Specificity Bio-identification of CNT-Based Transistor

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Abstract. In this research, we report a simple and general approach to π-π stacking functionalization of the sidewalls of CNTs by 1-pyrenebutanoic acid, succinimidyl ester (PSE), and subsequent immobilization of insulin-like growth factor 1 receptor (IGF1R) onto SWNTs with a high degree of control and specificity. The selection of PSE provides visualization and characterization of individual CNTs based on its strong luminescence. In addition, we designed a simple and efficient electrode with a staggered pattern to increase the effect of electrophoresis by using electric field for the macroscopic alignment of CNTs to complete a field-effect device for CNT-based biosensors. Scanning Electron Microscopy (SEM) was used to investigate the morphology of the biosensors. The results of four-point probe method demonstrated high selectivity and sensitivity of detection. The functionalization of SWNTs was investigated by Fourier transform infrared spectroscopy (FTIR). Experimental results imply that specific binding between IGF1R and its specific mAb results in a dramatic change in electrical current of CNT-based devices, and suggest that the devices are very promising biosensor candidates to detect circulating cancer cells.

1. Introduction

Functional carbon nanotubes (CNTs) exhibit a unique combination of excellent mechanical, electrical and electrochemical properties, which has stimulated increasing interest in the application of CNTs as components of biosensors. The use of CNTs for sensing technology has attracted intensive research interest in the last several years because interactions between target molecules and CNTs can significantly change the electronic properties of CNTs.¹² Carbon nanotubes are unique among solid-state materials in that every atom is on the surface. Surface chemistry could therefore be critical to the physical properties of CNTs and their applications.³⁴ Therefore, the sidewall functionalization of CNT in noncovalent ways is important to the purposes of soluble nanotubes, self-assembly on surfaces, and chemical sensors to preserve the nanotube structure and thus their electronic characteristics. On the other hand, immobilization of biomolecules on carbon nanotubes has been pursued in the past as well, motivated by the prospects of using nanotubes as new types of biosensor materials.⁵⁶ Recent reports have shown that nanoscale electronic devices can be used to detect a change in electrical...
properties when receptor proteins bind to their corresponding antibodies functionalized on the surface of the device. A prerequisite for research in this area is the development of chemical methods to immobilize biological molecules onto carbon nanotubes in a reliable manner. Nowadays, clinical cancer imaging technologies do not possess sufficient spatial resolution for early detection based on the molecular signatures of proteins that are overexpressed in cancer cells. Development of new technologies for reliable early detection of cancer from biological fluids via minimally invasive methods is still a high priority. In this research, we report a carbon nanotube field effect transistor array, with a simple and general approach to noncovalent functionalization of carbon nanotubes, and subsequent immobilization of various biological molecules onto nanotubes with a high degree of control and specificity.

2. Experiment

2.1. Functionalization of CNTs

The noncovalent functionalization involves a bifunctional molecule, 1-Pyrenebutyric Acid N-Hydroxysuccinimide Ester (PSE) (SIGMA–Alrich, Inc purity 95%, 114932-60-4), irreversibly adsorbed onto the inherently hydrophobic surfaces of CNTs in an organic solvent, isopropanol. The CNTs 1.5mg were soaked in 10ml IPA, and dispersed by 3 hrs sonification. And then 0.5mg PSE was added to the SWCNTs solution dispersed by 10mins sonification. This leads to the functionalization of CNTs (f-CNTS) with succinimidyl ester groups that are highly reactive to nucleophilic substitution by primary and secondary amines that exist in abundance on the surface of most proteins.

2.2. Immobilization of anti-protein

The nanotube-antibody device was prepared by modified f-CNTS with specific-IGF1R mAb and non-specific IgG mAb respectively. Specific anti-IGF1 receptor mouse monoclonal antibody (Life Span Ltd. LS-C41502) was mixed with phosphate buffered saline (PBS: pH 7.4) with 1:500 dilution and preserved at 4°C environment until use. Non-specific IgG mAb (Jackson ImmunoResearch Ltd. Antibody concentration 2.4mg/ml 115-005-003) was diluted 1:100 with sterile phosphate buffered saline (PBS: pH 7.6) and preserved at 4°C environment. MCF7 breast cancer cells was used in this study due to its high expression of IGF1 receptor. The CNTs functionalized with IGF1R-specific, which exhibits highly sensitive and selective sensing, interact with IGF1R overexpressed by MCF7 cell line.

2.3. Processing of electrode device

Carbon nanotube-based field effect transistor devices have been fabricated on a doped P-Type-Si that was coated with a layer of silicon dioxide. The staggered titanium metal electrodes, which were designed as metal arrays of source and drain separated with a spacing of 60μm, were patterned using microfabrication techniques such as photolithography, metal evaporation, and lift-off. Following that the nanotubes were integrated between the electrodes by electrophoretic self-assembly technique. This process was carried out using AC electrophoresis with a frequency of 60 Hz and voltage of 10.0V peak-to-peak.

2.4. Measurements and analysis

The electrical measurements were performed using a probe station attached to a semiconductor parameter analyzer after the electrophoretic self-assembly. The silicon substrate was used as back gate. Electrical conductance of each bare-CNTFET device was taken as a reference for each measurement to eliminate any possible variation between the device. The real-time electrical conductance measurements have taken place in three steps: starting with bare-CNTFET, then antibody exposure to the CNTFET device, and finally application of MCF7 breast cancer cells to the surface of the CNTFET device. The surface image of the device was analyzed by scanning electron microscope. The
CNTs functionalization and immobilization of antibody were investigated by Fourier transform infrared spectroscopy (FTIR).

