the cytotoxicity of other exogenous factors.

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