Full Paper

Electrochemical Biosensing for Cancer Cells Based on TiO$_2$/CNT Nanocomposites Modified Electrodes

Qin Shen, Sun-Kyung You, Soo-Gil Park, Hui Jiang, Dadong Guo, Baoan Chen, Xuemei Wang*

$^a$ State Key Lab of Bioelectronics, Chien-Shiung Wu Laboratory, Southeast University, Nanjing 210096, P. R. China
$^b$ Department of Industrial Chemical Engineering, Chungbuk University, Korea
$^c$ Zhongda Hospital and School of Clinic Medicine, Southeast University, Nanjing 210096, P. R. China

Received: July 2, 2008
Accepted: September 13, 2008

Abstract

Both TiO$_2$ nanoparticles and carbon nanotubes have been usually utilized to modify the electrodes to enhance the detection sensitivity of biomolecular recognition. In this research, novel TiO$_2$/CNT nanocomposites have been prepared and doped on the carbon paper as the modified electrodes. Subsequently, the redox behavior of the ferricyanide probe and the surface properties of the cancer cells coated on the modified electrodes have been investigated by using electrochemical and contact angle measurements. Compared with electrochemical signals on bare carbon paper and nanocomposite modified substrates, the significantly enhanced electrochemical signals on the modified electrodes covered with cancer cells have been observed. Meanwhile, different leukemia cells (i.e., K562/ADM cells and K562/B.W. cells) could be also recognized because of their different electrochemical behavior and hydrophilic/hydrophobic features on the modified electrodes due to the specific components on the plasma membranes of the target cells. This new strategy may have potential application in the development of the biocompatible and multi-signal responsive biosensors for the early diagnosis of cancers.

Keywords: CNTs and TiO$_2$ nanocomposites, Electrochemistry, Bioanalysis, Cancer cell detection

DOI: 10.1002/elna.200804351

1. Introduction

Cancer is among the most serious and difficult diseases to diagnose and treat. The efficient cure of cancers is still a hot topic in biomedical areas involving in the disease diagnostics and treatments as well as patient care. However, the detection and identification of pathogens are often pains-taking in clinic cases due to the low abundance of diseased cells in sputum, blood, and other clinical samples. Otherwise, the multidrug resistance is another major obstacle in cancer therapies. These challenges require effective methods for detecting cancerous cells and targeting in tissue. Beside the traditional methods, multifunctional nanocomplexes were reported as a new way for early cancer detection including tumor imaging, personalized diagnostics and targeted therapeutics.

Considerable attention has been drawn to the carbon nanotubes (CNTs) over the past decade because of their unique physical and chemical properties and potential applications in various research fields as well as industrial fields [1–4]. The subtle electronic properties suggest that CNTs are liable to promote electron-transfer processes and hence have been extensively used as the electrode material in electrochemical determinations. The larruping performance of the CNT electrode in comparison with other type of carbon electrode has been reported, which is mostly correlative with the electronic structures, the channels that are inherently present in the tubes, and the topological defects present on the surface of the tube [5–7]. In the recent years, increasing interests are being focused on the rational functionalization of the CNTs by some creative methods, e.g., to couple CNTs with biomolecules through a covalent or noncovalent interaction for miscellaneous bioassays [8–14]. However, the considerable toxicity of CNT is still a controversial issue and limits its biological application.

As one of the typical biocompatible materials, titanium dioxide (TiO$_2$) nanoparticles have been widely applied in biomedical and bioengineering fields due to their strong oxidizing properties, chemical inertness, and nontoxicity [15–17]. Interestingly, biomolecules with enediol, phosphate, and carboxylic groups have been known with the ability to bind to the surface of TiO$_2$ nanoparticles [18]. Good biocompatibility of TiO$_2$ nanoparticles may offer a chance to improve the biocompatible property of CNT by constructing TiO$_2$/CNT nanocomposites. Herein, in this contribution, we have explored the possibility to construct TiO$_2$/CNT nanocomposites with CNT and organic titanium precursors. Then the TiO$_2$/CNT nanocomposites were coated on the carbon paper substrates [19–22] to form the modified electrodes for high sensitive biorecognition. The electrochemical characterization of the TiO$_2$/CNT nanocomposites deposited on the carbon paper indicated that the new nanocomposites could efficiently promote the electron
transferability. Based on these observations, the redox behavior of the ferricyanide probe and the surface properties of the cancer cells coated on the TiO₂/CNT nanocomposites electrodes have been further investigated by using electrochemical and contact angle measurements, which provide a new strategy for the electrochemical biosensing of cancer cells and give direct evidence for the relative hydrophilic/ hydrophobic features and wetting properties as well as the redox-controlled transformations of the respective surfaces.

