Nanotube Biosensor Arrays for Detection of Molecular Surface Markers in Breast Cancer Cells

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ABSTRACT

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, in extracts from as few as ten lysed tumor cells. Here we report a single nanotube field effect transistor arrays, functionalized with IGF1R-specific and Her2-specific antibodies and which exhibits selective sensing of live, intact MCF7 and BT474 human breast cancer cells in human blood. We postulate that the free energy change due to multiple simultaneous cell-antibody binding events exerted stress along the nanotube surface, decreasing its electrical conductivity due to an increase in band gap. Because the free energy change upon cell-antibody binding, the stress exerted on the nanotube, and the change in conductivity are specific to a specific antigen-antibody interaction, these properties might be used as a fingerprint for molecular sensing of circulating cancer cells.

Key Words: Field effect transistor, Carbon nanotubes, antibody, Molecular targeting, IGF1R and Her2

1. INTRODUCTION

Identification and quantitation of numerous biological molecules to generate a complex molecular profile is required for diagnosis, monitoring, and prognostic evaluation of complex diseases such as cancer. Despite outstanding progress in the area of cancer biology, significant challenges remain in translating biological knowledge of cancer surface markers into clinically relevant devices that could be used as diagnostic or monitoring tools for cancer management. Developing high-throughput diagnostic cell and tissue analysis for disease detection has remained a challenge, however [1]. Techniques for multiplexed analysis of extracted proteins for disease monitoring can be divided into four different categories: (1) time-of-flight mass spectroscopy [5-6]; (2) radioactive, and fluorescent reporting of antigen-antibody binding [2-4]; (3) electrophoretic separation and antigen-antibody binding [7]; and (4) detection of changes in surface mechanical and electrical properties due to antigen–antibody binding [8-15]. Although they all have their individual strengths, none of these techniques can directly detect circulating cancer cells that overexpress characteristic surface receptors. Combining the knowledge of cancer biology, molecular targeting, and nanotechnology, however, might enable the design of portable or hand-held devices for diagnosis, monitoring, and treatment of cancer. Here we describe one such device that exhibits selectivity to breast cancer cells overexpressing IGF1 and Her2 receptors.

2. EXPERIMENTAL

The single wall carbon nanotube transistor arrays consisted of two columns of 10 transistors per column. One side of the array was used for detecting cells that overexpress IGF1R overexpression and the other side of the array is used to detect cells that overexpress Her2. This allows multiple cell recognition in a single hand-held chip about 1 cm × 1 cm. Such arrays were also used for control experiments. The electrodes in the arrays were patterned using optical lithography, gold metallization and lift-off processes widely used in modern semiconductor industries. Therefore the devices can be fabricated at low cost using batch fabrication techniques. The gap between the electrodes was ~1 μm and the length of the nanotubes that we used ranged from 1-10 μm.

20 mg SWCNT soot, commercially obtained from Nano-Lab (Lot Number FH-P071706), was dissolved in 100 mL methanol and agitated for 24 hours in an ultrasonicator (Fisher Scientific, FS60H) at 80ºC to separate the highly entangled SWCNTs to individual or small bundle. The solution was sedimented at 16,100×g for 30 minutes and the supernatant, primarily containing individual SWCNTs, was collected while the pellets were discarded. A spray nozzle driven by 65 psi compressed nitrogen with approximately 15000 sccm volume flow rate was exploited to airbrush 0.5 mL SWCNT solution onto a 1 cm × 1 cm SiO_2 wafer. After the evaporation of methanol, the sample was studied under Jeol JSM-7400F scanning electron microscope to evaluate the partial alignment of the carbon nanotubes.

One μm thick AZ5214 photoresist was applied to the airbrushed SiO_2 wafer and defined by standard photolithography and developing procedures. Excess SWCNTs not fully or partially covered by photoresist were disassociated by ultrasonication in distilled water for ~10 minutes (prolonged sonication was avoided to prevent

damage to photoresist). Titanium thin film of 10 nm thick was thermally deposited before the 90 nm thick biocompatible gold film to provide better adhesion than just pure gold. The metal contacts were lifted-off by the dissolution of photoresist in acetone. The wafer was again ultrasonically agitated to remove SWCNTs not anchored by either metal contact. Devices were then annealed at 400ºC for 5 minutes under the protection of nitrogen/argon to reduce the contact resistance and further improve the sensitivity of the device. Finally, the devices were covered with SU8 polymer to insulate the surrounding areas of the devices from biomolecules in liquids so as to minimize the noise. The devices were attached to an HP8466 semiconductor parameter analyzer on a Signatone S1160 probe station to detect and record the electrical signals.

