Talanta 77 (2009) 1009–1014

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid identification and high sensitive detection of cancer cells on the gold nanoparticle interface by combined contact angle and electrochemical measurements

Fang He[a], Qin Shen[a], Hui Jiang[a], Jian Zhou[a], Jian Cheng[b], Dadong Guo[a], Qingning Li[a], Xuemei Wang[a,*], Degang Fu[a], Baoan Chen[b]

[a] State Key Laboratory of Bioelectronics (Chien-Shiung Wu Laboratory), School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China
[b] Department of Hematology and Oncology, Affiliated Zhongda Hospital, Southeast University, DingJiaQiao 87, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 19 May 2008
Received in revised form 24 July 2008
Accepted 30 July 2008
Available online 14 August 2008

Keywords:
Gold nanoparticles
Cancer cell identification
Electrochemical detection
Contact angle measurement

ABSTRACT

In this study, we have proposed a novel strategy for the rapid identification and high sensitive detection of different kinds of cancer cells by means of electrochemical and contact angle measurements. A simple, unlabeled method based on the functionalized Au nanoparticles (GNPs) modified interface has been utilized to distinguish the different cancer cells, including lung cancer cells, liver cancer cells, drug sensitive leukemia K562/B.W cells and drug resistant leukemia K562/ADM cells. The relevant results indicate that under optimal conditions, this method can provide the quantitative determination of cancer cells, with a detection limit of $\sim 10^3$ cells mL^{-1}. Our observations demonstrate that the difference in the hydrophilic properties for target cellular surfaces and in the uptake efficiency of the anticancer drug daunorubicin for different cancer cells could be readily chosen as the elements of cancer identification and sensitive detection. This raises the possibility to advance the promising clinic diagnosis and monitoring of tumors with the aim of successful chemotherapy of human cancers.

1. Introduction

Cancer is one of the most serious and lethal diseases around the world. The research of fast identification and high-sensitive detection of cancer cells is extremely important for cancer diagnosis and therapy, which can be utilized to explore and monitor the relevant biological process of cancers and is critical for the early cancer diagnosis and clinical treatments.

The intensive interests and efforts in the early cancer diagnosis reflect the increasing demand of relevant biosensors with good sensitivity and selectivity, rapidness, and easy operation [1,2]. Several protocols have been developed to detect and identify cancerous cells, including those based on polymerase chain reaction (PCR) [3], quartz crystal microbalance (QCM) measurement [4], microarrays [5,6], monoclonal antibody-coupled ferromagnetic nanoparticles [7], aptamer-modified fluorescent nanoparticles [8], carbohydrate-mediated cell recognition using gold glyconanoparticles [9], immunophenotyping by means of flow cytometry [10], amperometric detection of enzymatic reaction products [11] chemiluminescence (CL) [12] or diffraction-based cell detection [13], etc. Many of the currently available detection methods require enrichment of the target cells in the sample or expression of fluorescent protein markers or antibodies in the cells, and tend to include additional steps and time-consuming assay procedures. For example, PCR-based methods are indirect ways of detecting cells and require prolonged isolation steps before analysis. Besides, the variable sensitivity of PCR can limit its effectiveness as a diagnostic technique and lead to false-negative results [3]. Moreover, the common immunophenotypic analyses on basis of flow cytometry, which require multiple antibody probes for accurate cell detection, are usually time-consuming, complex and costly. In view of these challenges, a simple detection system that can achieve high selectivity and sensitivity without the need for target amplification and labeling is highly desirable.

Recently, nanotechnology has been located in a unique position to transform cancer diagnostics and to produce a new generation of biosensors and medical imaging techniques for highly sensitive and precise recognition. Large quantities of biocompatible nanomaterials have attracted much interest of numerous scientists in various biomedical research areas due to their unique properties. Among them, gold nanoparticles (GNPs), the known biocompatible nanomaterials, have been widely concerned in the fields of biomedical imaging and biosensing applications during the last ten years [14–17]. Our recent study indicates that the relevant GNPs have

* Corresponding author. Tel.: +86 25 83792177; fax: +86 25 83792177.
E-mail address: xuewang@seu.edu.cn (X. Wang).

0039-9140/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2008.07.063

negligible effect on cellular drug uptake of daunorubicin (DNR) of normal cells but have significant effect on the intracellular drug accumulation of DNR on leukemia cancer cells [17]. Besides, the functional GNPs-based materials have been well adopted for the biosensing of biomacromolecules based on their unique mechanical and electronic properties [18–22].

Considering the plentiful merits of GNPs, in this study we have utilized them for highly sensitive detection of cancer cells. The strategy of a simple identification and sensitive detection of the drug sensitive and drug resistant leukemia K562 cells, lung cancer cells and liver cancer cells on the functionalized GNPs modified interface has been developed by using anticancer drug daunorubicin as the electrochemical probe. Meanwhile, according to the different hydrophilic characteristics of the relevant cancer cells on the GNPs modified interface, we could utilize the contact angle measurement to identify above different kinds of cancer cells. The researches have realized the cancerous cell detection by electrochemical and contact angle measurements with high sensitivity, selectivity and simplicity.

