Detection of Live Breast Cancer Cells Using Carbon Nanotube Devices

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ABSTRACT

Development of new technologies for reliable early detection of cancer from biological fluids via minimally invasive methods is still a high priority. Cancer cells often overexpress characteristic surface receptors, which provide an opportunity for early diagnosis of disease. This paper presents the first application of nanotube-based electronic devices for targeting overexpressed surface receptors in cancer cells upon interaction with specific monoclonal antibodies (mAb) adsorbed to single wall carbon nanotube (SWNT) devices. Monoclonal antibodies specific to cell surface antigens overexpressed on cancer cells can be adsorbed to single wall carbon nanotube (SWNT) devices, resulting in a drop in conductance. Application of human BT474 and MCF7 breast cancer cells increased the conductance of the insulin-like growth factor 1 receptor (IGF1R) specific mAB-SWNT devices. The degree of increase in conductance of the devices due to live cancer cell application was proportional to the number of overexpressed surface receptors in cancer cells.

Keywords: single wall carbon nanotube, Her2, IGF1 receptor, antibodies, conductance.

1 INTRODUCTION

Carbon nanotubes (CNTs) are fascinating synthetic nanomaterials with superior mechanical, chemical, and electronic properties [1-6]. CNTs are analogous to a 2D graphite sheet rolled into tubes of diameter 1-10 nm, and hence form hollow tubules of a single layer of carbon atoms, rendering them highly sensitive to changes of their sidewall surface properties [7]. Furthermore, the electronic transport properties of a nanotube is very sensitive to its surrounding and changes significantly with variations in electrostatic charges and surface adsorption of various molecules [8-10]. Moreover, the nanotube-based devices are fast and sensitive and the active detection area is comparable to the size of individual biomolecules. Therefore, these nanotube-based devices can open up new research fields and applications in biological and biomedical sciences.

Development of carbon nanotube-based nanoscale biosensors has been intensified by the successful immobilization of biological macromolecules, such as proteins and enzymes, either in the interior cavity or on the surface of nanotubes without any drastic conformational or bioactivity change [11, 12]. Recent advances in the fabrication of nanotube-based devices, such as field effect transistors (CNTFETs), have increased the expectations for the utilization of carbon nanotubes as superior biosensor materials [13, 14]. These CNTFETs have shown appreciable changes in electrical conductance, suggesting the possibility of using them for electronic biosensing applications. Meanwhile, recent advances in aqueous solubility of carbon nanotubes have accelerated biocompatibility evaluations toward biomedical applications, including the potential usage of carbon nanotubes as carriers in drug or gene delivery systems. Furthermore, conjugation of carbon nanotubes with biomolecules such as proteins, carbohydrates, and nucleic acids are likely to facilitate bio-applications of carbon nanotubes.

Circulating cancer cells often express characteristic cell surface markers, which could provide an opportunity for early diagnosis of progressive disease [15]. However, current cancer detection technologies do not provide effective early detection of cancer. Development of new technologies for reliable early detection of cancer from biological fluids via minimally invasive methods is still a high priority. This paper presents the first application of nanotube-based electronic devices for targeting overexpressed surface receptors in cancer cells upon interaction with specific monoclonal antibodies (mAb) adsorbed to single wall carbon nanotube (SWNT) devices.

The investigation of the interaction of breast cancer cells with mAb-SWNT devices has been conducted in four steps: 1) fabrication of nanodevices with SWCNT patterned between the source and drain electrodes, 2) measurement of electrical conductance of the SWNT as they were patterned and SWNT coated with mAb specific to IGF1R, 3) control experiments to determine charge transfer between adsorbed mAbs and BT474 or MCF7 cells, 4) measurement of electrical conductance of the devices during cellular interactions with non-specific mAbs and specific mAb.

2 RESULTS AND DISCUSSIONS

The Carbon nanotube based field effect transistor (CNTFET) devices have been fabricated on a doped silicon wafer that is coated with silicon dioxide. First, metal electrodes were patterned using microfabrication techniques

such as photolithography, metal evaporation, and lift-off. The electrodes consist of 10 nm titanium and 100 nm gold with a spacing of 1-4 µm between source and drain. Following that the nanotubes were integrated between the electrodes by electrophoretic self-assembly technique. Then, the devices were dried, and electrical measurements were performed using a probe station attached to a semiconductor parameter analyzer. The silicon substrate was used as back gate. After the measurements, the devices were annealed at 180°C for 15 minutes to reduce contact resistance between nanotube and the electrodes.

Fig. 1 shows the schematic of the SWNT device that was fabricated using photolithography, metal deposition, and lift-off. The conductance was measured continuously during mAb adsorption, and then during cell adsorption to the devices. The device was biased at 5 mV to minimize current due to ionic conduction.