Succinimidyl 1-pyrenebutanoate (AnaSpec Inc.) adsorbs onto the sidewalls of SWCNTs through π-stacking [16] and reacts with exposed amino sidechains of proteins by substitution of N-hydroxysuccinimide. Thus 5 mg of succinimidyl 1-pyrenebutanoate was dissolved in 50 mL methanol, in which the SWCNT airbrushed wafer was incubated for ~30 minutes at room temperature with gentle agitation to form a monolayer on the side wall of SWCNTs. After these incubation steps, devices were rinsed three times in DI water to remove any residual reagents.

Non-specific mouse anti-human myeloma immunoglobulin G (IgG) [Catalog Number 411550], anti-IGF1R mouse monoclonal antibody Ab-3 [33255.111, EMD Biosciences] and anti-HER2 monoclonal antibody [Cat. No. OP.39] (Merck Bioscience, Calbiochem Inc.) were dissolved in phosphate-buffered saline (PBS) solution (Mediatech Inc.) at concentrations from 0.5 to 3 µg/mL were used to incubate the transistors for 3 minutes at room temperature. To evaluate the binding effect of antibody, samples were studied using Jeol JEM-2000FX transmission electron microscope as shown in Figure 5. Poly(ethylene glycol) (PEG) (Acros, average mass 8000 Da), 5 mg, dissolved in 100 mL DI water was applied to SWCNT-mAb to form a PEG monolayer on unoccupied side walls to minimize nonspecific biomolecule immobilization during experiments.

The malignant human breast cancer cell lines MCF7 and BT474, which overexpress different levels of IGF1R and Her2 [21], and non-malignant MCF10A human breast cells were purchased from American Type Culture Collection (ATCC). Cells were incubated with 2 mM glutamine, 5000 U/ml penicillin, 50 µg/ml streptomycin and 10% fetal bovine serum in DMEM at 37ºC under 5% CO2 for 48 hours before experiments. MCF7 cells were incubated with additional 7.5 nM 17-□-estradiol. Cells released from culture dishes by EDTA were sedimented at 1000×g for 5 minutes. Cell pellets were re-suspended in PBS. 1 µL of cell suspensions containing about 100,000 cells was allowed to flow on the chip to make contact with the nanotube between the electrodes.

Experiments with blood were conducted with fresh human blood volunteered by one of the authors before each experiment. First a single nanotube device that was functionalized with anti-IGF1R mAb was exposed to fresh blood to investigate any potential bio-fouling of devices. For experiments with cells, 1 µL of the cell suspension was mixed with 1 µL of blood and the resulting suspension was used for experiments.

3. RESULTS AND DISCUSSION

Figure 1 is an optical image of an SWCNT FET chip array, consisting of 20 pairs of electrodes with a single carbon nanotube between each pair. The nanotubes were anchored between the pairs of electrodes by airbrushing [16]. Figure 2 is the SEM image of a nanotube anchored between pairs of electrodes. The spacing between the electrodes is 1 µm and the nanotube diameter is ~10 nm from the inset in Figure 2.

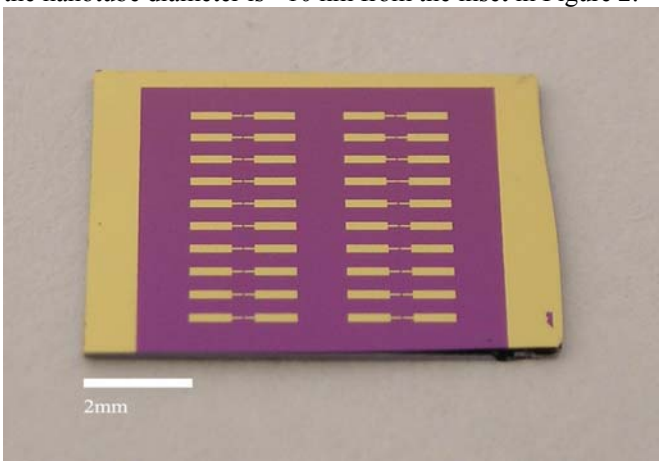

Figure 1: Optical micrograph of the chip between pair of electrodes for sensing molecular surface receptors.