2. Experimental

2.1. Reagents

Daunorubicin, mercaptopropionic acid (MPA) and hydroxyethylpiperazine ethanesulfonic acid (HEPES) were purchased from Aldrich. 1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide hydrochloride (EDC) was purchased from Sinopharm. Co. Ltd., China. Daunorubicin stock solutions were freshly prepared and stored in the dark at 4 °C. Phosphate buffer solution (PBS) and HEPES buffer was prepared with double distilled water. All other reagents were of analytical grade. The functionalized GNPs were synthesized according to the literature [23] by the ligand exchange reaction between triphenyl phosphine (PPh_3)-stabilized precursor nanoparticles and MPA. The concentration ratio between original Au (III) and MPA was adjusted at 1:100.

2.2. Cells and cell culture

Lung cancer cells, liver cancer cells, leukemia K562/ADM and K562/B.W cell lines were purchased from Institute of Hematology of Tianjin, Chinese Academy of Medical Sciences and cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. The drug resistant leukemia K562 cells (K562/ADM cells) were maintained with 1 μg mL^{-1} Adriamycin (Sigma).

The real leukemia cell lines were collected and separated from the mixture of peripheral blood of leukemic patients, PBS (0.1 M, pH 7.2) and lymphocytes separation medium with the ration of 1:1:2 (v/v/v) by centrifugation 2500 rpm for 30 min.

2.3. Electrode preparation

The glassy carbon electrode (GCE) was polished to a mirror using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by rinsing thoroughly with double distilled water. The electrodes were then pretreated electrochemically by applying a potential of +1.75 V in PBS (0.1 M, pH 5.0) 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 [24]. This process led to the formation of hydroxyl groups on the GCE surface and increased surface hydrophilicity of the GCE. Then the pretreated electrode was immersed thoroughly into the blending solution with 100 μL GNPs and 100 μL HEPES buffer solution (0.1 M, pH 7.2) containing 0.5 mmol EDC for about 10 h. The hydroxyl groups on GCE were linked to the MPA stabilized GNPs with the assistance of the EDC linker. The GNPs modified GCE was then rinsed thoroughly with double distilled water to remove the non-covalent nanoparticles before use.

2.4. Atomic force microscopy (AFM) measurements

The AFM measurements were performed through a simultaneous monitoring of both the amplitude and the phase of the oscillating cantilever in tapping mode. All AFM data were collected on a Digital Instruments using a Nanoscope III controller (Veeco Instruments, New York) and an E-scanner operating in tapping mode in air. Imaging was accomplished using diving board TESP tips with integral square pyramidal tips. All data were plane fit and flattened prior to analysis using the Nanoscope software.

2.5. Electrochemical investigation

The leukemia K562 cells at a concentration of 6×10^5 mL^{-1} with anticancer drug daunorubicin at a concentration of 5.3×10^{-5} M were firstly incubated at room temperature. After the targeted cells with DNR were pretreated for a series of incubated time, the suspensions were centrifuged for 10 min. Then all medium samples outside leukemia K562/ADM cells were collected and diluted with sterilized PBS (0.1 M, pH 7.2). The electrochemical signal of DNR residue in these samples was determined with differential pulse voltammetry (DPV) assay for each sample by CHI660C electrochemical analyzer. All measurements were performed at ambient temperature (22 ± 2 °C) in a three-component electrochemical cell consisting of the GNPs modified glassy carbon electrode (GNPs–GCE) as the working electrode, a Pt wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode.

2.6. Contact angle detection

Contact angle detection was performed 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. The droplets of solution were placed on the surface of the GNPs–GCE and the contact angle measurements were carried out at ambient temperature (22 ± 2 °C). To extract the precise contact angle values, the drop images were fitted using the Young–Laplace equation [25]. The contact angle values were determined with the precision of ±0.5°.

3. Results and discussion

3.1. Characterization of the GNPs–GCE

After the preparation of the GNPs–GCE according to the procedure described above, the GNPs–GCE was first characterized by AFM, which is an effective way to provide nanoscale surface topography and phase images. The AFM studies can afford direct evidence for the morphologies of the functionalized GNPs film covered on the GCE. As shown in Fig. 1, the typical topographic images of the bare GCE and the GNPs–GCE have been explored and characterized by AFM studies. The pretreated bare GCE exhibits a relatively planar interface (Part A, Fig. 1), while the GNPs film appears more rough and highlighted in the AFM graphs (Part B, Fig. 1). The average sizes of GNPs were about 15 nm and in coincidence with the sizes of dispersed GNPs obtained by TEM (not shown), indicating the assembly of GNPs on the surface produces little aggregation.

In addition, the electrochemical performance of the GNPs–GCE has also been investigated by cyclic voltammetry. As shown in