NANOELECTRONIC SENSOR FOR DETECTION OF PROSTATE CANCER BIOMAKERS

SUNFEST Summer Undergraduate Fellowship in Sensor Technologies
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ABSTRACT

Prostate cancer is the second cause of cancer death in American men; it is known that early detection of the disease is one of the most important tools for successful treatments. Unfortunately, current methods of diagnosis are either invasive or require high concentrations of prostate cancer biomarkers in order to detect the disease accurately. Here, we present an improved method for early detection, a nano electronic sensor for prostate cancer. This device relies on electrical sensing using a field effect transistor where the semiconducting channel is a functionalized single-walled carbon nanotube. These novel carbon structures have unique electronic and chemical properties that allowed us to fabricate a highly specific and highly sensitive nano sensor. Moreover, we designed a channel that allowed us to controllably deliver small amount of samples to the devices. This will allow real time detection since electrical measurements can be taken at same time the device is exposed to the biomarker. It is believed that this project could set up the platform to eventually have an array of these devices to detect several biomarkers in one step.

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1. INTRODUCTION

According to the American Cancer Society, from 1999-2005 only 68% of cancer patients survived. After heart disease, cancer is the second most common cause of death in the United States [1]. One of the most effective tools against cancer is early detection since successful treatments depend on how advanced the cancer is when diagnosed. Particular attention is drawn to prostate cancer since it is the second most common cause of cancer death in American men.

Unfortunately, the American Cancer Society anticipates 217,730 new cases of prostate cancer in 2010; therefore current research is focused on alternative ways for screening this disease [1]. Current methods of early detection such as prostate cancer specific-antigen (PSA) screening and digital rectal exam are either invasive or in the case of PSA screening, it requires high concentration of PSA in order to detect accurately. Clearly, there is a need for a detection method that is fast, reliable, non-invasive, and able to detect PSA at lower levels. For that reason, this project takes advantage of the semiconducting properties of carbon nanotubes to make a device that meets those needs.

The proposed device is a nano electronic sensor that relies on electronic readout from a single-walled nanotube field effect transistor that after functionalization is highly sensitive to PSA. Since this sensor is a molecular device, it will be able to detect PSA at lower levels than current detection methods. In addition, alternative prostate cancer markers are being researched by our collaborators, which will allow us to make the device more specific. This project is believed could be the platform to have an array of hundreds of these devices that will test for various biomarkers, all in one step.

This macro project consists of four major areas: Fabrication, Liquid Handling, Functionalization, and Final Testing. Due to time limitations, this paper will discuss only Fabrication, Functionalization and Liquid Handling in Sections 3, 4 and 5 respectively while Section 2, Background, reviews the main points about carbon nanotubes, synthesis of carbon nanotubes, single-walled carbon nanotube field effect transistors, biological underpinnings of the device, and microfluidics. The Fabrication, Functionalization and Fluid Handling steps will set up a platform for future work on the design of the nano electronic prostate cancer sensor. Finally, Section 6 is dedicated to discussion and conclusions of the project.

2. BACKGROUND

2.1 Carbon Nanotubes

Nanomaterials have drawn particular attention due to their various applications in a wide range of disciplines. Carbon nanotubes (CNTs) were discovered in 1991 and since then, research in their applications has grown significantly [2]. They are great tools for sensor technologies due to their exceptional electronic and chemical properties. These carbon structures can be thought of as highly ordered sheets of carbon atoms rolled into seamless cylinders [3]. Carbon nanotube dimensions vary; their diameter can be as small as 1-nm for single walled nanotubes and as large as hundreds of nanometers for multiple-walled tubes, and in each case still can have lengths up

to several millimeters [4]. Depending on their chirality (twists in the exact crystal structure) CNTs have different electrical properties from metallic to semiconducting [3]. Our focus is on single-walled, semiconducting carbon nanotubes, which make excellent electrical sensing devices.

2.2 Synthesis of Single-Walled Carbon Nanotubes

Synthesis of carbon nanotubes is a process that is still being improved given that current techniques provide only uncontrolled results. It would be ideal to be able to have control over the length, amount, and type of carbon nanotubes grown, but though some techniques are more advanced than others, synthesis of carbon nanotubes is still a challenging task. For this project, synthesis is done by chemical vapor deposition (CVD) since it is a simple and inexpensive method that provides high yield of single-walled carbon nanotubes [4]. The steps of the chemical vapor deposition process will be explained in Section 3.