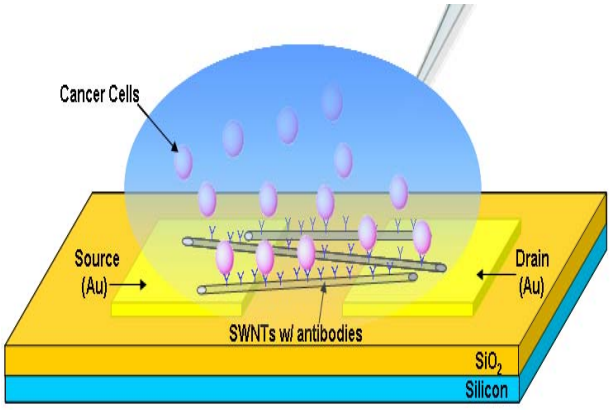

Figure 1. Schematic of the device used for detecting breast cancer cells by SWNT-mAb binding to surface receptors on live breast cancer cells.

Figure 2 is the optical micrograph of the device with live cancer cells.

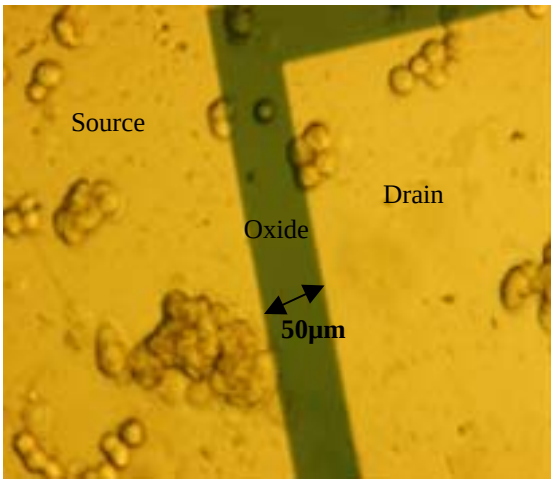

Figure 2. Optical micrograph of the device with live cancer cells.

Fig. 3a exhibits the effects of IGF1R-specific mAb adsorption, followed by adsorption of BT474 cells, which generated about 3.0-fold increase in the device conductance. Fig. 3b shows the conductance of the device during non-specific mAb adsorption (mouse myeloma IgG1), followed by adsorption of BT474 breast cancer cells, which generated two small spikes. It was apparent that BT474 cells interacting with the IGF1R-specific mAb-SWNT device produced a large increase in conductance, while the non-specific mAb-SWNT device yielded two small transients. We have previously reported that monoclonal antibodies specific to cell surface antigens overexpressed on cancer cells can be adsorbed to single wall carbon nanotube devices, resulting in a drop in conductance [16, 17].

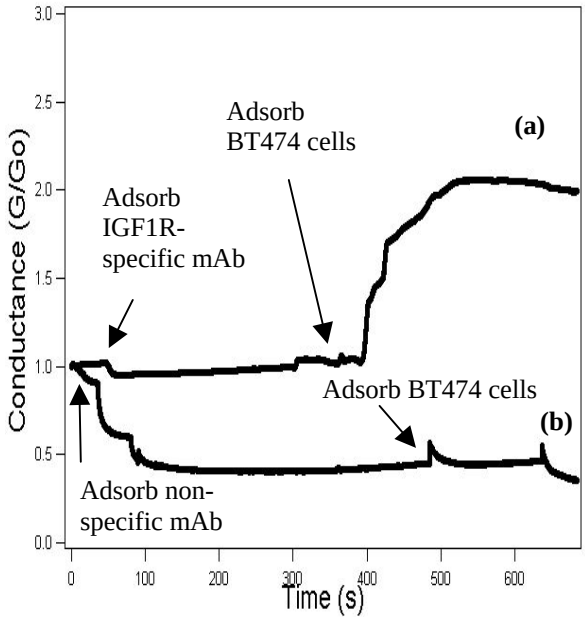

Figure 3. Change in normalized conductance for BT474 breast cancer cells applied to SWNT devices with adsorbed IGF1R-specific mAb **(a),** or non-specific mAb **(b)**, followed by BT474 breast cancer cell application.

Next, MCF7 breast cancer cells, which express a higher density of IGF1R than do BT474 cells [18, 20], have been used to investigate breast cancer cell surface protein binding to mAb adsorbed to SWNTs. Fig. 4a shows a large increase in the conductance of device, after IGF1R-specific mAb adsorption, followed by adsorption of MCF7 cells to the SWNT device. Conversely, Fig. 4b shows minimal change in device conductance after non-specific mAb adsorption (mouse myeloma IgG1) and MCF7 cell adsorption to the SWNT device. In fact, MCF7 cell binding increased device conductance about 8.0-fold, compared to non-specific mAb experiments.

