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Carbon nanotube thin film transistors for biomedical applications.

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CARBON NANOTUBE THIN FILM TRANSISTORS FOR BIOMEDICAL APPLICATIONS

By

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Thesis Director (Balaji Panchapakesan)

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ABSTRACT

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The application of carbon nanotubes (CNTs) has captivated the curiosity of today's experts due to the escalating potential in the field of electronic detection of biomolecules. Their extreme environmental sensitivity and small size make them ideal candidates for future biosensing technologies. Recent studies have shown that the binding of receptor proteins (biomolecules located at the membrane of cells) with their corresponding antibodies immobilized on a carbon nanotube surface causes changes in the electrical properties of carbon nanotubes and have been measured with a carbon nanotube field effect transistor (CNTFET). This specific molecular interaction and sensitivity is promising for the direct detection of live cells in blood.

In this study, a biosensor was developed based on carbon nanotube thin film transistors for the purpose of electrically detecting breast cancer cells (MCF-7) in blood. The electrical response of specific and non-specific interactions between anchored antibodies onto the carbon nanotube film surface and breast cancer cells mixed with

blood were monitored and recorded. The electrical measurements indicate that devices functionalized with specific antibodies (anti-IGF1R) experience large conductivity drops (~60 %). However for those device printed with non-specific antibodies (anti-IgG), small changes (~10 %) in conductivity are measured. It is postulated that the addition of increasing number of MCF-7cells mixed with blood on a CNT surface functionalized with specific antibodies (anti-IGF1R) acts as a chemical gate modulating the current flow. Biosensing mechanistic studies using a liquid gated CNTFET, confirmed that the specific antibody-receptor binding can be attributed to electrostatic gating effect by which cancer cells can be screened in blood.

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CHAPTER 1

INTRODUCTION

Carbon nanotubes (CNTs) have been the cornerstone of many research projects since their discovery by Japanese physicist, Sumio Iijima in 1991 [1]. Owing to their unique structure, mechanical, and electrical properties, they have been proposed for many applications. One of the most interesting carbon nanotubes applications is the electrical detection of biomolecules and biomolecular interactions for the development of new biosensors and biosensing techniques.

The electrical detection or monitoring of biomolecules has been accomplished by integrating carbon nanotubes, particularly, single-walled carbon nanotubes (SWCNTs) into field effect transistors (FETs) to form sensor platforms [2]. In these electrical devices, carbon nanotubes play the role of transducers by translating biological interactions into characteristic electrical signals. Upon the binding of electrically charged target molecules to ligands linked to the CNT surface, the device experiences a change in conductance [3]. Consequently, if specific biological interactions such as those that occur in malignant diseases are studied and characterized through carbon nanotubes field

effect transistors (CNTFETs), new low-cost and portable bio-analytical platforms would emerge.

In the past, CNTFETs have been used for the detection of various biological species such as proteins, DNA hybrids, viruses, bacteria and other analytes alike [4-7]. In this project, we developed thin film transistors to detect human breast cancer cells (MCF-7) mixed in blood. This was accomplished though the implementation of a technique that involves receptor proteins binding to their corresponding antibodies. Carbon nanotubes were functionalized with antibodies specific to cancer cell surface markers. At the onset of molecular interactions, the carbon nanotubes experience changes in their electronic properties which were measured by the CNTFETs. In the following, the motivation, the contributions of this research and a general overview of the thesis are presented.

1.1 Motivation

The spontaneous appearance of circulating tumor cells (CTCs) in the bloodstream is a signal of invasive behavior of cancer cells. The detection of such cancer cells has the possibility of providing a tool for cancer prognosis, diagnosis of minimal residual disease, evaluation of tumor sensitivity to anticancer drugs, and personalized cancer therapy and management [8]. As a result, the early diagnosis of invasive cancers could be possible with highly sensitive and specific detection of CTCs.

The motivation of this thesis id to develop innovative biomedical devices to detect cancer cells stems from the limitations of current diagnostic tools. Current methods in biomolecular detection have lengthy turn-over times, laborious sample purification procedures, and lack the sensitivity and specificity for single analyte screening [9]. The

electronic detection of biomolecules has shown the potential of becoming an effective alternative to conventional optical bio-detection methods [10]. Therefore, in order to address the problem, novel and cost-effective biomedical nano-devices such as CNTFET-based biosensors need to be studied and developed in order to achieve selective cancer detection.

1.2 Contributions by the Research

This work focuses on the design of biosensors based on an array of CNT thin film transistors to detect human breast cancer cells (MCF-7) mixed in blood. The biosensors designed use specific ligands (antibodies) functionalized on the surface of the CNTs to target physiological cancer surface markers. A biosensing mechanism that involves electrostatic interactions and charge transfer between CNTs and antibody–cell surface receptor binding is used to determine the specific biological interactions using carbon nanotube-based devices. The electrostatic interactions induce changes in the electrical properties of the CNTs, which were readily measured by the CNT thin film transistors.

1.3 Overview of the Thesis

This thesis is divided in five chapters. The first chapter presents a brief introduction and motivation of the research. The basic background of the present work is discussed in Chapter 2, which include a brief description on breast cancer cells, cancer surface markers, carbon nanotubes and why their integration in field-effect transistors holds the potential for the detection of cancer cells in blood. Chapter 3 includes the design and fabrication of biosensors based on CNT thin film transistors. Chapter 4

focuses on the experimental results obtained and discussion of such results. Finally, conclusions and future work are presented in Chapter 5.

CHAPTER 2

CARBON NANOTUBE TRANSISTORS FOR BIOSENSING APPLICATIONS

Recent discoveries in the field of cancer biology have achieved a better understanding of cancer surface markers (receptor proteins located on the surface of cancer cells). However, utilizing this biological information to develop relevant cancer diagnostic methods remains a difficult task. In order to address this challenge, in this work, CNT thin film transistors were developed to electronically detect human breast cancer cells in blood. Before continuing any further, it is necessary to present a basic background of the investigation. From herein, a brief description of circulating tumor cells, breast cancer cells MCF-7 and cancer surface markers are presented. Furthermore, carbon nanotubes and their properties are discussed, as well as, CNT functionalization methods. Lastly, carbon nanotube field-effect transistors and their biomedical applications are summarized.

2.1 Circulating Tumor Cells

Circulating tumor cells (CTCs) are cells that escape from a primary tumor site, travel in the bloodstream to a secondary site and begin to form a new tumor, causing metastasis. Metastasis is the spread of cancer from its initial location to other places in the body (see Figure 2.1-a) [11]. According to studies, circulating breast cancer cells are more frequently detected at advanced clinical stages rather than at early stages of disease [12]. The late detection of CTCs has been correlated to lethal metastases, one of the main factors that lead to cancer treatment failure. It was reported that women with primary breast cancer were screened for disseminated tumor cells (DTC; similar to CTC) found in bone marrow. Several years later, a check-up indicated that ~ 30 % of the women who tested positive for DTCs had larger number of deaths cases caused by cancer than those women who tested negative for DTCs [13-14]. As a result, the early detection of CTCs has the potential of playing critical role in future cancer diagnostic and management methods.

The detection of CTCs in peripheral blood has remained a challenge till this day. This is due to their low concentration compared to other components found in blood. It has been reported that a patient with metastatic cancer only has ~ 1 to 10 CTCs mixed with 10 million leukocytes and 5 billion erythrocytes in 1 mL of blood [15]. Moreover, distinguishing CTCs from normal epithelial cells and leukocytes also poses a great task. Techniques for CTCs screening have been proposed including immuno-cytometry, immuno-magnetic and reverse transcriptase polymerase chain reaction (RT-PCR) [16]. However, these usually require laborious sample preparation procedures.

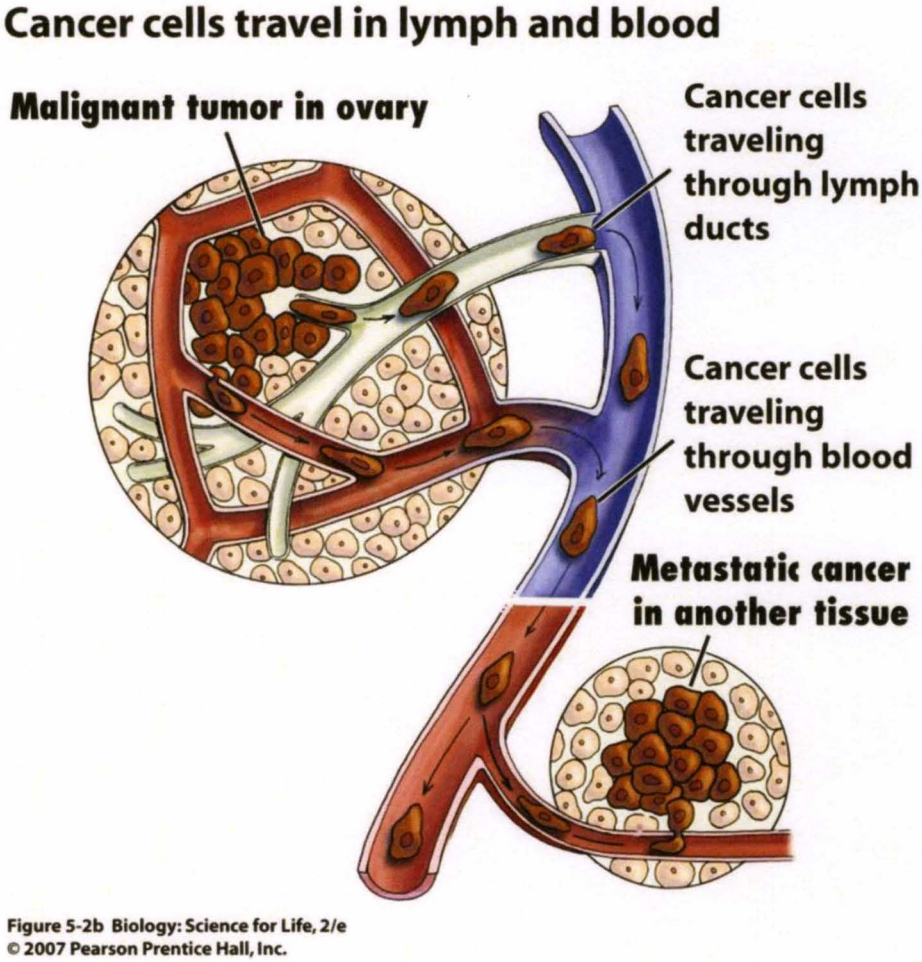

Figure 2.1-a Illustration of metastasis, when a cancer cell leaves the tumor site and enters the blood stream and moves to a secondary location to form a new tumor site [17].