2. Experimental

2.1. Reagents

The ultrapure water was prepared using Milli-Q purification system (Millipore Trading Co., Ltd) to a specific resistance of > 18 MΩ cm at 25 °C. Carbon nanotube was purchased from Shenzhen Nanotechnologies Co. Ltd.(NTP). Titanium isopropoxide, K₃[Fe(CN)₆], penicillin, streptomycin and Adriamycin were purchased from Sigma. Ketjen black, poly (vinylidene fluoride) and N-metyl-2-pyrrolidione were purchased from Aldrich. The carbon paper was purchased from Shanghai Hezen electric Co. Ltd. The RPMI 1640 medium was purchased from GIBCO. The fetal calf serum (FCS) was purchased from Hyclone, USA. All the reagents were analytical grade. For the following studies, all experimental measurements were completed at least three times in parallel.

2.2. Preparation of Modified Electrode

First, titanium isopropoxide and multiwalled CNT were dispersed in 2-propanol respectively with the ratio of 70:30 (w/w) and mixed evenly. Then the nano-TiO₂ were grown on the CNT using sol-gel procedures. Finally, the solvent was removed by evaporation to collect the composite powder. The coating material was prepared from a paste mixture of TiO₂/CNT powder, ketjen black (as an electronic conductor additive) and poly (vinylidene fluoride) (as a binder) in N-metyl-2-pyrrolidione with a ratio of 85:10:5 (w/w/w). The paste was coated on the carbon paper and dried in vacuum at 120 °C for 24 h. The morphologies of carbon paper before and after the modification were characterized by scanning electron microscope. To fabricate the cells covered electrode, the cells of a certain concentration (5 × 10⁶/mL) were collected and separated from 1.0 mL medium by centrifugation 1250 rpm for 10 min. The sediment was covered on the surface of modified electrodes and air dried for 15 min.

2.3. Scanning Electron Micrograph Study

Scanning electron micrographs were obtained with a scanning electron microscopy (SEM, LEO 1550, Germany) at 20 kV. The typical SEM image of the carbon paper substrate before and after the modification was illustrated in Figure 1.

2.4. Cells and Cell Culture

Leukemia K562 cell lines and normal cell lines were cultured in a flask in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 μg mL⁻¹), and streptomycin (100 μg mL⁻¹) at 37 °C in a humidified atmosphere containing 5% CO₂. Additionally, the drug resistant leukemia K562 cells (K562/ADM cells) were maintained with 1 μg mL⁻¹ Adriamycin.

2.5. Static Contact Angle Measurement

Static contact angle measurements were performed on the respective modified carbon paper substrate using a CAM200 optical contact angle analyzer (KSV Instruments, Finland). For all systems, the images of the drops were recorded about 10 s after it was dropped on the respective interface, which allows the drop to adjust its shape. To extract the precise contact angle values, the drop images were fitted using the Young – Laplace equation. The contact angle values were determined with the precision of ±0.5°.

2.6. Electrochemical Study

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on CHI 660b electrochemical workstation (CH incorporation, USA) to detect the electrochemical response of the ferricyanide probe on different modified electrodes. All electrochemical measurements were carried out at ambient temperature (20 ± 2 °C) using three-component electrochemical system consisting of a piece of modified carbon paper as the working electrode, a Pt wire as the counter electrode and an Ag wire as the reference electrode. All potentials are reported here versus the Ag-wire reference electrode. We found that the reference potential of a SCE and the Ag wire in 0.1 M phosphate buffer, pH 7.2, have the relationship of V_{SCE} = V_{Ag wire} + 0.07 V.
3. Results and Discussion

3.1. Characterization of TiO$_2$/CNT Nanocomposites

Initially, the morphologies of the electrodes were characterized with scanning electron microscope (SEM). Figure 1a shows that the paper substrate made of carbon fibers (10 μm in diameter). When the paper was modified with the TiO$_2$/CNT nanocomposites, the composites were evenly coated on the substrate (Fig. 1b), with an estimated width of 50 nm (see supporting information). The width is much higher than that of the CNTs (20 nm), indicating that the CNTs were enwrapped by the TiO$_2$ gel to form the composite.

3.2. Electrochemical Characterization

The electrochemical performance of the TiO$_2$/CNT nanocomposites modified carbon papers was characterized by the ferricyanide probe. The results indicated that the redox response of K$_3$[Fe(CN)$_6$] at the modified electrodes (Fig. 2b) was about 10-fold enhanced than that at the bare carbon paper (Fig. 2a). Besides, the peak potential difference ($\Delta E$) for the modified carbon papers was smaller than that for the bare carbon papers, indicating the relatively good redox reversibility at the modified carbon papers. The dependence of reduction peak current of K$_3$[Fe(CN)$_6$] on the scan rates were also studied. The excellent linear relationship between peak current and square root of scan rate ($R = 0.998$) indicated that the redox electron transfer was a diffusion-controlled process.