2.3 Single-Walled Carbon Nanotube Field Effect Transistor

Single-walled carbon nanotubes (SWCNTs) are an excellent tool for sensing devices because due to their cylindrical geometry, every carbon atom is exposed to its surroundings. This characteristic makes CNTs very susceptible to nearby bio-molecules; this trait can be employed to fabricate a highly-sensitive device. Moreover, the small size of CNTs facilitates sensing at the molecular level, which is desirable in many fields, but particularly in biomedical applications.

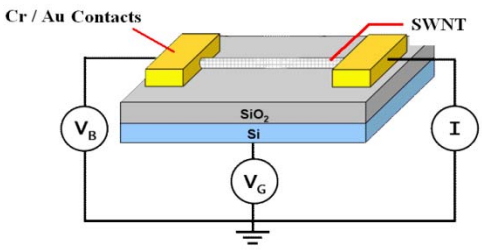

Figure2.3.1 Schematics of a single-walled carbon nanotube field effect transistor. [1]

In order to make use of the SWCNTs properties for a sensor device we are interested in semiconducting SWCNTs since these can be used as the semiconducting channel in a field effect transistor (FET) (Figure1.) Transistors have been the focus of attention for the microelectronics industry with miniaturization being one of the limits that the semiconductor industry has encountered due to the intrinsic quantum effects of semiconductors at a small scale [4]. Nano-electronic devices such as, carbon nanotube FETs, have given optimism that a solution can be found to miniaturization limits [6.] However, the fabrication of these devices is not a simple task because, as explained previously, synthesis of carbon nanotubes yields a mixture of metallic, semiconducting, single-walled, and multi-walled CNTs.

2.4 Biological Underpinnings

One of the important biological aspects for this project is the specificity by which antigens and antibodies attach. Antigens are foreign molecules that when entering the body stimulate the production of antibodies. Examples of antigens are toxins, viruses and bacteria. Antibodies are proteins that have, at the tip of their structure, an

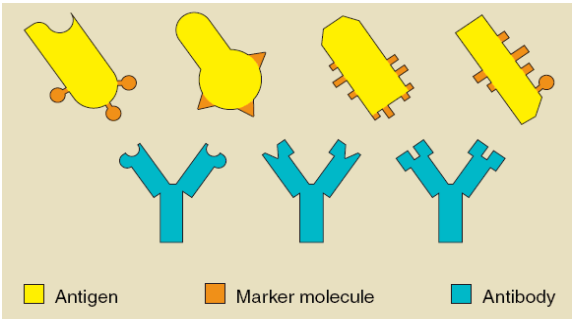

Figure 2. 4.1 Schematics of the antibodies and antigens key/lock system. [2]

antigen site. This site is specific for only one type of antigen (Figure2.4.1) This biological trait is desirable for advancements in sensor technology since it could be use as a highly-specific detection method. However, there are a few concerns about this technique such as the biological-electronic interface. Specifically, we are concerned about the hydrophobic characteristics between proteins (antibodies) and carbon nanotubes [6]. In addition, the device must have the capability to test complex fluids such as a blood sample, which could compromise the device's electronic characteristics.

2.5 Microfluidics

The field of microfluidics has to do with the control of small volumes of liquids in a channel of dimensions on the micron scale. The promising ground of microfluidics has been used as an interface among subsystems in devices that require measurement or control of complex fluids [7]. This, of course, is ideal because it would allow a multi-step process to be done in one step, all within one device. Moreover, microfluidics techniques are ideal for disease diagnosis because they provide straightforward analysis of complex fluids such as glucose and DNA. Common methods of detection that involve microfluidics rely on optical detection after the fluid has been marked (usually with dyes.) However, here we are dealing with a more complicated set up since the sensing relies on electronic readout, which could be hindered by the liquid environment on the device.

