Fabrication and Characterization of Nano-Field Effect Transistor for Bio-safety

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ABSTRACT

Various nano Field Effect Transistors (FET) were designed and fabricated with doped semiconductor nanowires (30nm-500nm) on silicon/silicon dioxide wafers to optimize the best feature size and stable output. The polyimide coated nano-FET surfaces were covalently modified with in-lab synthesized specific peptide nucleic acid (PNA) as well as specific antibodies. When targeted DNA or bio-agents were captured, the constant overall impedance increased or decreased depending on the charge on target molecule. Current data indicates the strong influence of the resistance, capacitance and mass transfer as a result of DNA binding. Current data indicates that the nano-FET will be the basis for a robust, very sensitive hand held tool for bio-threat and clinical detection.

***Keywords*:** nano-FET, electrochemical impedance spectroscopy, nanowires, peptide nucleic acid, DNA, RNA.

1 INTRODUCTION

The advances in development of semiconductor nanotechnologies have provided fabrication means to achieve a new sensing philosophy that merge chemical as well as biological events for "lab on a chip" silicon based devices [1]. The nano-device, included in the respective set-up of a chip, play the key role with regard to their sensitive biochemical behavior [2, 3]. New research in the field of nanowires has provided evidence that nanowires based methods [4] may be the next great leap in clinical tools. Recently, there has been an increasing demand for detection of specific nucleic acids and bio-threat agents. The detection of specific DNA or RNA sequences is of importance because of the large number of known inherited diseases. Many detection techniques in biology are based on hybridization of DNA. Single-stranded DNA binds to its complementary strand present in a sample, and a double helix structure is formed. This hybridization is a measure of the amount of specific sequence in the target samples. Most current methods of DNA sensing [5] are based on optical detection by labeling a target DNA with fluorescent material at specific wavelength. But these methods require labeling, long chain reaction and PCR provide amplification of a sample. Our nano-field effect biosensors are very sensitive, label free, and operate for real time detection of bio-agents. The charge carrier in the nanowires can be varied with the molecules captured on top of the nanowires surface. Also, the utilization of low cost semi-conductor fabrication using polyimide, which are chemically and physically stable in a working atmosphere, also possess the advantage of biocompatibility to attached bio-agents. In this work polyimide film was formed over the low doped semiconductor nanowires. Prior to target DNA hybridization, a specific recognition PNA is functionalized onto a thin polyimide surface.

2 METHOD

2.1 Device Fabrication

A nanowire based gold nano-electronic device for bio-detection was fabricated at the Cornell Nanoscale Facility (CNF), Cornell University, Ithaca NY (Fig 1). The photo-mask for designed large gold contact pad, gold pad opening mask after dielectric layer and zero layer alignment mark for the e-beam were made from a Heidelberg DWL66 laser pattern generator. A four-inch p-type silicon (500µm)/silicon dioxide (50nm) wafers were first processed in TFT polysilicon furnace to deposit 50nm of a P+ type semiconductor film and later annealed. The wafers were then put on a HDMS (hexamethyldisilazane) vapor prime oven for 35 min for better adhesion to the resist layer. The SPR 220-3.0 i-line photo-resist was then spun on HDMS primed wafers at 4000rpm for 1 min and wafers were pre-baked at 115ºC for 90 sec. Developed wafers then subjected to deep etch in Oxford 100 plasma. After etch, wafers were then placed in hot resist bath for 2 hrs, cleaned and inspected on a microscope. Wafers were then spin coated with PMMA (poly methyl methacrylate) in anisole safe solvent e-beam resist at 1000-5000 rpm for 60 sec and pre-baked at 170ºC for 15 min. The wafers were then exposed on a JEOL e-beam, developed in MIBK: IPA (1:1) for 90 sec, rinse in IPA and dried under