2.2 Breast Cancer Cells (MCF-7)

MCF-7 is a breast cancer cell line. Figure 2.2-a depicts an image of MCF-7 cells. It was first isolated in 1970 from a patient with metastatic breast cancer [18]. MCF-7 is a great model for cancer research because it is one of the most used and best characterized of all human breast cancer cell lines. Additionally, this cell line can be used to study malignant progression. Work by Bullinger *et al*, showed that under appropriate

endocrinologic and physiologic pressures, variants of MCF-7 with more advanced phenotypes are observed [19].

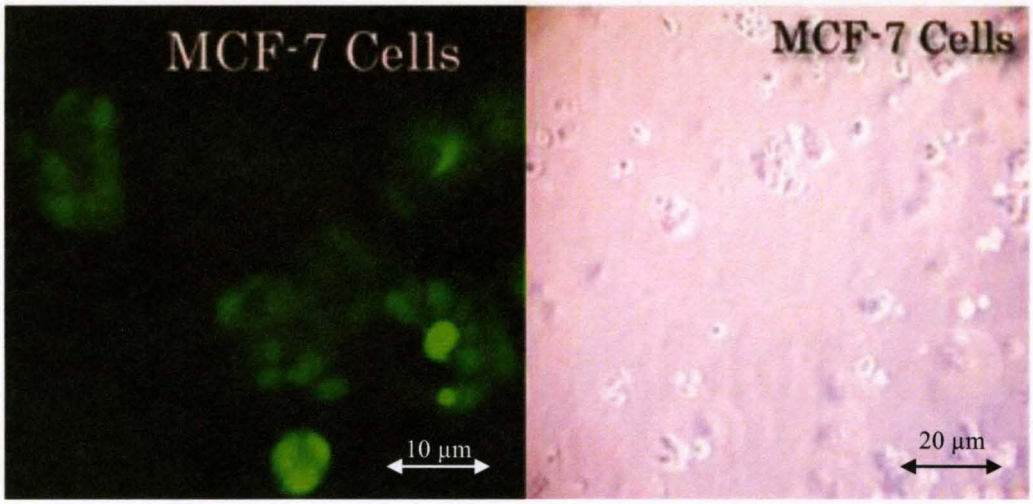

Figure 2.2-b A fluorescent microscopy image and optical microscope image of MCF-7 cells [20].

2.3 Cancer Surface Markers

Cancer surface markers are receptor proteins located at the membrane of a specific cancer cell type. Receptor proteins (IGF1R and HER2) bind specifically to their respective antibodies (anti-IGF1R and anti-HER2) versus binding to non-specific antibodies such as IgG. (see Figure 2.3-a) [21]. Advances in cancer biology has lead to the identification of membrane proteins of cultured breast cancer cell line MCF-7 and has been discovered that insulin-like growth receptor (IGF1R) and human epithelial growth factor receptor 2 (HER2) are overexpressed by breast cancer cells [22-25]. Studies have indicated that MCF-7 cells express IGF1R at higher levels than other known human breast cancer cell lines [22]. Consequently, the use IGF1R antibodies to target IGF1R

proteins and detect circulating breast cancer cells hold incredible potential for the development of powerful cancer diagnostic tools.

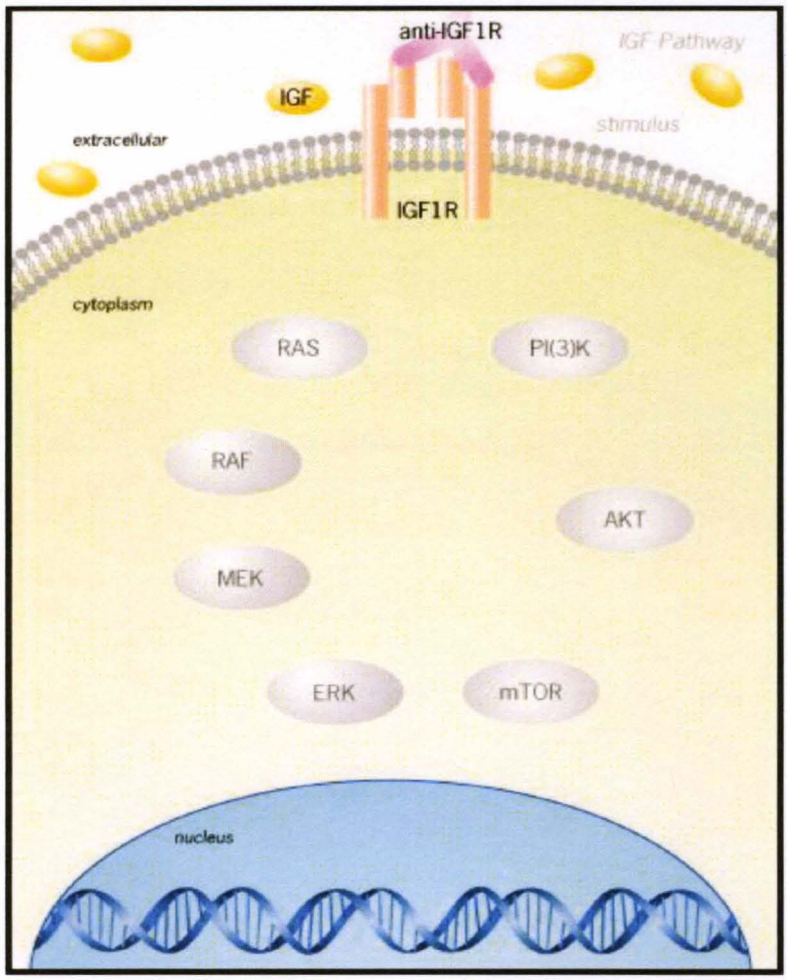

Figure 2.3-a An illustration of IGF1R antibodies binding to IGF1R proteins at surface of cell membrane [26].

2.4 Carbon Nanotubes

Owing to the novel properties that carbon nanotubes exhibit, they are excellent candidates as tranducing elements in biosensors. Carbon nanotubes are graphene sheets

seamlessly wrapped into cylindrical tubes, with nano-scaled diameters often acknowledged for having extremely high aspect ratios compared to other materials. It has been reported that typical diameters of SWCNT range between 0.4 nm to 2 nm, while their lengths can be up to 1.5 cm [27]. CNTs come in two forms multi-walled or single-walled. Single-walled carbon nanotubes (SWCNTs) consist of a one atom thick planar sheet of carbon atoms organized a honeycomb crystal lattice, while multi-walled carbon nanotubes (MWCNTs) are composed of several layers of graphene sheets (Figure 2.4-a). In the context of this project, when CNTs are discussed, it will be referring to SWCNTs.

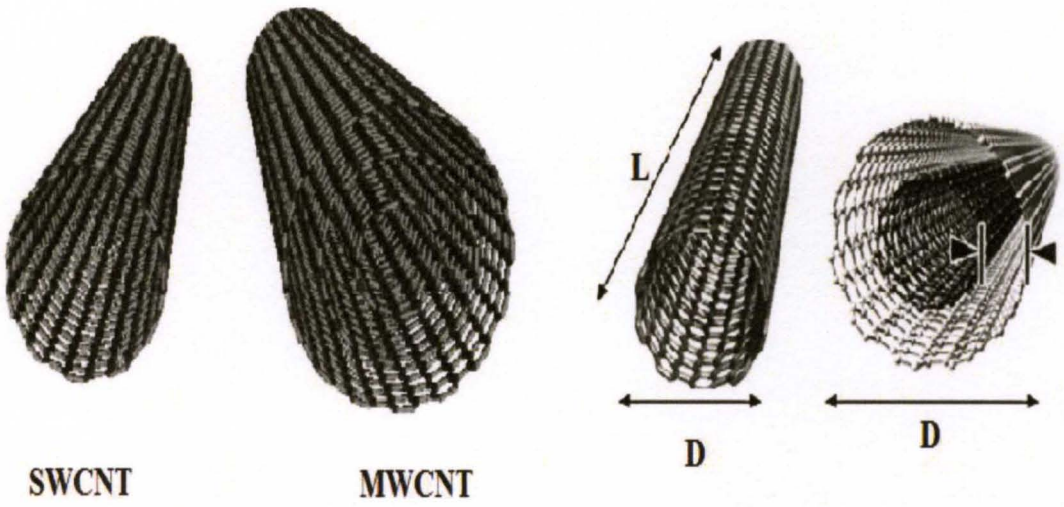

Figure 2.4-a Schematic representation of the SWCNT, MWCNT and the aspect ratio parameters for a carbon nanotube [28].

The structure of SWCNT is usually described by the chiral vector (C_h), which is shown in Figure 2.4-b. The chiral vector depends on a pair of integers (n,m). These integers express the relative position of the atoms in a graphene strip when they are rolled onto each other to form the tube. When integers n=m, the carbon nanotubes have an

"armchair" configuration. When n or m=0, carbon nanotubes have a "zig-zag" configuration. Any other configuration is denoted as chiral [1].

The chirality of CNTs has been linked to their electrical behavior. Armchair nanotubes present metallic behavior, zig-zag nanotubes are semiconducting and chiral nanotubes can be metallic or semiconducting depending on the diameter of the tube and wrapping angle [29-30].

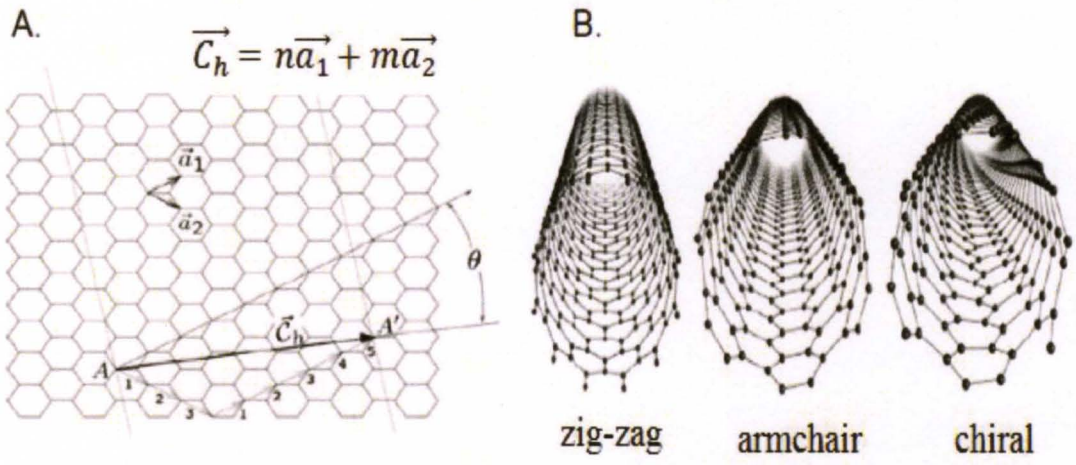

Figure 2.4-b (A) Graphene honeycomb network with lattice vectors a_1 and a_2. The chiral vector represents the possible wrapping of the 2D graphene sheet into a tubular form. The direction perpendicular to C_h is the tube axis. (B) The 3 different configurations of CNTs [31].