3. FABRICATION OF FIELD EFFECT TRANSISTOR

3.1 Wafer Preparation

Minimal contamination during this process is extremely critical to obtain reliable nano-devices. We started with a 4-inch diameter silicon/silicon dioxide wafer, which was cleaned by oxygen plasma and then covered with a metal catalyst. For this experiment, the catalyst used is iron nitrate dissolved in isopropanol, which is then spun onto the wafer (Recipe 1). The catalyst step provides control over the synthesis CNTs since the diameter of CNTs is proportional to the size of the catalyst particles. However, there are many other variables that affect the random process of carbon nanotubes self-assembly and not always we obtained the desired results.

3.2 Synthesis of Carbon Nanotubes

The CVD process consists of heating up the furnace to 900° C where the wafer is kept inside a quartz tube. At this temperature a process gas, nitrogen, and a carbon-containing gas, methane, are flowed into the tube (Recipe 2). The flow of these two gases is done for two minutes, time in which CNTs will grow at the catalyst sites. After the synthesis is completed, we examined the wafer by atomic force microscope (AFM) in order to find CNTs (Figure 3.2.1)

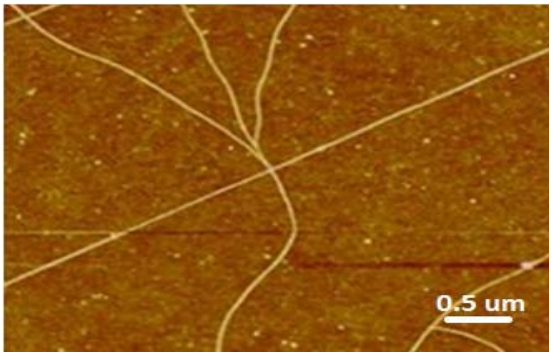

Figure 3.2.1 AFM image of CNTs on wafer after CVD growth. Image obtained by lab member.

3.3 Patterning and Deposition of Electrodes

Subsequently, the electrode sites were patterned by photolithography using a chrome mask (Recipe 3.) After that, the metal deposition of the electrodes was done by evaporating a layer of 5 nm thick of chrome, and a 40 nm thick layer of gold (Recipe4). After evaporation a lift of process was performed to remove the unwanted metal from the wafer (Recipe 5.) Keep in mind that the whole wafer is patterned with several pairs of electrodes without knowing the location of the CNTs. Figure 3.3.1 shows a pair of electrode trenches after photolithography.

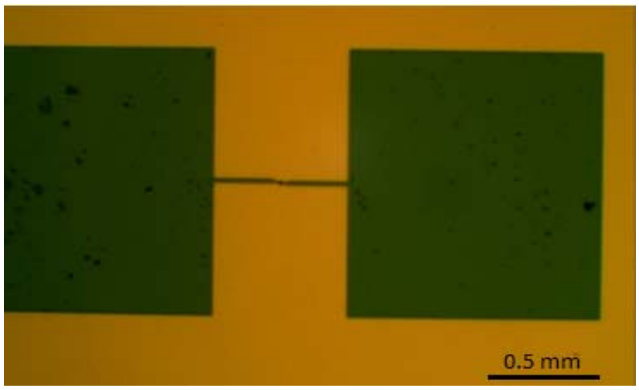

Figure 3.3.1. Pair of electrodes trenches formed by photolithography. Image obtained by lab member

3.4 Current vs. Voltage measurements

The final step of fabrication is to measure the current through each pair of electrodes of each device when a 10 mV voltage is applied to the gate. As explained previously, we are looking for semiconducting SWCNTs with an IVg curve (current vs. Gate voltage) as shown in Figure 3.4.2. The probing set up used is shown in Figure 3.4.1. After probing all the devices, we determined the yield of carbon nanotube FETs.

