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Selective determination of drug resistant cancer cells on indium tin oxide electrode modified with nano titanium dioxide

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ABSTRACT

A new electrochemical cell sensor, with low cost, simple fabrication, high selectivity and sensitivity was developed in this study. Titanium dioxide nanoparticles (nano-TiO_2) were assembled on the disposable indium tin oxide (ITO) electrodes for the immobilization of the drug sensitive leukemia K562/B.W. cells and drug resistant leukemia K562/ADM cells to fabricate the relative cell sensors. The different electrochemical behaviors of the probe allowed us to differentiate one type of leukemia cells from another. Furthermore, the results of electrochemical impedance spectroscopy indicated that the detection limit of the new cell sensor is 1.3×10^3 cells ml^{-1} with a linear range of 1.6×10^4 to 1.0×10^7 cells ml^{-1}. These results suggested the promising application of this nano-TiO_2 interface to construct the non-labeling potential-discriminative cell biosensors for clinical uses.

1. Introduction

Malignant tumor, also called cancer, has become one of the most frequently-occurring diseases currently. Although there are many other tumor therapeutics, chemotherapy is still the mainstream to a large extent at present. However, the multidrug resistance is a major obstacle in the process of cancer chemotherapy [1]. In order to make accurate judgment to the patient's condition after chemotherapy and work out an individual treatment ulteriorly, it is necessary to find an effective test method to distinguish the drug sensitive cancer cells from the drug resistant ones and determine them with high sensitivity. Numerous recent reports [2–8] suggested that electrochemical cell sensor in combination with nanotechnology could take bright future for this issue.

In the fabrication of cell sensors, the selection of the biomimetic interface has attracted much attention recently [9]. Considering the significant affection of the support surface properties, such as surface topography, roughness, hydrophobicity and specific protein or cell surface interactions [10], several kinds of biomaterials were conventional to be adopted, including nanocomposites [2–4], gold nanoparticle [5–8], and sol-gel silicon dioxide [11]. Nano-TiO_2 bears many admirable properties such as biocompatibility, environmentally benign, porous structure and nontoxicity, which make it be wildly investigated for various applications in photocatalysis [12], biomedical fields [13], and electrochemical sensors [14,15]. Due to the prominent electrochemical and physical characteristics, as well as low cost, the ITO electrodes were accustomed to be used as the substrate for fabricating the disposable biosensors [16,17].

This work proposes a new approach for the cell distinguishability and cancer early diagnosis. In this contribution, K562/B.W. and K562/ADM cells were immobilized on the same piece of nano-TiO_2 modified ITO (nano-TiO_2/ITO) electrode for the preparation of the novel cell sensors, respectively. The scanning electron microscopy and contact angle measurement suggested that the nano-TiO_2 interface was much rougher and hydrophilic making for cells adhesion, while the voltammetry manifested that it could efficiently promote the electron transferability under optimal conditions. Meanwhile, the two types of target cells, i.e., drug sensitive leukemia K562/B.W. cells and drug resistant leukemia K562/ADM cells, could be discriminated one from the other and the enumeration of cells could also be determined individually with high sensitivity. This work suggests a new strategy for the realization of early diagnosis of cancer.

2. Experimental

2.1. Materials and reagents

TiO_2 nanoparticles (P25) were purchased from Degussa Co. Ltd. (Germany). ITO conductive glass (square resistance $\leq 40\ \Omega/cm^2$) was kindly provided by Kangdake Applied Film Center (Jintan, China) and was cut into stripes of 3 cm × 0.5 cm, setting a fixed area of 0.25 cm^2 (0.5 cm × 0.5 cm) as the conducting surface of the electrode. Cetyl

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trimethyl ammonium bromide (CTAB) was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Both the CTAB solution (2.0 mM) and phosphate buffer solution (PBS, 0.02 M, pH 7.2) were prepared with ultrapure water. All other reagents were of analytical grade.

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 drug resistant leukemia K562/ADM cells 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. Preparation of nano-TiO_2/ITO electrodes and cell sensors

Nano-TiO_2 were ultrasonically dispersed in 2.0 mM CTAB solution for 20 min to obtain an ivory-white TiO_2 colloidal sol, with a final concentration of 0.33 mg ml^{-1}. The ITO electrodes were ultrasonically cleaned sequentially with acetone, absolute ethanol, and doubly ultrapure water for 5 min, respectively, and dried with nitrogen gas. Then a certain volume of TiO_2 colloidal sol was cast on the surface of ITO electrode to form the even nano-TiO_2 film and dried in the silica gel desiccator. Afterwards, different types of leukemia cells were collected and separated from the culture medium by centrifugation at 1200 rpm for 5 min, followed by washing twice with PBS and suspending in PBS to obtain a certain concentrations of cell suspension. Finally, 10 μL of different cell suspension was dropped onto the modified electrode and placed in the incubator at 37 °C for 2 h.