Carbon nanotubes are composed of sp^2 bonds which are stronger than sp^3 bonds found in diamonds. In carbon nanotubes, three out of the four electrons found in the outer shell of carbons participate in the bonding with neighboring carbons. The fourth electron is located in the p-orbital perpendicular to the hexagonal lattice. These p-orbital

electrons are distributed in the valence (π) and conduction (π^*) energy levels [32]. These levels are separated by an energy band gap (E_g). The energy band gap is representative of the amount of energy it takes to free an electron and become a mobile charge carrier. The value of the band gap is indicative of the electrical conductivity behavior of the nanotube. In the case of insulating CNTs, the band gap is larger than ~ 5 eV [33]. Semiconducting nanotubes have slightly lower energy band gaps while metallic nanotubes have no gap between the conduction and valence bands. The nanotube's diameter plays an important role in their electrical behavior as well. According to Heller *et al,* nanotubes with smaller diameters usually have larger band gaps [34].

Additionally, the position of the Fermy energy is yet another parameter used for the classification of CNTs as metallic or semiconducting. For undoped carbon nanotubes, the E_F is at the charge neutrality point (E_F=0). However, when the carbon nanotube undergoes electron (n) or hole (p) doping the E_F shifts up or downward. When Fermi level shifts exceed the energy separation between subbands, a semiconducting nanotube becomes metallic [35].

One of the most interesting properties of SWCNTs, especially for biosensing applications, is the fact that all their atoms lie at the surface and have the ability to participate in electrostatic interactions. CNTs are extremely sensitive to their environment. The presence of any external substance causes changes in their electrical properties. Thus, SWCNTs have the ability for highly sensitive detection of biomolecules through the depletion or accumulation of charge carriers caused by the binding of molecules at the surface [36].

2.5 *Antibody Functionalization of Carbon Nanotubes*

The immobilization of antibodies over the surface of the carbon nanotubes is fundamental in the development of a biosensor. In the past, antibodies have been functionalized either by covalent or non-covalent attachment methods [37]. In this project, a non-covalent approach was used for the immobilization of IGF1R, HE2 and IgG antibodies on the surface of the CNTs. Covalent methods provide chemical stability and functionality; yet have been shown to hamper electrical and mechanical properties of SWCNTs [37-38]. Non-covalent attachment, although not as robust as covalent immobilization, preserves the electrical and mechanical properties of carbon nanotubes [39].

Investigations show that supramolecular forces play a significant role in the physical adsorption of proteins (such as antibodies) onto CNTs [40]. Hydrophobic interactions are one of the major sources of carbon nanotube and protein binding [41]. Protein surfaces with high degree of hydrophobicity display a stronger affinity to the surface. On the same note, an increased protein adsorption is also observed when the surface is more hydrophobic [40-42].

2.6 *Carbon Nanotube Field-Effect Transistors (CNTFETs)*

CNTFETs are electronic devices where the conduction of current occurs along the surface of carbon nanotubes [2]. Figure 2.6-a showcases an illustration of a typical CNTFET. It consists of three electrodes denoted as the source, drain and gate. Voltage is applied to both the source and gate electrode, while the drain electrode is held at

ground. The application of a voltage on the gate electrode produces an electric field that alters the amount of current that travels through the conducting channel.

There are two main gate electrode configurations used for biosensing with CNTFETs: a "back" gated architecture and an electrolyte (or liquid) gated architecture. Back gated CNTFETs are those which the gate terminal is located on back of the substrate (usually silicon) as presented in Figure 2.6-a. In this architecture, a silicon oxide layer (or other dielectric material) acts as an insulator between the Si substrate and SWCNTs. When a voltage is applied to the back gate, an electric field is produced across the dielectric layer. By modifying the electric field, the density of mobile charges on the SWCNTs can also be altered.

Electrolyte gated transistors function in a liquid environment. For electrolyte gated CNTFETs, solution containing an analyte is placed in direct contact with the SWCNTs and a probe is inserted into the liquid, as shown in Figure 2.6-a. The electrochemical potential of the solution is controlled with the gate voltage [42]. When a gate voltage is applied, the interface of CNT-electrolyte solution becomes polarized. As a result, the double layer forms a gate capacitance, which can be large [35]. This type of experimental setup can be ideal for biosensing. Resulting from the facts that most biological processes take place in liquid environments and real time molecular interaction can be observed [43]. Moreover, Krüger *et al,* and Rosenblatt *et al,* have demonstrated that highly performing CNTFETs were achieved through electrolyte gating [35, 43].

The performance of a CNTFET for detecting applications is usually described by the transfer characteristic (or device characteristic, DC) of the device [9]. This is the amount of current that flows between the drain and source as a result of the application of

different gate voltages (V_g) at a fixed source-drain voltage (V_{sd}). It is typically displayed as a current versus gate voltage plot (see Figure 2.6-b). Another way that transistor performance is expressed derives from the DC and it is referred as the ON/OFF current ratio. The ON/OFF ratio is attained by dividing the current at the peak of the DC and valley of DC. An excellent performing FET usually has an ON/OFF of several magnitudes.

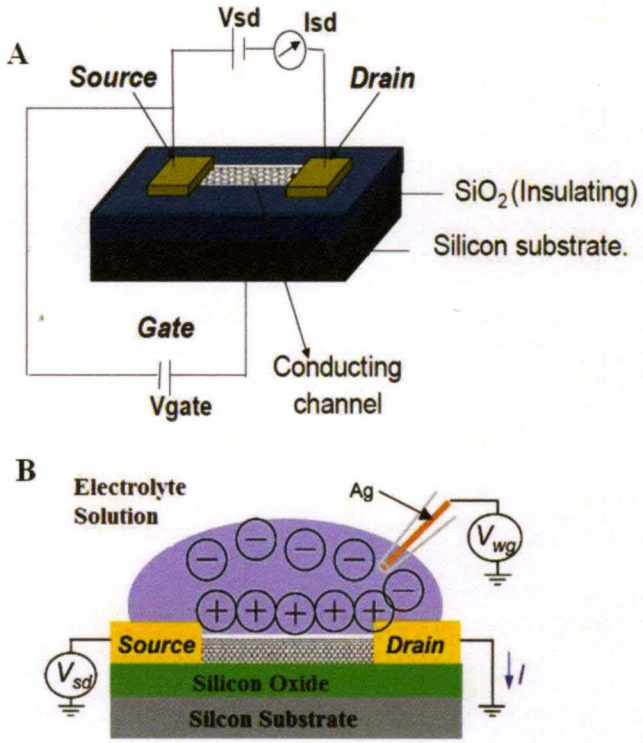

Figure 2.6-a Schematic of a typical (A) back gated CNTFET and its components. (B) a liquid gated CNTFET where a probe is interested into a liquid and used as a gate terminal [41].

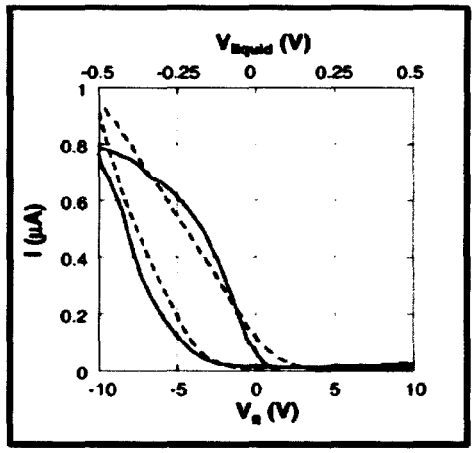

Figure 2.6-b A diagram of the transfer characteristic of a p-type CNTFET with both back gate and liquid gate configurations. The solid line is the performance of the device measure in air and the dashed line is the performance of the device in water [44].

Similar to silicon based FETs, CNTFETs display n-type or p-type behavior. N-type CNTFETs are those that at negative gate voltages conduct little to no current and are said to be at the "OFF" state. While, at positive gate voltages, electrons flow more easily and the transistor is said to be at the "ON" state. P-type CNTFETs, on the other hand, behave in the opposite manner. At negative gate voltages, they are at an "ON" state, but are "OFF" at positive voltages. In normal ambient conditions, CNTFETs behave mostly as p-type transistors [45]. This is due to the fact CNTs are doped with oxygen molecules when exposed to air. N-type behavior in CNTFETs has also been observed, but only when they have been subjected to chemical doping. Reports have indicated that adsorption of electron-donating molecules or charge transfer from alkali metals lead to n-type CNTFETs [46].

A parameter that influences the performance of a CNTFET is the Schottky barrier (SB). The SB is the rectifying barrier at the interface of the metal electrode and CNT. It

represents the mismatch in the energy position of the majority carrier band edge of the CNT and the metal's Fermi level across the interface. Usually the CNT and metal have different work functions. Therefore a donation of electrons from the metal to the partially depleted valence band of the CNT occurs when equilibrium at the Fermi level is achieved [47]. The SB reduces the transport of holes from the metal electrode to the valence band of the SWCNT at positive gate voltages. This leads to a small current flow and the device is in the "OFF" state. At more negative gate voltages, the SB increases the hole transport into SWNCT. Consequently, the flow of current increases and the device is in the "ON" state [2].

Two different types of CNTFETs have been developed and studied. One consists of a single SWCNT anchored between the source and drain electrodes. This type of device is very sensitive displaying ON/OFF current ratios of several orders of magnitudes [48]. Despite their outstanding performance, single CNTFET are not practical for mass production. There is significant performance discrepancies between devices resulting from variability of chirality and diameter of the nanotube used. Furthermore, the Schottky barrier varies substantially from device to device [10].