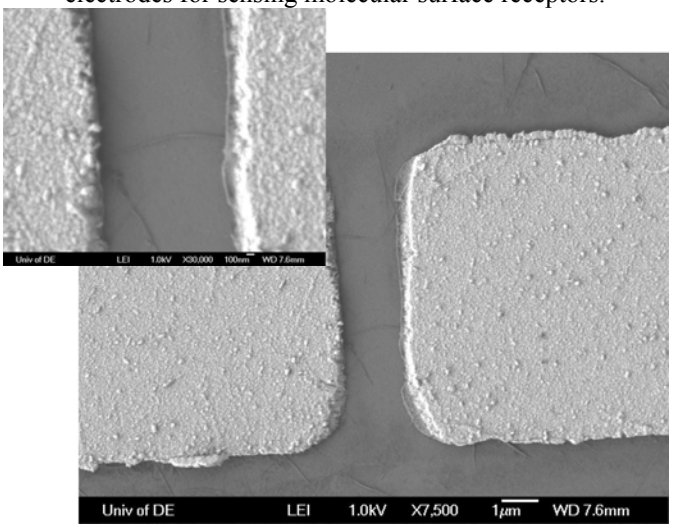

Figure 2: SEM image of nanotube anchored between the pairs of electrodes. Inset is the high magnification of the device

Following fabrication and SEM characterization of the transistors, the I-V characteristics were measured. The transfer characteristics of the devices fit the criteria for a p-type SWNT transistor, showing a strong decrease in source-drain current with increasing gate voltage as shown in Figure 3. This also shows that the transport in SWCNT is through holes (positive charge carriers). This is in line with previous reports [17].

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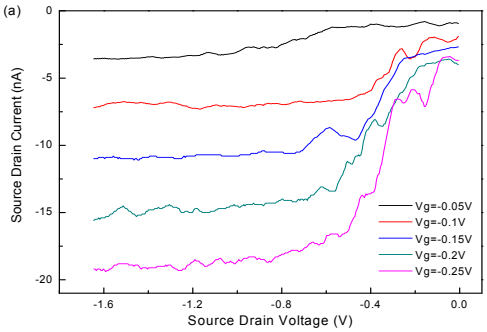

Figure 3: Source to drain current of nanotube transistors for different values of gate voltage.

Figure 4 presents the effects of increasing antibody concentration on the nanotube FET. The device behaves like a p-type semiconducting FET due to the depletion of current at all antibody concentrations tested. The negatively charged antibodies deplete the current with increasing source to drain voltage. There is a strong decrease in conductance of the channel with increasing antibody concentration showing the modulation of transfer characteristics. Figure 5 shows the set of experiments conducted on cancer cells. It can be seen that the cells expressing characteristic surface receptors underwent greater change in conductance compared to the cells that did not overexpress the receptor. The binding of antibodies to their corresponding antigens creates a change in free energy that in turn can generate stress on a surface [18]. This has been used to create bioassays of prostate specific antigens using deflections of a micro-cantilever [19]. Our results show that cancer cell binding to cognate antibodies on a nanotube device alters the electron transport properties of the nanotube. Since the nanotubes are not free to deflect and constrained between electrodes, one is left with the explanation that the nanotubes undergo strain that changes the electron transport properties. This hypothesis is supported by the theory of electron transport in single wall carbon nanotubes that predicted that strain can have a large effect on the band structure of a nanotube [20], which, in turn, has an influence on its electron transport properties.