It should be emphasized that compared to BT474 breast cancer cells (Fig. 3), the MCF7 breast cancer cells produced a greater increase in device conductance upon binding to

IGF1R-specific mAb-SWNTs. This could be attributed to the larger number of surface receptors in MCF7 breast cancer cells compared to BT474 cancer cells. It should also be mentioned that the non-specific mAb-SCNT conductance data for the BT474 and MCF7 breast cancer cells were quite similar.

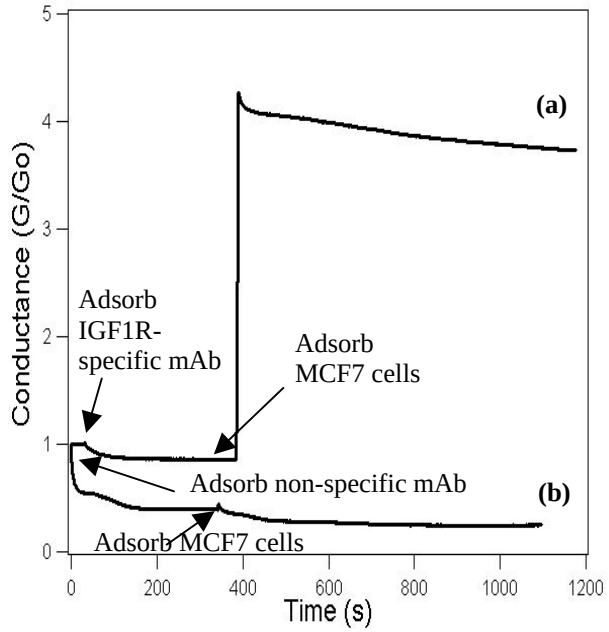

Figure 4. Change in normalized conductance for MCF7 breast cancer cells applied to SWNT devices with adsorbed IGF1R-specific mAb (a), or non-specific mAb (b), followed by MCF7 breast cancer cell application.

As stated earlier, when antibodies or other proteins adsorb to the surface of p-type SWNT, they decrease the conductance of the SWNT devices [16, 17, 21]. In this study, we found that subsequent adsorption of BT474 breast cancer cells (expressing significant IGF1R) or MCF7 breast cancer cells (overexpressing IGF1R) to IGF1R-specific mAb on the surfaces of the SWNT dramatically increased the conductance of the SWNT devices. These experimental results suggest the existence of a charge transfer process between SWNTs and breast cancer cells. It is believed that the charge transfer takes place through the mAbs that connect the SWNTs to the cells. In general, the amount of charge transfer is larger for antigen-specific mAb than non-specific mAbs as determined by conductance measurements upon breast cell application. The nature of the charge transfer process is explained in the next paragraph.

As the cell surface proteins interacted with the specific mAbs, electrons were transferred from the surface of the p-type SWNT to the bound cell, thereby increasing the conductance of the device. When proteins interact with their target receptors, they usually bind to these receptors through the amine groups, which are electron donors. When the cell surface receptors bind to the proteins that are adsorbed on the surface of the nanotube, it creates a path for the transfer of electrons from the nanotube to the cell. Charge injection takes place by the flow of electrons from the SWNT to the cell through the antibody.

Control experiments were conducted to determine the role of the SWNT in the charge transfer process. One of the control experiment was to monitor the electrical conductance during mAb and subsequent breast cancer cell adsorption without any SWNT on the device. It was found that no dramatic modulation of the device was observed upon adsorption of IGF1R mAb and BT474 breast cancer cell on the device. This experiment demonstrated that the charge transfer process that resulted in an increase in conductance of the device for the interaction of antigen-specific mAb with their specific targets on breast cancer cells was mediated by the SWNT.

3 CONCLUSIONS

We have presented the charge transfer process between adsorbed monoclonal antibodies and their corresponding surface protein targets on breast cancer cells using SWNT devices. We observed that adsorption of antigen-specific and non-specific mAbs to SWNT resulted in a decrease in the conductance of the SWNT device. Subsequent adsorption of BT474 breast cancer cells or MCF7 breast cancer cells to IGF1R-specific mAb on the surfaces of the SWNT considerably increased the conductance of the devices. Non-specific mAbs, followed by BT474 or MCF7 breast cancer cell adsorption, did not yield a large increase in conductance. The increase in conductance was approximately proportional to the density of IGF1R expressed on the surfaces of the breast cancer cells. These results therefore imply that it is the specific interaction between the IGF1 receptor and its specific antibody that results in a large change in conductance. Consequently, these findings suggest applications of mAb-SWNT to detect a wide variety of surface markers on diseased cells, which could lead to sensitive nanotube-based biosensors for detecting specific cell surface antigens on circulating cells.

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