The second type of CNTFET consists of a network (or film) of CNTs operating as the conducting channel, as shown in Figure 2.6-c. Carbon nanotubes in a typical network usually consist of a mixture of semiconducting and metallic nanotubes. Nonetheless, new approaches in CNT synthesis and filtration have made it possible to produce CNT soots with 99 % of either metallic or semiconducting CNTs. For the CNTFETs fabricated in this project, the CNT thin film was composed of both semiconducting (70 %) and metallic (30 %) CNTs. Hence, for the sake of not loosing focus on this thesis, from

herein when a network CNTs is discussed, it consists of both semiconducting and metallic CNTs.

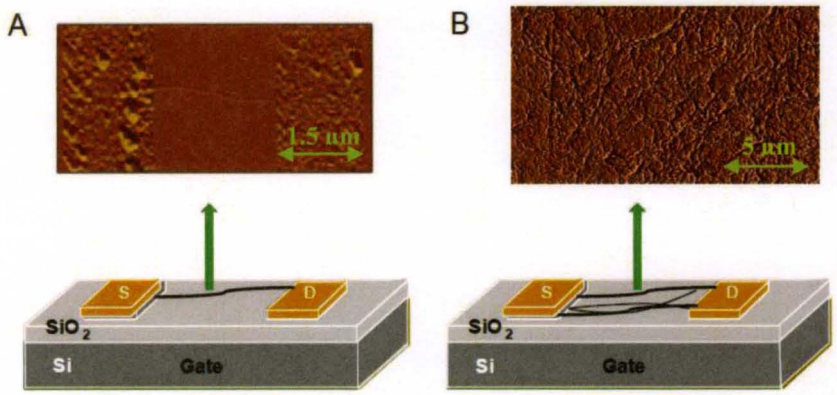

Figure 2.6-c CNTFETs based on (A) a single CNT and (B) a network (film) of CNTs. Inserts are AFM scans that illustrate the topography of the devices [10].

Carbon nanotube thin film devices consisting of a mixture of metallic and semiconducting CNTs tend to be less sensitive but are more reproducible than single CNT devices. Their overall performance depends on the collective properties of each carbon nanotube. Thus, the density of the CNTs plays a critical role on how the device behaves. For example, in dense CNT films, metallic CNTs tend to screen the gate voltage and lower the ON/OFF ratio of the device [46]. In thin CNT film devices, percolation issues increase the ON/OFF ratio. Hu *et al.* explains that in low density networks, there are only a small amount of paths for current to flow and the possibility of those paths being non-continuous is high. This can lead to higher ON/OFF ratios than those observed for thick films [49].

2.7 CNTFETs for Biomedical Applications

For years now, CNTFETs have been proposed for many biosensing applications. In CNTFETs, a current flows through the surface of the nanotube structure and is in direct contact with the environment. Therefore, at the occurrence of any changes in the proximity of the CNT surface, a change can be measured in the devices' transfer characteristic.

When a molecule approaches the CNT surfaces, four main phenomena can take place: electrostatic gating, Schottky barrier effect, reduced gating efficiency, or reduced carrier mobility (scattering effect) [49-50]. However, the most reported biosensing mechanisms have been those of electrostatic gating and scattering effects. Charge transfer involves the molecule donating a hole or an electron to the CNT. The device characteristic shifts toward more positive gate voltage when the molecule donates a hole and shifts toward more negative gate voltages when an electron is donated (see Figure 2.7-a). Alternatively, scattering effects due to the presence of the molecules cause a decrease in mobility. In this case, the DC shifts downward as seen in Figure 2.7-a. This type of behavior is also observed when CNT undergo mechanical distortion [10].

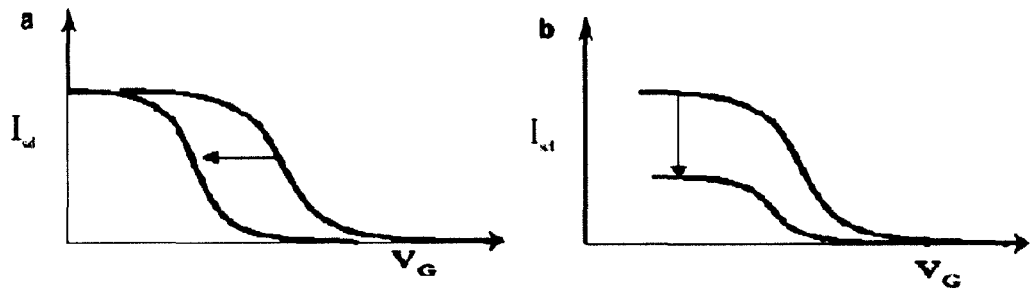

Figure 2.7-a Transfer characteristic of (a) charge transfer between biomolecule and CNT and (b) scattering effects caused by the presence of molecules [10].

In the past, CNTFETs have been used to detect a large range of biomolecules such as glucose, proteins like IgG, DNA, and viruses, among others [3-6]. In 2008, Ning Shao reported the detection of CTCs in blood using a field effect transistor composed of small bundle CNTs [51]. Although, he successfully showed the detection of a single CTC in blood, single and small bundle CNTFETs are difficult to reproduce making them ineligible for mass production. Carbon nanotube thin films transistors on the other hand are easily fabricated and tend to behave more consistently from film to film. Carbon nanotube thin films transistors have not been used yet in the detection of CTCs. Thus, in the present work, thin film carbon nanotube field effect transistors were developed for the purpose of detecting breast cancer cells (MCF-7) in whole blood.

CHAPTER 3

METHODS AND MATERIALS

In this chapter, a comprehensive description of the materials, reagents, equipment, and protocols utilized during the development and testing of the CNTFET based biosensor are discussed. The experimental approach, CNTFET fabrication and functionalization methods, as well as, biomolecule detection are presented. This chapter provides a detailed procedure that can be utilized to reproduce the findings of this document, while offering a foundation for future developments.

3.1 Experimental Approach

In the present research, an array of CNT thin films transistors was developed for the detection of circulating breast cancer cells in blood. Although single CNTFETs have shown to be more sensitive, CNTFETs based on CNT thin films offer some advantages. They are easier to fabricate and reproduce and have more surface area for biomolecule immobilization and target binding. During the course of the project, two designs were implemented. The first design consisted of a large CNT thin film (with a surface area of $\sim 4mm^2$) anchored between two chromium (Cr)/ gold (Au) electrodes separated by a gap of $\sim$ 1mm as in Figure 3.1-a. In this design, a back silicon gate configuration was used. These devices were functionalized with three different antibodies (specific and

non-specific) and the interaction of 5 μL of blood mixed with different MCF-7 cells concentrations was examined.

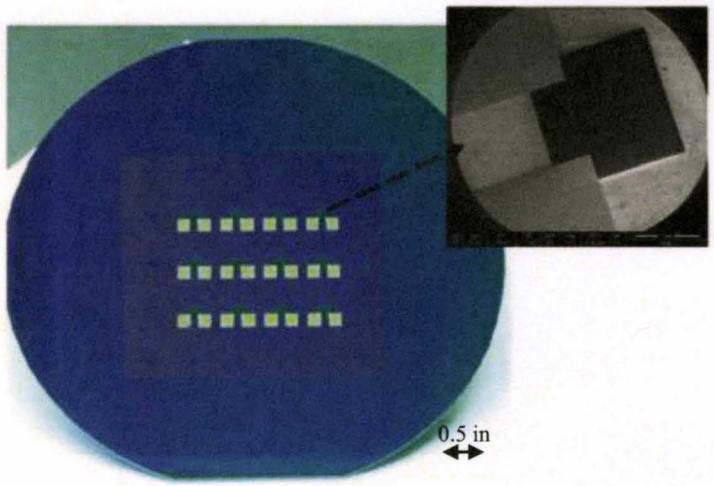

Figure 3.1-a An image of the wafer containing the array of the first design of CNTFETs. A total of 12 devices were fabricated in a single substrate. The inset is an SEM image of a single device.

A second design of CNTFETs was manufactured in order to investigate the sensing mechanisms and gain a better understanding of the phenomena that occurs during CTCs screening with the CNT thin film devices mentioned above (refer to Figure 3.1-b). For this design, a localized liquid gate configuration was utilized to modulate the current in the conducting channel. According to Dekker *et al*, insight on sensing mechanisms can be achieved by observing the effects of protein adsorption or binding on the conductance of the device at different liquid gate potentials [50]. These CNTFETs were scaled down to a smaller film area of ~0.008 mm^2 with only ~ 100 μm gap between patterned electrodes in order to observe any charge transfer and minimize diffusive

behavior of charged particles. A more detailed description regarding the biosensor structure, antibody functionalization methods and electrical characterization is provided below for both biosensors.

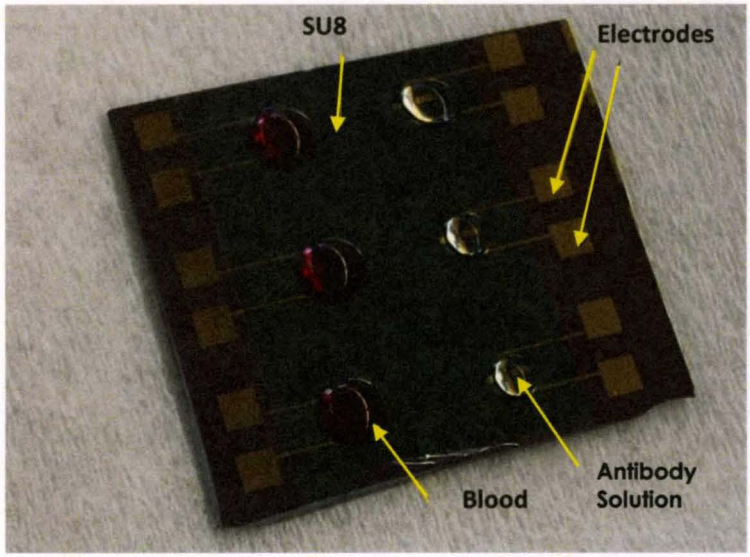

Figure 3.1-b An image of a chip with an array of the electrolyte gated devices presented in this research.

3.2 CNTFET-based Biosensor Architecture

The structures of both CNTFET-based biosensors are presented in Figure 3.2-a. The biosensor consists of a silicon oxidized substrate used as both an insulator (in the case of the oxide) and back gate (in the case of the silicon). A semiconducting channel was formed by a patterned thin CNT film, located on top of the dielectric film. Two metal electrodes separated by ~1 mm gap for the first design and ~100 μm gap for the second

design that act as the drain and source and anchor the thin CN T film. A localized liquid gate consisting of 1 μL of ~10mM NaCl solution that sits on top of the nanotube conducting channel. Finally, a SU-8 layer was deposited to isolate the electrodes from the sensing area and avoid any electrochemistry between the solution, electrodes and probes.