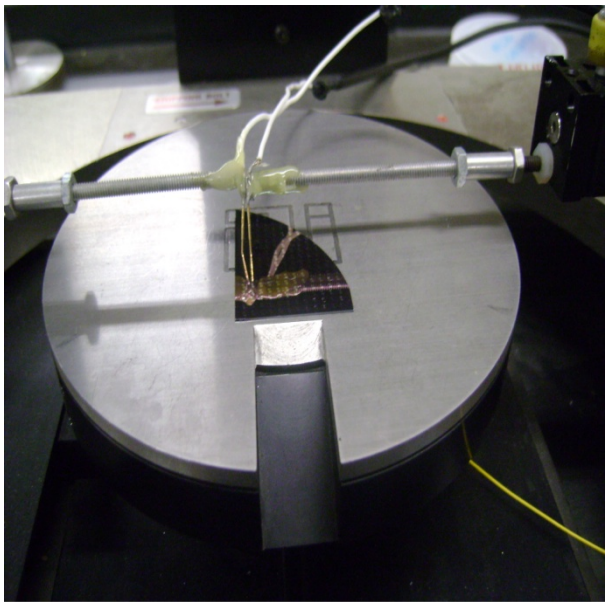

Figure 3.4.1. Probing set up at the Johnson's lab. Picture taken by lab member

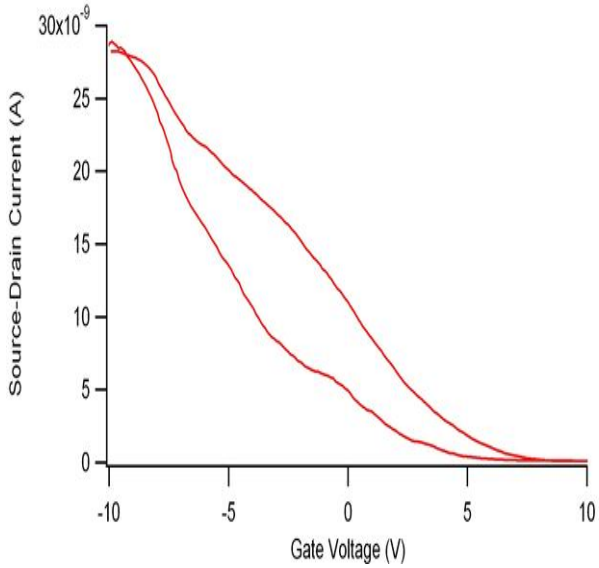

Figure 3.4.2. Graph showing current vs. gate voltage curve of single walled carbon nanotube transistor. Image obtained by lab member

4 FUNCTIONALIZATION

The devices were then functionalized to detect prostate cancer. The carbon nanotubes were chemically processed, first with an oxidation step done by diazonium, followed with a bath in a solution of EDC, NHS and MES buffer (Recipe 6.) As shown on Figure 4.1, this process generates a chemical bonding site for the prostate cancer antibody. The devices were exposed to the prostate cancer antibody for 45 minutes in a moist environment to mimic in vivo conditions. After that, the functionalized devices were exposed to the prostate cancer biomarker for 10 minutes, again mimicking humid conditions. Throughout this process electronic measurements were taken before and after functionalization, and after antigen contact. Results are shown in Figure 4.2.

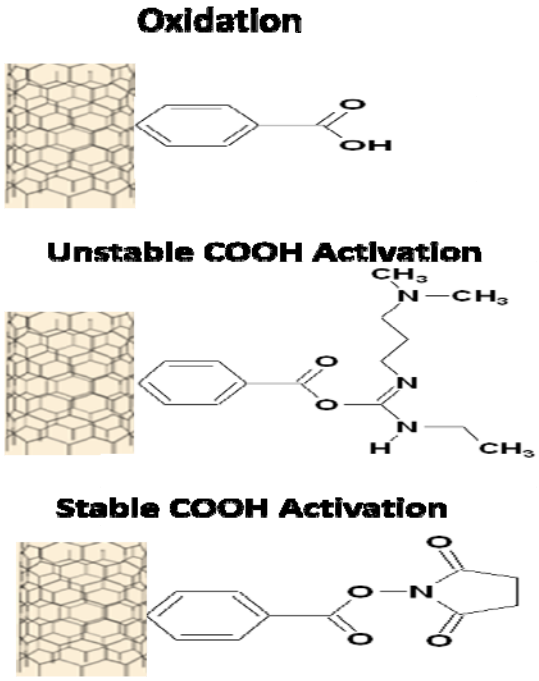

Figure 4.1. Schematics of the chemical processing that a device goes through to form a bonding site for the antibody. Graph was made by a lab member