Based on these observations, we have further explored the electrochemical behavior of the probe and the relevant surface properties of the cancer cells on the TiO$_2$/CNT nanocomposites modified electrodes. Heretofore numerous reports indicated that the presence of cells could block the electron transfer by the insulated of the cell membrane and lead to an increase in the electrode resistance [23, 24]. However in our studies, after the TiO$_2$/CNT nanocomposites modified electrode was covered with cancer cells, the electrochemical response of the ferricyanide probe was observed to obviously increase by compared to that without the cancer cells. It is noted that the peak current became ca. 10-fold higher than that without the cancer cells (Fig. 3a) and also bigger than that of the electrode covered with the noncancerous (normal) cells (Fig. 4b). Furthermore, the electrochemical behaviors of K$_3$[Fe(CN)$_6$] on the leukemia K562/ADM cells (Fig. 3b) and K562/B.W. cells (Fig. 3c) covered electrodes were also different, where the electrochemical response of the probe on the K562/B.W. cells film is apparently stronger than that on the K562/ADM cells film, accompanying with a 30 mV negative shift of the peak potential.

![Fig. 2. Cyclic (left) and differential pulse (right) voltammetric study of 5 mM K$_3$[Fe(CN)$_6$] at the bare carbon paper (a) and TiO$_2$/CNT nanocomposite modified carbon paper (b). For cyclic voltammetry: scan rate: 50 mV/s. For differential pulse voltammetry: increment: 0.004 V, amplitude: 0.05 V, pulse width: 0.05 s.](image1)

![Fig. 3. Cyclic voltammetric (CV) study of 5 mM K$_3$[Fe(CN)$_6$] at the TiO$_2$/CNT nanocomposite modified carbon paper before (a) and after covering the leukemia K562/ADM cells (b) and K562/B.W. cells (c). The cell concentration is 5 x 10$^6$/mL. Scan rate: 50 mV/s. Inset: amplification of curve a.](image2)
3.3. Static Contact Angle Measurement

Meanwhile, the contact angle measurement illustrates that the TiO$_2$/CNT nanocomposites modified electrode is hydrophobic with a contact angle of about 135° (Fig. 5a). After this modified electrode was covered with cancer cells, it became more hydrophilic with a contact angle of 53° (Fig. 5b), which was also smaller than that covered with the noncancerous cells (Fig. 5c). Therefore the contact area between the droplet and the electrode sharply increases.

3.4. Possible Mechanism of the Enhanced Effect by Nanocomposites

Based on these studies above, it appears that the TiO$_2$/CNT nanocomposites modified electrode could accelerate the heterogeneous electron transfer rates and thus enhance the relevant detection signal. This significant increase could be attributed to the amplification in the surface area of the electrode by the modified nanocomposites. Meanwhile, the electrochemical response of K$_3$[Fe(CN)$_6$] could be obviously increased when the electrode was covered with cancer cells. Scheme 1 illustrates the possible mechanism of the enhanced effect on the relevant electrochemical response. This unexpected phenomenon could be ascribed as following: the nanocomposites modified electrode with a hydrophobic interface limited the electron exchange between the electrode and hydrophilic ferricyanide probe and subsequently led to the lower electrochemical response. After the interface was covered with cancer cells, it became much more hydrophilic and the contact area between the droplet and the electrode sharply increases. Moreover, the relevant surface is also advantageous for the approach of the hydrophilic probe and promotes the electron exchange. Thus, the significantly enhanced electrochemical responses were detected on the cancer cells covered interface. As is known, the plasma membranes of the drug resistant leukemia K562/ADM cells are anchored with over-expressed proteins, such as P-glycoprotein (P-gp). The proteins are usually affinity towards hydrophobic substrates [25]. Thus the hydrophobic TiO$_2$/CNT modified interface will make the K562/ADM cells adsorbed onto the carbon paper’s surface more easily than K562/B.W. cells. Compared with the K562/B.W. cells, the higher adsorption amount of K562/ADM cells at the carbon papers will somehow block the electron transfer, and that is the reason of the peak current and peak potential difference of the two kinds of leukemia cells.

4. Conclusions

In summary, in this study the new TiO$_2$/CNT nanocomposites were prepared and coated on the carbon paper substrates to form the modified electrodes, which were applied in the respective biorecognition and detection of cancer cells. The electrochemical observations demonstrated that these nanocomposites could accelerate the heterogeneous electron transfer rates and lead to the significant enhancement of the relative detection sensitivity. Thus, it is obvious that a considerable increase in the electrochemical response of the ferricyanide probe appeared on the K562/ADM and K562/B.W. cells covered TiO$_2$/CNT electrodes. Moreover, the relevant modification interfaces could show the distinguishable contact angle and electrochemical signals for the different target cells. These results suggest that the TiO$_2$/CNT nanocomposites modified electrodes could be potentially used in the bioanalytical applications such as fabrication of whole-cell biosensors with enhanced detection sensitivity.

5. Acknowledgements

The support from National Science Foundation of China (90713023, 20711140391, 20675014, & 60121101), and Min-
istry of Science & Technology of China (2007AA022007) is greatly appreciated.

6. References


Scheme 1. Schematic illustration of the TiO2/CNT nanocomposite modified carbon paper covering the leukemia K562 cells. The contact angle images of aqueous droplet on modified electrode before (a) and after (b) the covering of cancer cells.