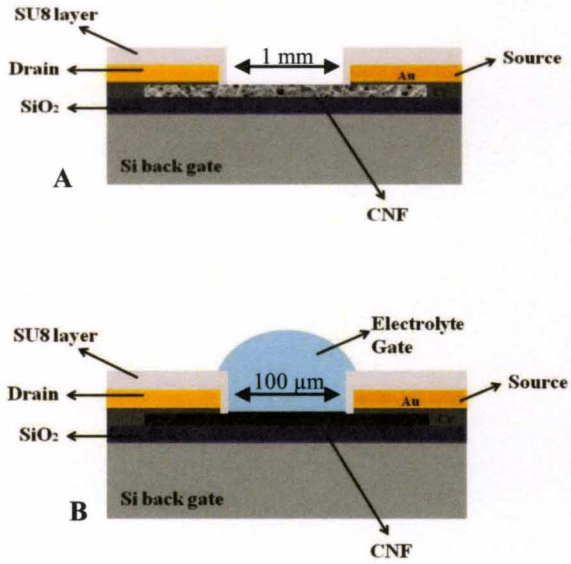

Figure 3.2-a Schematic illustration of both biosensor designs based on CNTFETs. (A) First design consisting of a large CNT film anchored between two electrodes at a distance of ~1 mm. (B) Second design used for sensing mechanistic studies by applying a liquid gate potential. A smaller CNT film area is anchored between two electrodes set to ~ 100 μm apart.

3.3 Fabrication of CNTFET-based Biosensor

A batch fabrication process was used for both designs. An advantage of batch

fabrication is that multiple testing can be conducted on a single substrate while concurrently limiting large fluctuations in device electrical characteristics.

Transistor fabrication consisted of three different lithography steps and a metal deposition step. A 4" n-type doped silicon substrate was used as a platform to build the biosensors. A ~500 nm thick oxide is grown by thermal oxidation. The substrate was then placed in the MARCH RIE system for an oxygen plasma clean. Treating surfaces with O_2 plasma has been known to change the surface energy and improve the wettability of a surface [52].

A carbon nanotube solution was prepared from commercially obtained single wall carbon nanotubes dispersed in isopropyl alcohol to a concentration of ~0.007mg/ml. The solution was placed in an ultrasonication bath for ~ 24 hours to separate the highly entangled SWCNTs into individual or small bundle SWCNTs (refer to Figure 3.3-a).

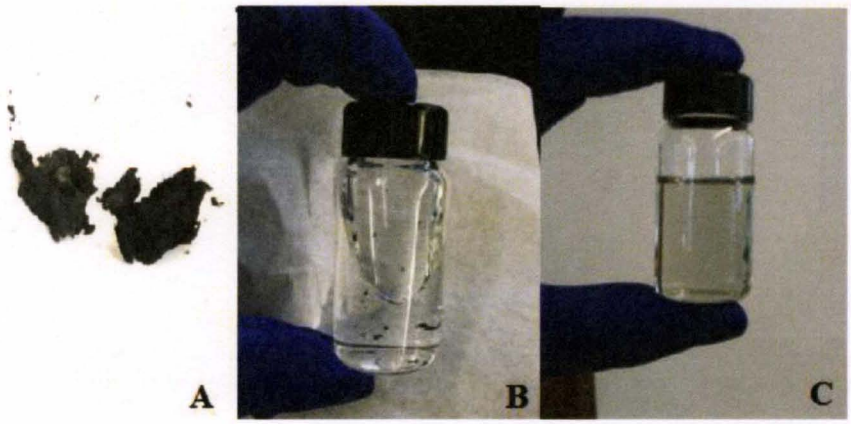

Figure 3.3-a Images of (A) CNT soot, (B) undispersed CNT solution and (C) dispersed CNT solution.

A vacuum filtration process was used to produce the carbon nanotube thin films. 30 mL of the dispersed CNT solution was vacuum filtered through a Millipore mixed cellulose ester (MCE) membrane. The CNT film was then transferred and bonded to a silicon oxide substrate by a procedure reported previously [53]. After vacuum filtration, the wet CNT film and membrane were transferred onto the silicon oxide substrate and subjected to compressive loading between two heavy flanges (cushioned by porous filter paper) for ~ 20min. During compressive loading, the CNT film and membrane dries and adheres to the substrate. The MCE membrane is then dissolved by placing the wafer/ nanotube/membrane composite in an acetone vapor bath. The condensed acetone washes the membrane off, leaving a wrinkle free CNT film on the substrate.

The CNT film thickness was controlled by the CNT solution concentration and volume used during vacuum filtration. Figure 3.3-b illustrates different CNT films attained from two different concentrations. In this work, visibly transparent thin films were produced for the biosensor as shown in Figure 3.3-b.

After the CNT film was thoroughly rinsed and baked to remove any residual impurities, the film was patterned. Defining the carbon nanotube conducting channel was accomplished by photolithography and a successive step of O_2 plasma etching in an inductively coupled plasma system (ICP), as explained in Shaoxin Lu *et al,* and Ashkan Behnam *et al,* work [54-55]. A 2.7μm thick photoresist and photolithography were used to mask the desired resulting pattern. The thickness choice of photoresist layer was important since the O_2 plasma etching does not selectively etch the carbon nanotubes and also removes the photoresist. The ICP system was set to a power of 200 W, bias power of 100 W and O_2 flow rate of 50 sccm, resulting in an etch rate of ~ 5.4nm/s. After

etching, the substrate is gently washed with acetone, methanol, and isopropyl alcohol to remove the etching mask and remove any other impurities resulting from the photolithography process. The etching process produces anisotropic etch profiles necessary for carbon nanotube integration in microfabricated devices.

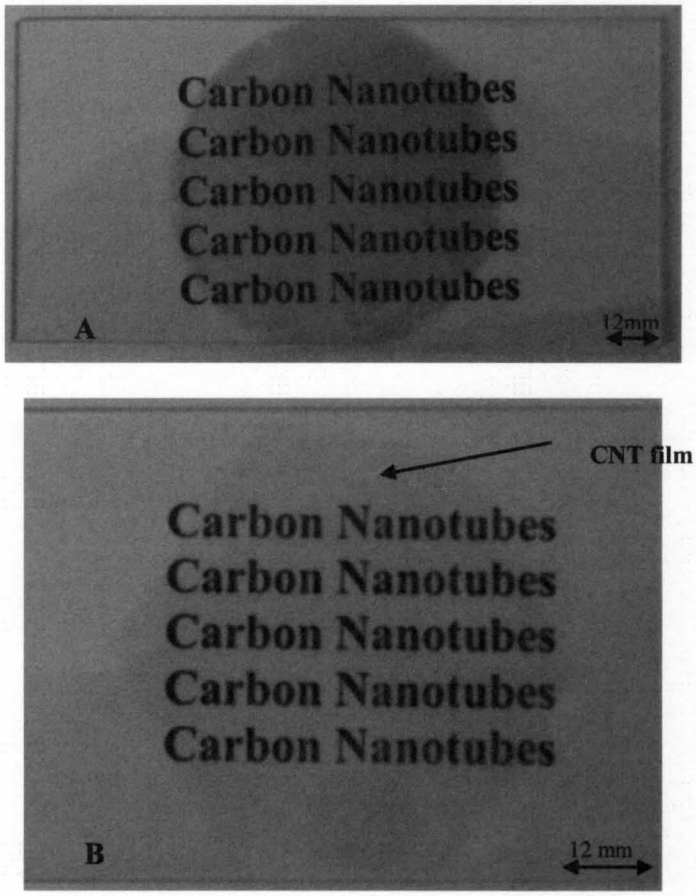

Figure 3.3-b Images of CNT films of different thicknesses. (A) A dense CNT film while (B) is a thinner film. The ultra thin film was used in this research.

A second lithography step defined the metal electrodes for the source and drain. The substrates were prepared for metal deposition by applying a 1.3 μm thick positive

photoresist (Shipley 1813) and carrying out standard photolithography steps. A chromium film of ~10 nm was deposited as an adhesion layer followed by ~100 nm biocompatible gold film. The polymer and metal that did not pertain to the electrodes was lifted off by placing the substrate in an acetone bath. Following, the substrate was rinsed with isopropyl alcohol and then dried with nitrogen. Finally, a 2 μm thick SU8 layer was added and patterned only exposing the carbon nanotube area between the electrodes to the environment. The SU8 layer helped to isolate the electrodes from the biomolecules and solutions and limit the possibility of any unwanted reactions or noise.

3.4 CNT Film Characterization

CNT characterization is usually accomplished through different microscopy methods. In this present work, Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) were used to analyze the CNT films. SEM and AFM scans as in Figure 3.4-a were used to determine the topography and density of CNT network, verify the presence and coverage of antibodies on the CNT surface. During SEM imaging a maximum voltage of 10 kV was used for bare CNT films. For biomolecule functionalized CNT, a range of 2 to 6 kV was used. Finally, a working distance of 5-15mm was used at all times. AFM scans were performed by Ben King, a lab mate in the small systems laboratory. He was able to image bare CNTs as well as antibody coated CNTs.

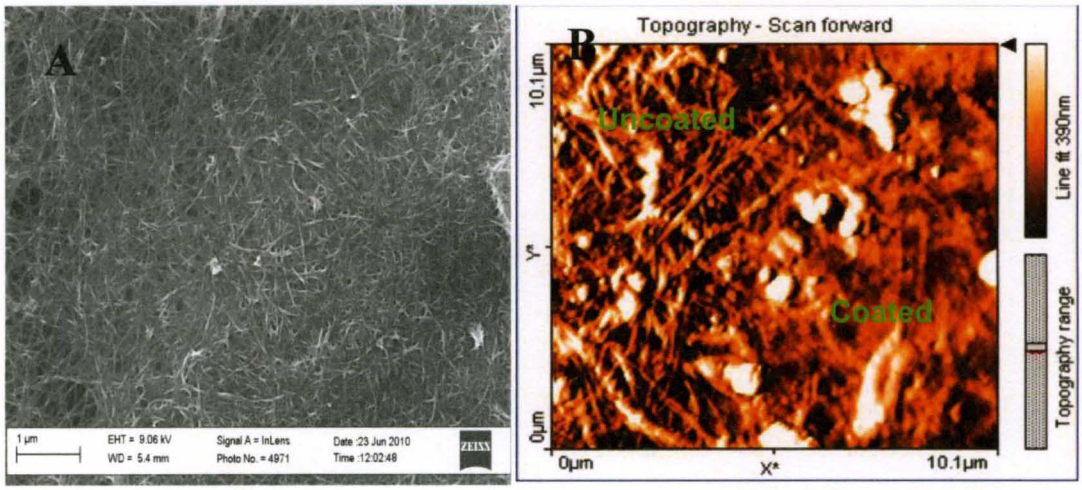

Figure 3.4-a (A) An SEM image of bare CNT film. (B) An AFM scan of the interface between uncoated and antibody coated antibodies.