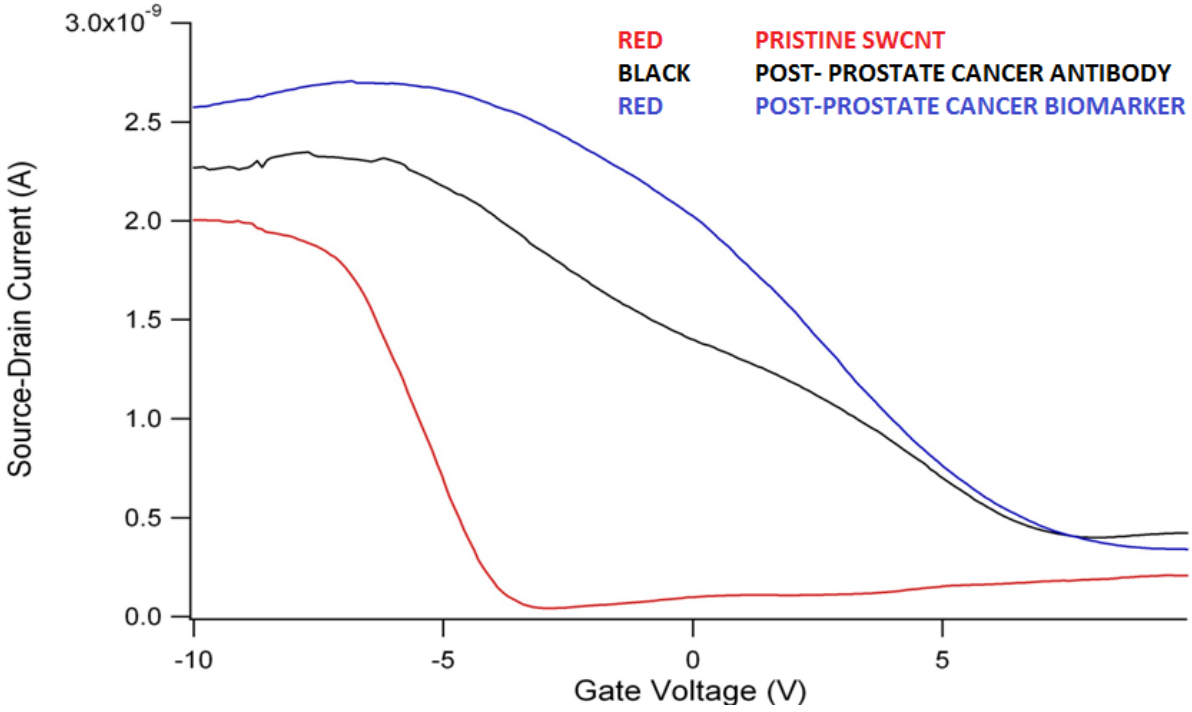

Figure 4.2.Electrical data taken during the functionalization steps Image obtained by lab member

5. FLUID HANDLING

The purpose of this step was to design a channel that allowed us to take electrical measurements while the fluid being tested is passing through the semiconducting channel (CNT) of the device. This is, of course, important for a real time detection, which will provide faster diagnosis than current methods. In addition, having this whole process happening within a channel prevents contamination or any effects that having the devices exposed to ambience, which happens during functionalization, could cause. Also, the channel eventually will allow us to test complex fluids such as blood instead of purified proteins, which is what we are using currently. The initial experiments were performed on non-functionalized devices since the first step is to flow fluid through the semiconducting channel while being able to acquire electrical data. That is, a fluid needs to pass through the small area (2.5 mm) between the electrode pads of the FET so that the electrical data is not hindered by surrounding liquids. The schematics of the proposed channel are shown in Figure 5.1.

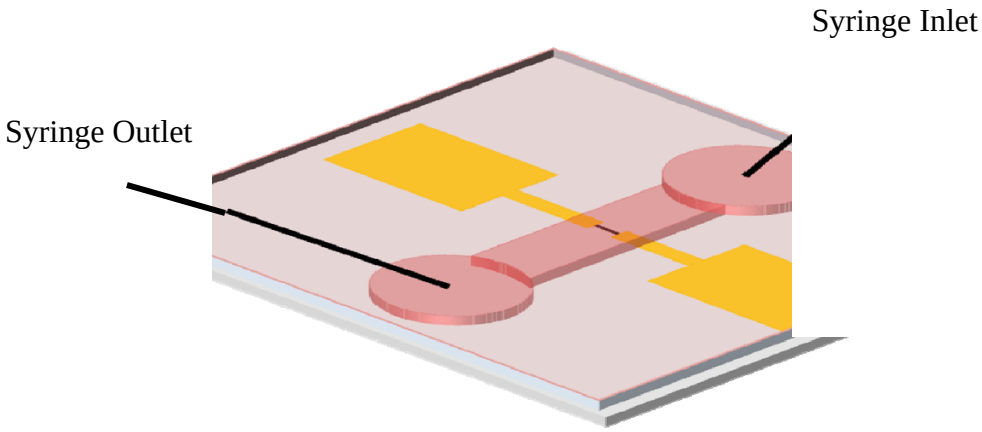

Figure5.1. Schematics of the proposed channel (pink). Graph was drawn by lab member using power point Microsoft office 2007.