We postulate that the binding of cancer cells simultaneously to multiple antibodies anchored to a nanotube creates a surface strain that changes the band gap and the conductivity of the nanotube. The putative increase in band gap due to cell binding effect would result in a decrease in conductivity. It has been seen in recent reports that for all semiconducting nanotubes, application of strain <0.1% leads to an increase in band gap, thereby decreasing the conductivity of the device [20]. Our results support this hypothesis, implying that the strain originates on the surface of the nanotube due to biomolecular interactions that are specific and therefore can be used as a "fingerprint" for molecular recognition. Specific antibodies create much higher change in free energy upon binding to their antigens compared to non-specific antibodies [1, 18, 19]. This has also shown to be true for ligand-receptor interaction using Atomic Force Microscope [44]. Therefore the stress on the surface of the nanotube and the accompanying change in the electron transport properties must be higher for specific pairs compared to non-specific pairs as our results indicate (60% reduction in conductivity for specific pairs compared to ~10% reduction for non-specific pairs). The origin of stress and the change in conductivity can therefore be used as a "fingerprint" for sensing various different types of biomolecules without labeling. The nature of stress remains unknown. It could be linear or torsional depending on how the antibodies interact with their receptors and their geometric shape. Such understanding requires detailed investigations using an AFM or STM.

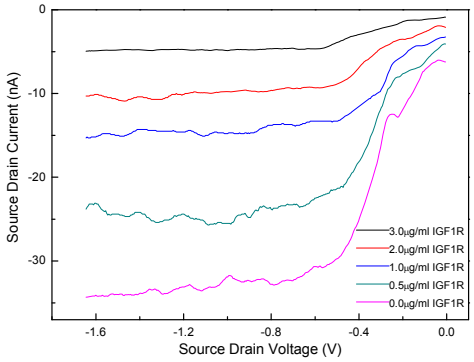

Figure 4: I-V characteristics of the transistor for various antibody concentrations.

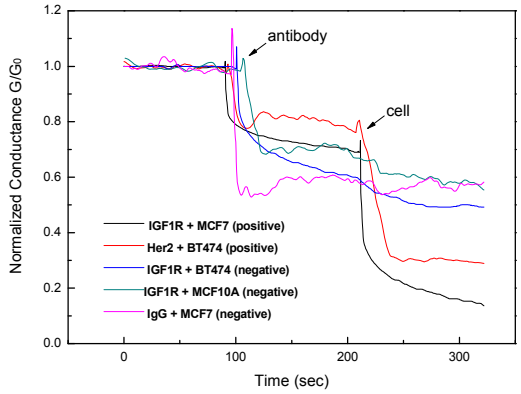

Figure 5: Change in normalized conductance when antibody and cancer cells mixed in blood were applied to the device. Antibodies and cells were applied at about 100th second and 220th second. Two specific pairs of antibody and cell showed conductance reduction and three non-specific pair had little impact.

4. CONCLUSIONS

There are many innovations in the work that is presented here: First, the single nanotube electronic device that we report here for cancer cell detection is fabricated by airbrushing, rather than by e-beam lithography, which is time

consuming and expensive. The nanotubes are aligned between lithographically patterned electrodes using the torque generated by the flow of air, prior to subsequent lithography and metallization steps, to ensure reliable and stable contacts. Such a protocol ensures both high yields, low cost, and acceptable performance.

Second, the cancer cell that is detected here, ~10 μm in diameter, is 10-15 times larger than the spacing between the nanotube contact electrodes. While we use live cells in our experiments, it is only the proteins that are precisely sensed that makes this technology viable for sensing DNA, proteins as well as cells. The electrodes are completely isolated from the cell by an insulating coating. Therefore, when a cell comes in contact with the device, only the antibodies on the surface of the nanotube can contact the surface of the cell, as all the other areas of the device are isolated. The change in conductivity can therefore only arise from the interaction of cell surface proteins with antibodies that are functionalized on the nanotube surface.

Third, the device sensitivity is high due to the use of a single nanotube device, as opposed to lower sensitivity nanotube bundles that were reported in the past. Nanotube bundles tend to be more conductive compared to single nanotube devices, thereby masking the actual biological events and producing low signal to noise ratio.

This technology for live cancer cell detection is label-free, displays single cell sensitivity, high selectivity, high reproducibility, easy fabrication, and low cost compared to nanoscale top-down manufacturing. We postulate that the change in free energy associated with multiple simultaneous cell-antibody-nanotube interactions generates a stress on the surface of the nanotube that decreases its conductivity.

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