3.5 Electrolyte Solution Preparation

For the CNTFET-based biosensors, a liquid gate configuration was used in the mechanistic studies for the detection of cancer cells in blood. In the past, Rosenbaltt *et al,* has reported on high performing electrolyte gated transistors and stated that a NaCL solution was used for most of their experiments [43]. Therefore, a ~ 10mM NaCL solution was prepared and used as the liquid gate in the experiments executed in this research.

3.6 Blood Sample Preparation

Blood samples were obtained from a healthy male collaborator in Thomas Jefferson University. MCF-7 cells were cultured at Thomas Jefferson University as well. Samples were prepared by mixing the cultured cell with blood at different concentrations.

Samples were sent and arrived the same day. Experiments were performed upon the arrival of samples.

3.7 Antibody Functionalization

A non-covalent approach was implemented for the immobilization of antibodies onto the surface of the CNT film. The functionalization process was as follows: commercially purchased antibodies were diluted in phosphate buffered saline (PBS) to a concentration of ~ 10 μg/mL. A 5 μL (for large CNT film transistors) and 1 μL (for mechanistic study transistors) droplet of antibody solution was placed onto the surface of the CNT film and incubated for 3 min at room temperature, allowing the molecules to diffuse down to the surface and bond with the CNTs. Then the surface was flushed with PBS. At this point, the large CNT film transistors were ready for an electrical measurement. For the liquid gated transistors, a droplet of NaCl was placed on the CNT surface for electrical measurement. The transfer characteristic was taken and the surface was ready for the blood sample mixed with breast cancer cells. Then, a 5 μL (for large CNT film transistors) or 1 μL (for mechanistic study transistors) drop of blood was placed on the surface allowed to settle for 3 min and then it was removed, rinsed, and another electrical measurement was taken. Physical adsorption of the antibodies and blood with CTCs was driven by hydrophobic interactions between the random network of CNTs and antibodies [40].

3.8 Electrical Characterization

The CNTFETs were electrically characterized with an Agilent 4156 precision semiconductor parameter analyzer and probe station, as depicted in Figure 3.8-a. The source and drain electrodes were probed using gold-coated tips. The gold tips were used to avoid any corrosive reaction during the liquid gating. After probing the device, a resistance measurement was performed by applying a source-to-drain voltage (V_{sd}) and sweeping from -1V to 1V, while keeping the gate voltage (V_g) at a value of 0. At the same time, the source-to-gate current or leakage current (I_{sg}) was monitored. After these initial measurements were completed, 1μL of electrolyte solution was placed on the CNT surface and the gate probe was immersed into the droplet. Next, the baseline of the device characteristic was recorded by sweeping the gate voltage (V_g) and holding the V_{sd} at a constant value. This was executed again after antibody functionalization and after exposure of blood.

The flow of current through the conducting channel at the addition of antibodies and blood was also recorded. These experiments were performed by once again probing the source and the drain. Then, a fixed V_{sd} was applied and the source-to-drain current (I_{sd}) was measured with respect to time. To initiate run, the device was allowed to stabilize for two minutes, then a 1μL drop of antibody or blood was added to CNT surface. Another three minutes was allowed to elapse and a second drop would be added.

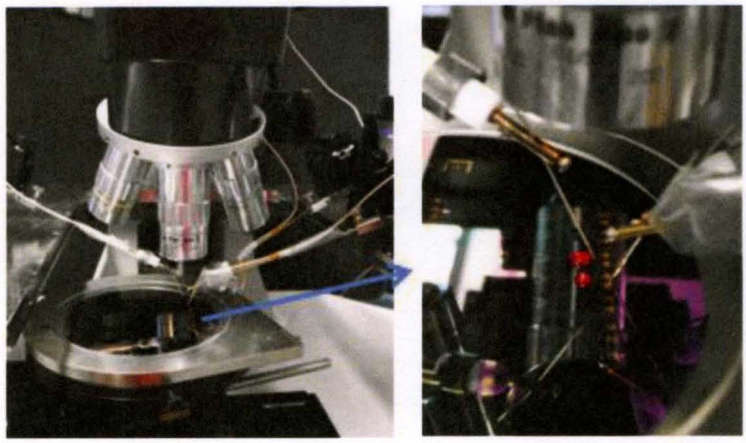

Figure 3.8-a An image of the device during experimental measurements. The devices were placed in a probing station and probes with gold coated tips.

CHAPTER 4

RESULTS AND DISCUSSION

The application of carbon nanotube thin films transistors for the detection of circulating breast cancer cells was achieved by incorporating CNT thin films into an electrical device configuration, functionalizing monoclonal antibodies on the surface of the carbon nanotube networks and monitoring the electrical signatures during interaction of blood mixed with breast cancer cells on carbon nanotube networks.

4.1 Biosensor Design Parameters

Preliminary evaluations indicated that the performance of the CNT thin film transistor was dependent on the CNT film thickness. As a result, the electrical characterization of several devices with an assortment of CNT densities was completed. Experiments indicated that thicker films displayed a metallic behavior and required significantly large gate voltage ($V_g > 30V$) in order to create an electric field that would modulate the current. This is in accordance to previous observations performed by *Hu et al*, where he attributed low ON/OFF current ratios of dense films to conductive CNT that act to screen the gate voltage [49]. As a result, ultra thin films were used for the

construction of the biosensors. An AFM scan of the CNT film determined a thickness of ~180 nm for the film.

4.2 Antibody Functionalization

Monoclonal antibodies were functionalized onto the surface of CNT film through physical adsorption. The physical adsorption is attributed to hydrophobic interactions between CNTs and antibodies as previous reported by Allen *et al,* [42]. Figure 4.2-a shows the contrasting behavior of the hydrophobic interactions of a drop of blood and antibody between a CNT film and silicon oxide surface.

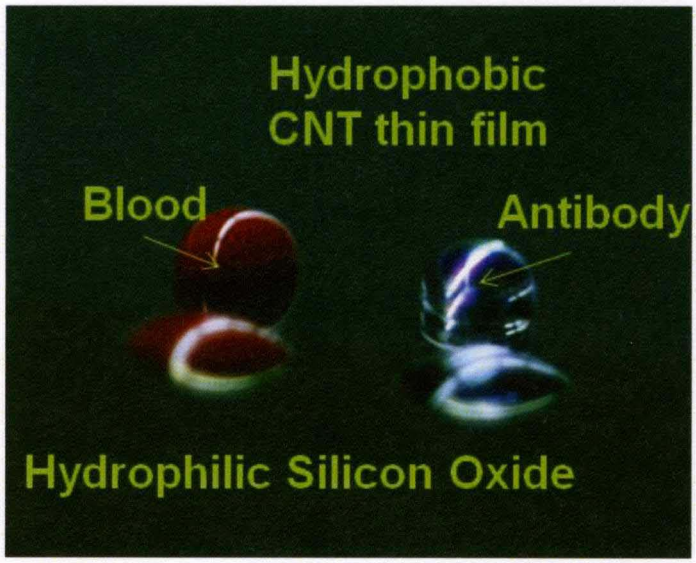

Figure 4.2-a Image depicting the hydrophobic interactions of a drop of blood and antibody between a CNT film and silicon oxide surface.

The density and uniformity of the immobilized antibodies over the CNT film was investigated with the use SEM and AFM imaging. Three different concentrations namely 1 μg/mL, 5 μg/mL, and 10 μg/mL and three different volumes namely 1 μL, 5 μL, and 10 μL were used in an array and imaged using AFM and SEM. Figure 4.2-b shows the representative images of the antibodies on the surface of carbon nanotube films. From the images, a uniform coating was noticed for 5 μL of antibody solution of ~10 μg/mL. As a result, a dose 5 μL of antibody solution at a concentration of ~10 μg/mL was used for detection circulating breast cancer cells in blood.

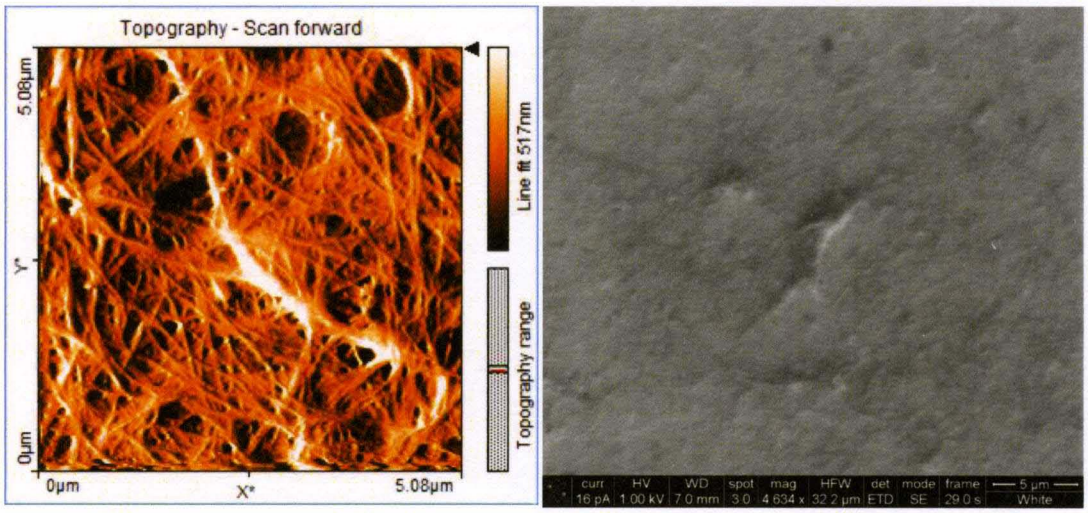

Figure 4.2-b AFM and SEM images of a uniform coating of 5 μL antibodies.

4.3 Detection Circulating Breast Cancer Cells with CNT Thin Films

The electrical response (I vs. V_{ds}) of thin CNT films functionalized with different antibodies (specific and non-specific) were recorded after adding 5 μL of blood mixed with 1-300 MCF-7 breast cancer cells. Upon adding biological components, a noticeable

change in conductance was observed, as shown in Figure 4.3-a. The reduction in conductance was negligible for PBS wash. However, a 50 % drop in the current of the device was observed after the adsorption of 5 μL of anti-HER2. After antibody adsorption, the addition of blood mixed with cancer cells also resulted in an additional decrease in device conductance.