5.1 Channel Mold Design

Experimentally, we decided that the channel should have the following characteristics:

Proposed Channel for Fluid Handling			
Physical Dimensions			
	Material	**Dimensions**	**Height**
Petri Dish	Plastic	80 mm diameter	10 mm
Channel Pattern	Teflon tube	4 mm length, 1 mm diameter	1 mm
Inlet/Outlet	Epoxy	~1.5 mm diameter	~1.2 mm

Table 5.1.1 Details of the proposed channel.

Figure 5.1.1 shows the end result of the proposed PDMS channel placed between electrode pads. The method used to make this channel is explained below. Keep in mind that this is an inexpensive and fast method of making a microfluidics channel that did not require the use of a clean room. Though there are more advance techniques to fabricate a microfluidics channel, our main focus was to have small-metered amount of fluids through the CNT without affecting the electronic readout, which we believed can initially be tested with this channel.

Figure5.1.1 Results of the proposed channel. Picture taken by a lab member.

The channel fabrication started with a mold made on a plastic Petri dish. A 1 mm diameter and 4 mm length Teflon tube was placed on the Petri dish; the tube served as the mold for the channel walls. For the inlet and outlet wells, a sphere profile was desired since we needed enough space to penetrate the syringe needle to pump the liquid through the channel. This profile was achieved by dispensing ~1.5 mm diameter drops of epoxy; this material was used because in addition to hardening as a drop, epoxy serves as glue between the Teflon tube and the Petri dish. (Figure 5.1.2 and 5.1.3)

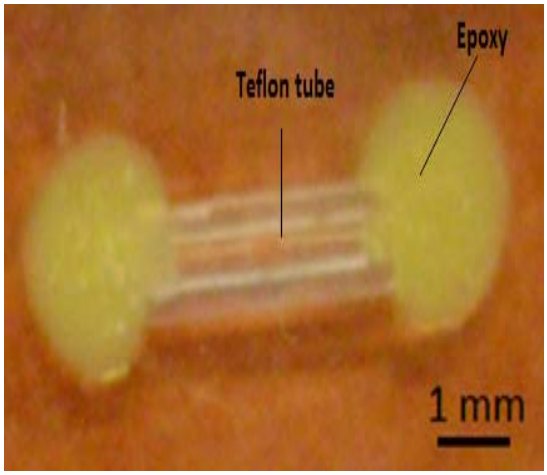

Figure5.1.2 Picture of a single channel taken by lab member.

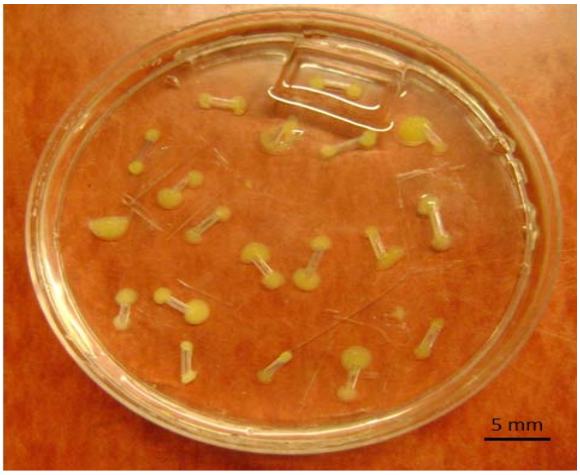

Figure 5.1.3 Channel mold showing 16 channels. Picture taken by lab member

5.2 Elastomeric Channel

After the channel mold was done, we needed an elastomeric material to conform around the shape of the channel (Figure 5.1.2) to form the channel walls. The elastomeric used is polydimethylsiloxane (PDMS) for several reasons as shown in Table 5.2.1