2.4. Apparatus

The cyclic voltammetry (CV) and the differential pulse voltammetry (DPV) were performed on a CHI660B electrochemical workstation (CH Incorporation, USA), using a Pt wire as the counter electrode and an Ag wire as the reference electrode. The reference potential of the Ag wire was calibrated *vs* a saturated calomel electrode (SCE), according to the relationship of $V_{Ag\ wire} = V_{SCE} - 0.07$ V. The electrochemical impedance spectrum (EIS) measurements were carried out on an Autolab PGSTAT302N system (Eco Chemie, Netherlands) using a conventional three-electrode system (a Pt wire and a SCE as the counter and the reference electrode, respectively). The scanning electron microscopic (SEM) images were obtained on an ultra plus field emission SEM (Zeiss, Germany), with an acceleration potential at 15 KV. The contact angle measurement was conducted with a CAM2000 optical contact angle analyzer (KSV Instruments, Finland) using a CCD video camera and a horizontal light source to illuminate the liquid droplet. All experiments were performed at 22 ± 2 °C.

3. Results and discussion

3.1. Characterization of nano-TiO_2/ITO electrode

The morphologies of the different electrodes were characterized with the SEM. The bare ITO electrode possesses the somewhat complanate and hydrophobic surface (Fig. 1A), with a contact angle of about 77° (Fig. 1C). When the nano-TiO_2 with a mean particle diameter of 25 nm has been well modified on the bare ITO electrode (Fig. 1B), it produces a much rougher, but much hydrophilic surface (a contact angle of about 20°, Fig. 1D), which makes it possible for the nano-TiO_2 interface to promote cells adhesion and reserve their activity.

3.2. CV behavior of electrochemical probe on the nano-TiO_2 interface

It was noted that the redox response of $K_3[Fe(CN)_6]$ at the nano-TiO_2/ITO electrodes enhanced by ca. 60% over that at the bare ITO electrode (Fig. 2A), indicating that the nano-TiO_2 interface could observably promote the electron transferability between the electrode and the electrolytic solution. Both the reduction and oxidation peak currents at the nano-TiO_2/ITO electrode increased linearly with the increase of the square root of the scan rates (from 25 to 300 mV/s) (Fig. 2A inset, $R = 0.999$ and 0.999, respectively), verifying that this redox reaction was a typical diffusion-controlled process.

3.3. Optimization of nano-TiO_2/ITO electrodes

The deposition volume of nano-TiO_2 (0.33 mg ml^{-1}) shows an important effect on the DPV peak current of $K_3[Fe(CN)_6]$. The peak currents increased with the increasing volume of nano-TiO_2 and reached a summit at a volume of 6 μL (Fig. 2B), but then it decreased

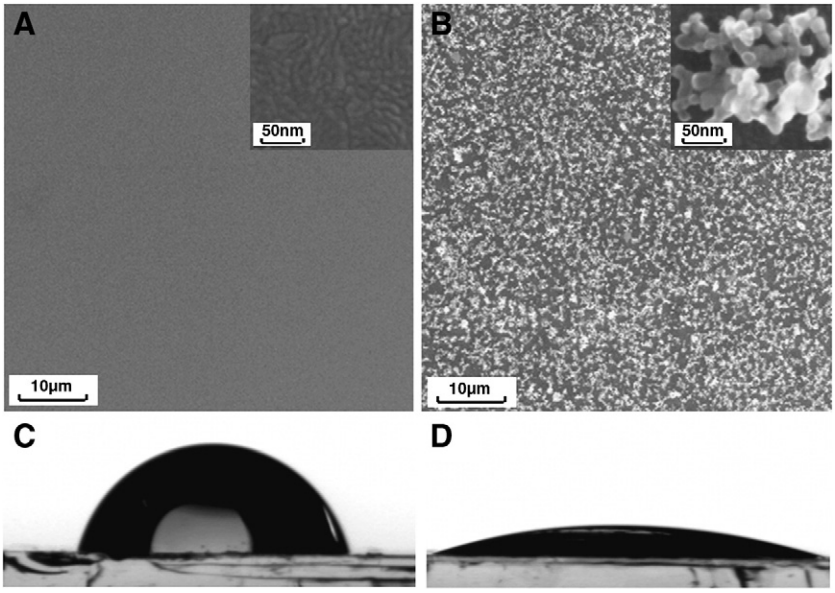

Fig. 1. SEM (A, B) and contact angle (5 μL aqueous droplets) images (C, D) of the bare ITO electrode (A, C) and nano-TiO_2/ITO electrode (B, D).