Comparison studies between the electrical behaviors of CNT thin films functionalized with specific (anti-IGF1R) and non-specific (anti-IgG) antibodies were conducted in order to investigate whether sensitive and specific detection of molecular surface receptors is possible in a sample of unaltered blood. 5 μl of anti-IGF1R and anti-IgG were immobilized on the surface of the CNT networks followed by the addition of blood with different MCF-7 cell concentrations. Figure 4.3-b shows that devices printed with IgG experiences less than a ~10% change in conductivity while devices printed with IGF1R exhibits ~60 % drop in conductivity with increasing number of MCF-7 breast cancer cells in blood. Taking a closer look, the electrical characteristics attained from IGF1R are similar to that of a field effect transistor, where the increasing concentration of cancer cells act as a chemical gate modulating the current flow between source and drain. The binding of IGF1R receptor proteins to their respective antibodies reduces the charge carrier concentration in the carbon nanotubes which leads to a reduction in the conductivity of the device. Similar results were also observed by Li *et al,* and his colleagues in University of Southern California where they detected prostate-specific antigen using In_2O_3 nanowires and carbon nanotubes [56-57].

The distinguished drop in conductivity for specific antibody and receptor interactions can be further observed from a real time current measurement of

functionalized CNT film transistor with anti-IGF1R upon the addition of a blood control sample and blood mixed with MCF-7 cells, as shown in Figure 4.3-c.

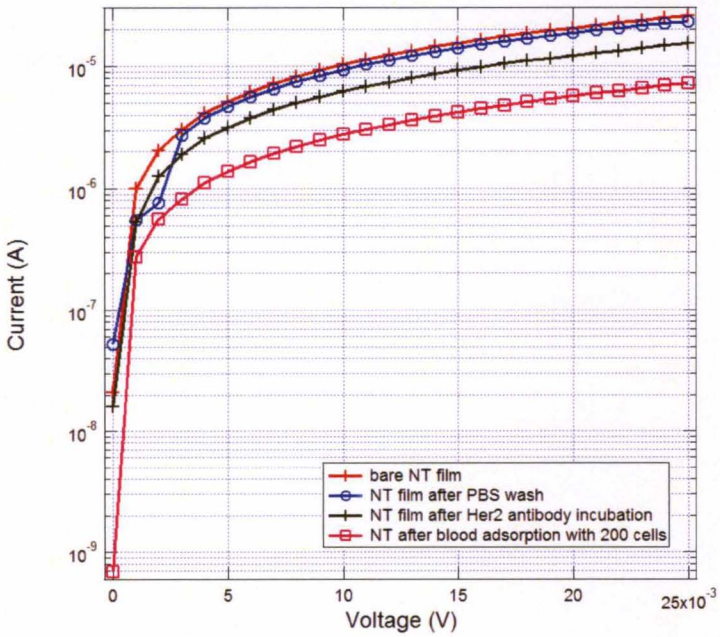

Figure 4.3-a A change in the current flow through CNT conducting channel is observed after functionalizing the CNT film, with antibodies and the current is further reduced after adding 5µL of blood with a concentration of 200 MCF-7 cells/ µL.

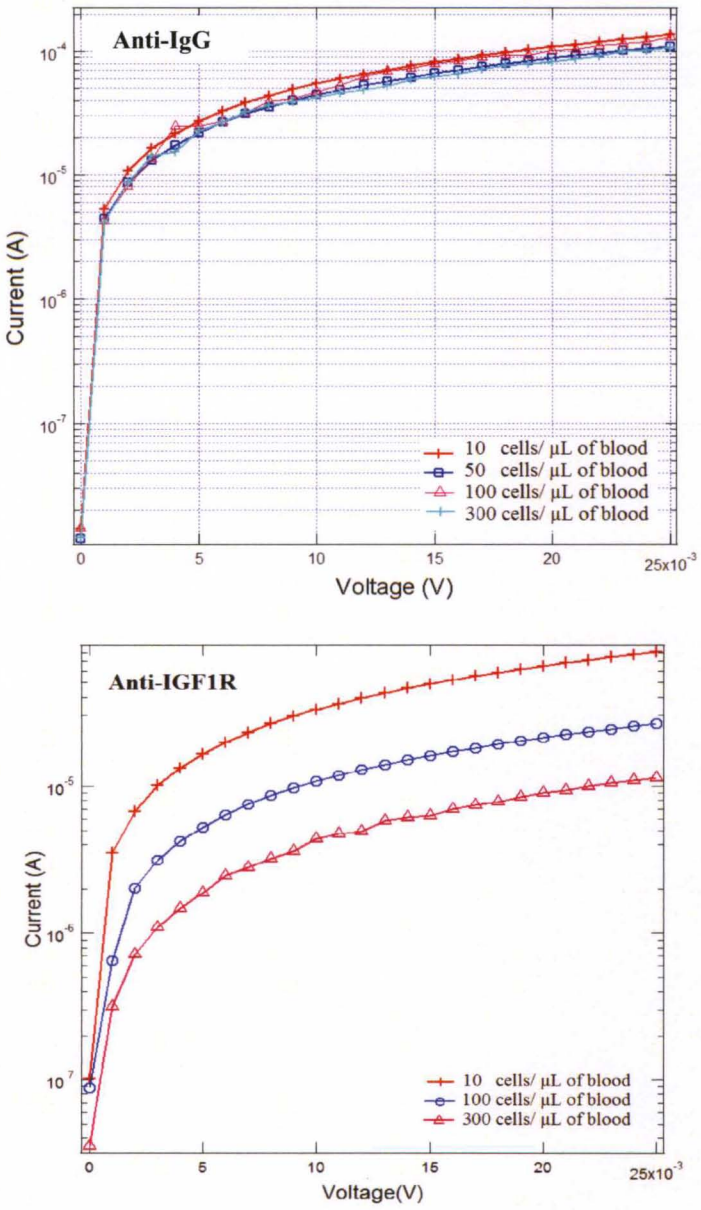

Figure 4.3-b Portrays the difference of device behavior between specific and non-specific binding. IgG is a non-specific protein and it shows minimal changes in conductance. On the other hand, IGF1R, a specific protein receptor found in breast cancer does show a noticeable current suppression.

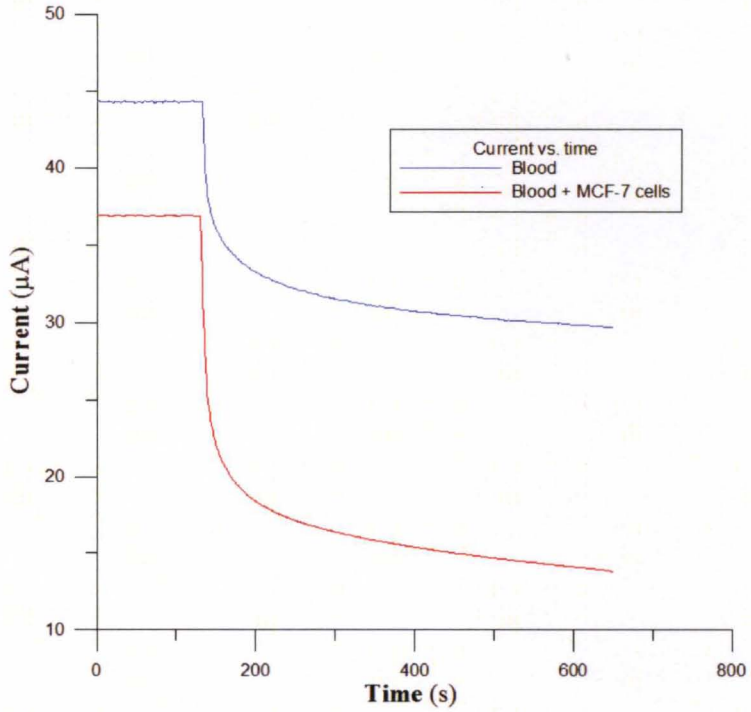

Figure 4.3-c Displays real time current measurements upon the addition of 5 μL blood control sample and a blood sample that contained MCF-7 cells.

4.4 Mechanistic Studies

In order to understand the phenomena occurring in the biosensors based CNT thin film transistors above, mechanistic studies were carried out with liquid gated CNTFETs. The goal of these experiments was to identify the interactions between the CNTs and the antibody-receptor binding that lead to the charge carriers being reduced.

The transfer characteristics (I vs. V_g) of liquid gated transistors were monitored upon the addition of 1 μL of antibodies and 1 μL MCF-7 cells mixed with blood in order to identify the electrostatic interactions taking place between CNTs and the binding of surface receptors with antibodies. For these experiments, only one single concentration of MCF-7 cells (5 MCF-7 cells/ μL) of blood was used.

The transfer characteristics were recorded for devices functionalized with IgG and IGF1R antibodies as shown in Figure 4.4-a. It can be observed that there is a distinct difference between electrical characteristics of non-specific and specific interactions of MCF-7 surface receptors. For the device printed with IGF1R antibodies, there is obvious shift in the threshold voltage (~250 mV), whereas a device printed with anti-IgG has no distinguishable shift. Biosensing mechanistic studies performed by Dekker's group, indicate that the adsorbed charged species induced a partial charge transfer between the carbon nanotubes and biomolecules [50]. Thus, CNT is doped and leads to a shift in the I- V_g curve. In the case of the results attained in this project, the threshold voltage shift towards more negative gate voltages originates from an electron donation to the CNTs when the binding of IGF1R receptor to immobilized ligands take place. However, the biosensing mechanism can not only be attributed to gating effect. From Figure 4.4-a, one can observe that the current is also suppressed after adding the blood mixed with cancer cells. Current suppression is often observed when the CNTs experience mechanical distortion [10]. In the context of this project, it is thought that the current suppression is caused by the blood medium. Several components in blood can cause stress on the functionalized surface and minimize the mobility of charge carries.

The transfer characteristic corresponding to non-specific IgG interactions with breast circulating cancers is one that is similarly observed for a change in charge mobility as explained by Kauffman *et al,* (refer to Figure 4.4-a) [2]. As the circulating cancer cells and blood components approach the functionalized CNT surface, they inhibit the acceleration of electrons causing collisions and scattering effects. Similarly to those caused by impurities in semiconductors.