Advantages and Disadvantages of PDMS	
Advantages	**Disadvantages**
Low cost	Collapsing of channels
Low surface tension	Shrink 1% during curing
Transparent	Sagging of structures
Pliant	
Reliable	
Easy and Fast manufacture	
Biocompatible	
Permeable to oxygen	
Chemically inert	
Homogeneous	
Low water absorption	
Variable hydrophobicity	

Table 5.2.1. Benefits of PDMS material

There are various ratios for PDMS mixture. After various experiments, we concluded that 10:1.1 ratio produced the necessary properties for this task (Figure 5.2.1.) The PDMS mixture was poured onto the channel mold, and then it went through a degassing and curing process (Recipe 7.) After that, we cut around the channel, making sure that there was enough surrounding area (~ 10 mm) and carefully peeled the PDMS from the Petri dish. The PDMS channel was placed directly onto the wafer between the two electrodes of the device. It was critical to keep the PDMS surface clean because any contaminants or moisture would hinder its adherence properties.

Figure 5.2.1 PDMS Materials. Picture taken by lab member

5.3 Channel Testing

At this point we tested the channel with the set up shown in Figure 5.3.1.

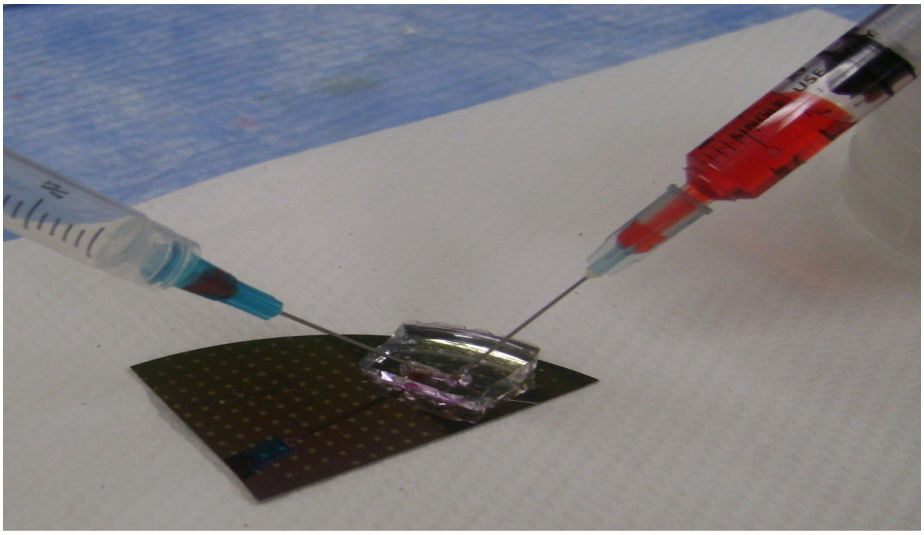

Figure 5.3.1 Set up that was created to test the channel. Picture taken by lab member

We introduced fluid to the channel with a syringe. We observed that the fluid went through the channel and it came out on outlet syringe without leaking.

6. DISCUSSION AND CONCLUSIONS

We were able to fabricate SWCNT field effect transistors successfully. When we probed each device we ended up with approximate 10-20% yield of devices, which is good considering that the growth of CNTs is an uncontrolled process, which yields a mixture of metallic, semiconducting, single-walled, and multi-walled CNTs. Then, we functionalized these devices by attaching prostate cancer antibody and exposed them to the prostate cancer biomarker. After that, we probed the devices to see if we could electrically sense the presence of prostate cancer biomarkers. This step is still in progress, but as we can see in Figure 4.2 there is a change from the measurement pre-prostate cancer biomarker exposure to post-prostate cancer biomarker, but more experiments need to be performed to characterize and understand this change. Finally, we designed a PDMS channel, which was placed between the two electrodes of the SWCNT FET (Figure 5.1). This channel passed fluid through the CNT without any leakage (Figure 5.3.1); eventually this will allow us to take electrical measurements while fluid was passing through the CNT. Future work will be focused on bringing the functionalization and fluid handling parts together in order to have real time, highly sensitive and highly specific detection method.