3. Results and Discussion

The optical micrograph and scanning electron microscopy images of a fabricated CNTFET device is shown in Fig.1(a). By electrophoretic self-assembly technique the well separated f-CNTs network was obtained in between source and drain Ti-electrodes, which is clearly identified in the Figure 1(b). The measured width of the source drain channel was about 62μm.

The electronic transport properties measured, using Si substrate as back gate, before immobilization of anti-protein is shown in figure 2. The device exhibited significant gate effect as determined by $I-V_G$ measurements. The source-drain current of the devices increased with increasing gate voltage indicating that the dominant conduction process is due to electron transport, i.e. n-type semiconducting CNTs. This result indicating the conductance of semiconducting CNTs could be modulated by gate voltages. A CNTFET can act as ambipolar device, both an n-type and a p-type transistor, depending on the FET voltage. The conductance in the n-type region is larger than the p-type region due to the work function of Ti electrodes. The Fermi level of the Ti electrodes lies close to the conduction band of the CNT, making a path for the injection electrons. Figure 3 shows the FTIR spectra of CNTFET before and after functionalization and immobilization of CNTs. Shown in curve (a) is the absorption spectra of bare-CNTs as the control group. Curve (b) shows the absorption peaks of PSE functionalized CNTs in IPA. It can be seen from the figure that the two less intense peaks at 2850 ~ 3000 cm$^{-1}$ are C-H stretching vibration modes of functional groups. The 1592 cm$^{-1}$ peak is generated from stretch aromatic hydrocarbon carbon sp$^2$ orbital vibrations. There is a multi-frequency observed around 1600 cm$^{-1}$ absorption peak indicates that the Benzene group of pyrene butyric acid attached on the SWNT sidewall via physical adsorption, resulting π -π stacking interactions and makes sp$^2$ hybrid orbital of carbon and carbon benzene ring oscillating between the apparent absorption frequencies in the range of the benzene ring. The broadening of IPA
peak in the range of 700 cm\(^{-1}\) ~ 800 cm\(^{-1}\) owing to the C-C bending vibration and C-H stretching vibration of the tar-based structural brain benzene ring suggests a considerable amount of pyrene butyric acid adsorbed on the SWNT sidewall.

Curve (c) shows the immobilization of specific-IGF1R mAb on the pyrene butyric acid modified f-CNT. The biological characteristic of the amino bonds after IGF1R protein molecules reaction is obvious. The stretching vibration modes of the amino functional groups of N-H, C-N and C=O bonds are found at 3450 cm\(^{-1}\), 3000 cm\(^{-1}\), and 1600 cm\(^{-1}\) respectively. Three characteristic peaks of amide I, II, and III appeared at frequencies 1700 cm\(^{-1}\), 1500 cm\(^{-1}\), and 1300 cm\(^{-1}\). This result confirms the existence of specific-IGF1R anti-protein functional groups which acts as the biological probe on CNTFET device. Curve (d) represents the FTIR spectrum of MCF-7 cell reacted with specific-IGF1R mAb immobilized f-CNTs for 2 hrs. The signals at 2800-3000 cm\(^{-1}\) are dominated by symmetric and asymmetric stretching vibrations of CH\(_2\) and CH\(_3\) groups, mainly contained in fatty acids of cells\(^{[10]}\).

The amide I stretching of carbonyl from peptide bond was observed at 1641 cm\(^{-1}\), a band sensitive to secondary structure of the protein. Deformation of protein amide N-H bond (Amide II) results in a signal at 1540 cm\(^{-1}\). The signals in the region between 1450-1400 cm\(^{-1}\) result from various amino acid side chains and some lipids. The absorptions bands at 1238 cm\(^{-1}\) and 1080 cm\(^{-1}\) are characteristic of asymmetric and symmetric phosphodiester vibrations of nucleic acids\(^{[11]}\).

The electrical conductance measurements of modified f-CNTs were carried out in real-time at room temperature by four point probe method. To monitor breast cancer cell binding events, a 10 \(\mu\)l of the...
24 μg/ml mAb solution was applied on the surface of f-CNT device, while the current was monitored continuously. Once the current stabilized, 5 μl of MCF-7 breast cancer cells (about 10000) were applied to the device and the current was monitored continuously. Figure 4 demonstrates the effects of specific-IGF1R mAb adsorption, followed by MCF-7 cell, which generated about 3-fold increase in the device conductance. Similar result was obtained when the measurement was taken again after one day. Figure 5 shows the conductance of the device during nonspecific adsorption of MCF-7 breast cancer cells. The conductance rises immediately and dropped to the same level of the conductance before MCF-7 adsorption. These results suggest a charge transfer between mAb modified f-CNTs and breast cancer cells. Furthermore, specific binding between IGF1R and its specific mAb results in a dramatic change in electrical conductance of the device.

4. Conclusions

This research has demonstrated the label-free detection of MCF-7 breast cancer cells using specific-IGF1 receptor mAb modified CNTFET device. Experimental results indicated that it is the specific interaction between the IGF1R and its specific antibody that results in a large change in conductance. These observations imply a pathway, with high sensitivity and selectivity, that the mAb-CNTFET approach could be used to sense overexpressed protein biomarkers on breast cancer cells. Furthermore, these findings suggest applications of mAb-CNTFET to detect a wide variety of surface markers on diseased cells.

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