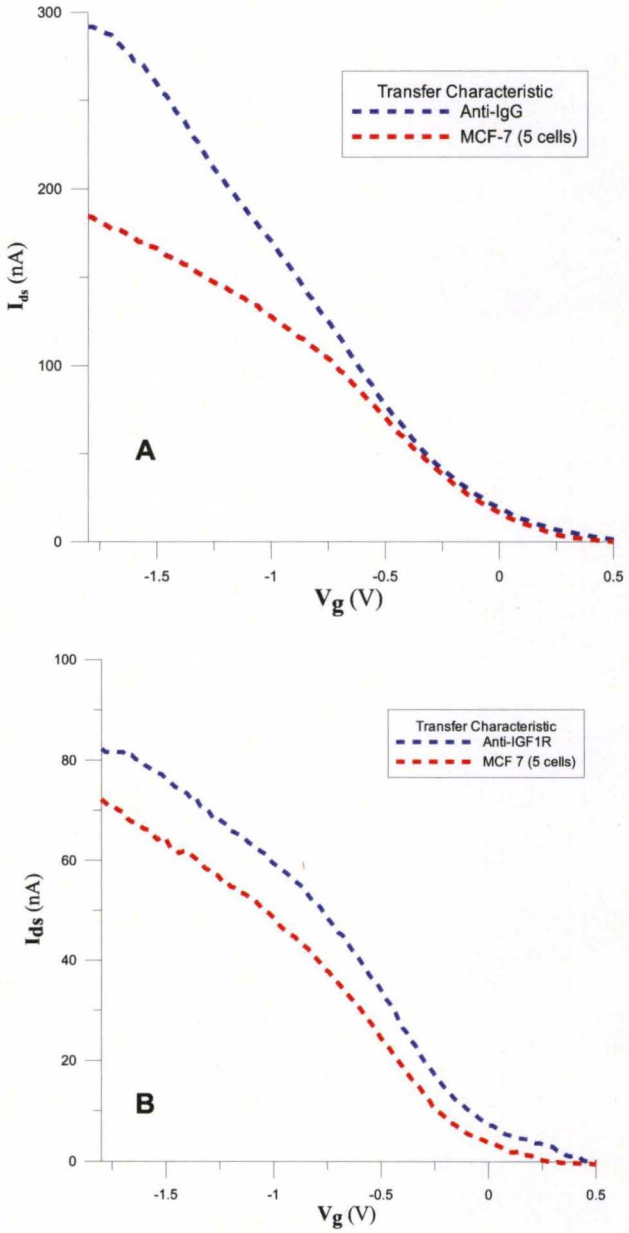

Figure 4.4-a Comparison of transfer characteristics upon adding a 1μL drop of 5 MCF-7 cell/ μL of blood (A) a device functionalized with non-specific IgG antibodies and (B) device functionalized with specific antibodies IGF1R antibodies .

CHAPTER 5

CONCLUSIONS AND FUTURE WORK

In conclusion, the preliminary results from this research indicate that the detection of circulating breast cancer cells in blood medium may be possible using biosensors based on CNT thin film transistors. By functionalizing the surface area of the CNT film with ligands that specifically bind to surface receptors on breast cancer cells, specific protein interaction can be detected on the surface of the CNTs. Owing to the sensitivity of CNTs, the binding of cancer surface markers to their respective antibodies participate in a charge transfer with the CNTs whereas non-specific interactions do not. Further investigations are required for future implementation of such detection technique, such as control experiments with blood containing no cancer cells and other cancer cell types. As well as, fluorescent imaging to confirm the immobilization of cancer cells on CNT surface. Nonetheless, the detection of biomolecules with carbon nanotube thin film sensors based on transistors shows great promise for selective detection of biomolecules that can be implemented into relevant biosensors.

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Education

M.S.
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Mechanical Engineering
University of Louisville, Louisville, KY 40292
Thesis: "Carbon Nanotube Thin Film Transistors for Biomedical Applications"
Advisor: Prof. Balaji Panchapakesan

B.S.
April 2008

Chemical Engineering & Biomedical Mathematics
Florida State University, Tallahassee, FL
Senior Project: Design and Simulation of Membrane Separation

Experience

08/ 2009- Present

University of Louisville, Louisville, KY
Micro/Nano Technology Center
Research Scientist

- Mentoring cleanroom clients in the operation and performance of photolithography equipment and

methods, deep reactive ion etching systems, and material deposition systems such as sputtering and evaporation.

- Developing and updating standard operating procedures for micro-fabrication systems in the cleanroom facility at the University of Louisville.
- Characterization of thin film deposition, deep reactive ion etching systems and lithographic systems for MEMS and nanotechnology applications.
- Design, fabrication and characterization of micro-electro mechanical systems and nano-structured interfaces and devices.

07/ 2009

Thomas Jefferson University, Philadelphia, PA
Kimmel Cancer Center
Visiting Research Assistant

- Culture and characterization of cell lines such as BT474 and MCF7 breast cancer cells, LnCAP prostate cancer cells and AsPC1 pancreas cancer cells.
- Optical microscopy of cell cultures.
- Immobilization of antibodies on carbon nanotube films for capturing circulating cancer cells on nanotube surfaces.

08/ 2008- Present

University of Louisville, Louisville, KY
Small Systems Laboratory
Graduate Research Assistant

- Design and development of carbon nanotube biosensors for capturing circulating breast cancer cells in blood.
- Design and fabrication of carbon nanotube field effect transistors for highly specific biosensor applications.
- Design, fabrication and characterization of nanotube cantilevers for bioassay of prostate specific antigen.
- Carbon based material characterization techniques such as scanning electron microscopy, atomic force microscopy and scanning probe microscopy.
- Training incoming undergraduate and graduate students on all the equipment in laboratory.
- Conduct laboratory maintenance and duties.
- Writing reports, conference and journal publications.

08/ 2007-04/ 2008

FSU-FAMU College of Engineering, Tallahassee, FL
Undergraduate Research Assistant

- Observation of animal sedation and dissection.

- Developing and testing probes used in animal neurology studies.

08/ 2007-01/ 2008 ACS, Affiliated Computer Services, Tallahassee, FL
Bi-lingual Customer Service Representative

- Interfaced with current and perspective clients.
- Provided information about a Florida health care program for children.

Skills

- Well versed in multi-disciplinary computational design and simulation software (ANSYS, CoventorWare, ChemCAD, Maple, MATHCAD)
- Qualified and skilled operator of several micro-fabrication equipment (such as Sputtering, Electron beam Evaporation, Deep Reactive Ion Etching, March Reactive Ion Etching, Lithography systems)
- Knowledgeable and experienced in wet micro-etching techniques
- Knowledgeable in Scanning Electron Microscopy, Scanning Probe Microscopy Atomic Force Microscopy imaging techniques
- Experienced in Raman Spectroscopy
- Able to work effectively and efficiently alone or as part of a team
- Excellent leadership and communication skills
- Able to perform reliably and ethically in fast-paced environments
- Bilingual, fluent in Spanish and English

Scholarly Activities

Book Chapters:

1. V. Velasco, E. Wickstrom and B. Panchapakesan, "Applications of nanoparticles for molecular targeting and selective destruction of breast cancer cells" in Nanoparticle synthesis, characterization and applications, Edited by R.V. Chaughule and R.S. Ramanujan, American Scientific Publishers, 2010.

Journal Publications:

1. S. Lu, S. Ahir, V. Velasco, B. King, P. Xu, E. Terentjev and B. Panchapakesan, "Photomechanical actuation of carbon nanotubes: mechanisms and applications in micro and nanodevices", Journal of Micro and Nanomechatronics, 5 (1-2) 29-41, 2009.

Journal Publications-In Preparation :

1. B. King, V. Velasco, P. Xu, E. Wickstram and B. Panchapakesan, "Immobilization of antibodies on thin carbon nanotube films for batch fabricated biosensors", Nanotechnology, in review, 2010.
2. V. Velasco, B. King, P. Xu, E. Wickstrom and B. Panchapakesan, "Bioassay of prostate specific antigen using carbon nanotube micro-cantilevers", Nanotechnology, in review, 2010.
3. V. Velasco, B. King, P. Xu, E. Wickstram and B. Panchapakesan, "Nanotube antibody biosensor arrays for the detection of circulating breast cancer cells in blood", manuscript in preparation to Nature Biotechnology, 2010.
4. V. Velasco, B. King, P. Xu, E. Wickstram and B. Panchapakesan, "Nanotube antibody biosensor arrays for the detection of circulating prostate cancer cells in blood", manuscript in preparation to Nanotechnology, 2010.
5. P. Xu, V. Velasco, B. King, M. Yazdanpanah, R. W Cohn and B. Panchapakesan, "Nano-optomechanical actuators", manuscript in preparation to Applied Physics Letters, 2010.

Conference Publications:

1. V. Velasco, E. Wickstrom and B. Panchapakesan, "Nanotube Biosensor Arrays for Detection of Molecular Surface Markers in Breast Cancer Cells", Proceedings of Nanotech 2009, May 3-7, Dallas, TX, 2009.
2. V. Velasco, E. Wickstrom and B. Panchapakesan, "Carbon nanotube as molecular nanocarriers for antibody delivery and photothermal ablation of breast cancer cells", Proceedings of Nanotech 2009, May 3-7, Dallas, TX, 2009.

Conference Publications-In Preperation:

1. B. King, V. Velasco, P. Xu, E. Wickstram and B. Panchapakesan, "Immobilization of antibodies on thin carbon nanotube films for batch fabricated biosensors", Proceedings of Nanotech 2010, June 1-4, Anaheim, CA.
2. V. Velasco, B. King, P. Xu, E. Wickstram and B. Panchapakesan, "Nanotube antibody biosensor arrays for the detection of circulating breast cancer cells in blood", Proceedings of Nanotech 2010, June 1-4, Anaheim, CA.

3. V. Velasco, B. King, P. Xu, E. Wickstrom and B. Panchapakesan, "Bioassay of prostate specific antigen using carbon nanotube micro-cantilevers", Proceedings of Nanotech 2010, June 1-4, Anaheim, CA.
4. P. Xu, V. Velasco, B. King, M. Yazdanpanah, R. W Cohn and B. Panchapakesan, "Nano-optomechanical actuators", Proceedings of Nanotech 2010, June 1-4, Anaheim, CA.

Professional Affiliations:

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- Institute of Electrical and Electronics Engineers (IEEE)-*Member*
- Biomedical Engineering Society (BMES)- *Member*
- American Society of Mechanical Engineers (ASME)- *Member*