7. METHODS

Recipe 1. Wafer preparation

- Turn on plasma etcher
- Turn on nitrogen and oxygen tanks
- Vent the chamber with nitrogen and open
- Place silicon wafer inside
- Turn vacuum on to evacuate the air from chamber
- Set up voltage to 60 Watts
- As the pressure in the chamber is going down flow oxygen twice
- Once the chamber has reached low pressure the processing gas, oxygen is introduced, power is turn on at the same time to excite plasma, maintain for 5 minutes
- Turn off power and oxygen
- Vent the chamber with nitrogen
- Open and take wafer out
- Prepare the catalyst solution by mixing iron nitrate and isopropanol to form a 50 mg/L concentration
- Place silicon wafer on spinner
- Dispense the catalyst on the silicon wafer
- Spin the wafer at 3000 rpm for 1 minute (until wafer is visible dry)

Recipe 2. Carbon Nanotube Synthesis

- Cleave the silicon wafer so that they are smaller than 2-inch
- Place wafer in a 2inch diameter tube
- Close the seals on each end (ensure everything is tightly closed)
- Place tube in the furnace
- Set up furnace to heat up to 900°C
- Flow 320 sccm of hydrogen, 600 sccm of argon, and 300 sccm of methane for 10 minutes
- Turn off the methane and flow hydrogen and argon for 5 minutes
- Turn on furnace and wait until it reaches 900°C (approximately 1 hour)
- When it reaches 900°C turn on 2500-sscm for 2 minutes
- Turn off furnace
- Turn off methane
- Wait until the furnace temperature goes down to 400°C
- Open furnace and turn off hydrogen
- When furnace temperature reaches 100°C turn off argon and take sample out

Recipe 3. Lithography

- Pre-bake at 200 °C for 2 minutes
- Spin PMGI at 4000 rpm for 45 seconds
- Bake at 150° C for 5 minutes
- Spin 1813 photo resist at 5000 rpm for 45 seconds
- Bake 130° C for 2 minute
- Expose to 405 nm light at 15 mW/cm^2 for 3.3 sec
- Develop in MF-319 as needed, usually about 2 min
- Rinse with DI water
- Dry wafer with nitrogen gun

Recipe 4. Evaporation-Deposition of Electrodes

- Using double sided tape attach the wafer to the evaporator stage
- Vent the chamber and open
- Place the chrome and gold pellets on the designated boats
- Place the stage in chamber and close
- Turn on rough pump
- Wait until chamber reaches 30 mT pressure
- Turn off rough pump
- Turn on high vacuum
- Wait until chamber reaches 8x10^-7 mT pressure
- Turn on power so current starts flowing
- Wait until the reading stays at 0.100 nm
- Open the shutter and hit “process”
- Monitor thickness that is being deposited to 40-nm
- Turn off power
- Wait for ~5 minutes to cool down
- Turn off high vacuum
- Vent chamber

Recipe 5. Liftoff

- Prepare a four bath system with acetone, CD-26 developer, isopropanol, and DI water
- Immerse chip in glass container filled with acetone, shake constantly until visually the metal has come off.
- Change chip to the second bath, CD-26 developer, inspecting that all the residue from the first bath has left the chip
- Change chip to the acetone bath just for a few seconds
- Immerse the chip in DI- water, at this point very little residue should be visible in the DI-water
- Dry with nitrogen gun

Recipe 6. Functionalization

- Immerse the devices in a solution of 25mg/L of diazonium and DI water
- Heat up device in diazonium solution for one hour at 45°C
- Take the chip out of the diazonium and perform a three bath system with acetone, methanol, and DI water
- Dip chip in a solution of 16 mg of NHS, 6 mg of EDC and 15 mL of MES buffer for 15 minutes
- Perform bath with MES buffer and DI water
- Blow dry with nitrogen
- Pipette a drop of prostate cancer antibody on the device and keep chip in a moist environment for 45 minutes
- Wash chip with DI water and blow dry with nitrogen
- Pipette a drop of the prostate cancer biomarker for 10 minute
- Wash chip with DI water and blow dry with nitrogen

Recipe 7. PDMS

- Mixed the PDMS base curing agent 10:1.1 ratio respectively
- Degas PDMS by placing in a desiccator connected to a rough pump to create vacuum.
- Cure PDMS for 2.5 hours at 75°C

8. ACKNOWLEDGEMENTS

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