nitrogen. All wafers were exposed on oxygen plasma (descum) to remove any resist residue. The patterned wafers were then subjected to specific chlorine etches to obtain desired shape and size of nanowires. The PMMA was spun again onto wafers to get small gold bond pad on each end of nanowires and expose on JEOL e-beam, developed and descum. The titanium (15 nm) and gold (50 nm) films were deposited on the wafers, using a CVC SC4500 E-gun evaporator. After deposition, the wafers were left in methylene chloride and acetone solvent to lift off unwanted gold and cleaned. The P20 (HDMS) primer and followed by OiR 620-7i resist were spun at 4000rpm for 60 sec and prebaked at 90ºC for 60 sec. After resist bake, wafers were exposed using an auto-stepper to obtain large gold pattern transfer for 0.5 sec and post baked at 115ºC for 90 sec. The wafers were developed in a MF 300 developer and descum in oxygen plasma. The CVC SC4500 E-gun evaporator via E-beam used again to deposit titanium (15 nm) and gold (50 nm) films. The wafers were then placed in methylene chloride and acetone solvent to lift off unwanted gold and cleaned. Photoneece® PWDC-1000 positive tone polyimide was spun on the patterned glass wafer and soft-baked for 1 min at 110ºC. The GCA AS200 auto-stepper was used to expose the polyamide for 0.5s. The insulating layer was patterned to expose the opening of contact pads only, descum and cure in a YES 450PB polyimide oven for 8 hr.

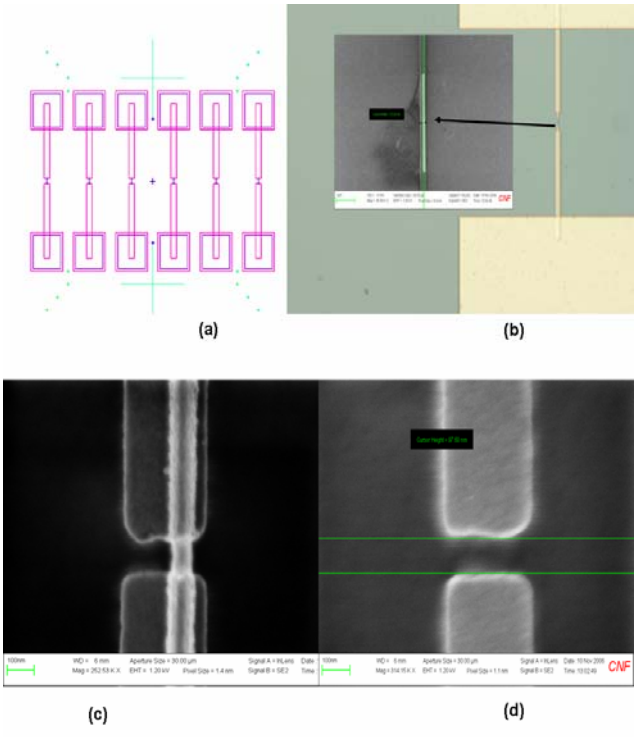

Figure 1: (a) Layout of fabricated device, (b) a 20x magnification optical view of nano-device with SEM image of nanowire area, (c) SEM image showing sub 30nm nanowires and (d) SEM image showing sub 100nm gap between two gold contact.

2.2 Chemicals and Instrumentation

All chemicals and bio-chemicals used in experiments were ACS analytical grade. The PNA was synthesized and purified by Shimadzu SPD-10AVP HPLC instruments, USA. The modified nano-biosensor surface images were taken on an Olympus BX60 UV microscope. Electrochemical impedance spectra were recorded using a Reference 600 from Gamry Instruments USA.

2.3 Nano-FET Characterization

Field effect behavior of nano-biosensor was characterized by the electro-chemical impedance spectroscopic (EIS) method. The AC voltage from 10-250 mV was applied through two electrodes linked with semi-conductor nanowires under varying DC voltage 0-2.5 V on each sweep. The EIS Bode and Nyquist plot were recorded from 0.01Hz-100 kHz frequency range in grounded faraday gauge. The spectra were analyzed with Gamry's Echem software.

2.4 Surface Modification Procedure

Self assembled monolayer of long carbon chain alkanoic acid was formed by using 1 µL of 5mM solution in ethanol of Nano-Thinker (11-mercaptoundecanoic acid, MUA) on plasma cleaned polyimide coated nanowires surface and incubated more than 14 hrs in a humid environment at room temperature. After incubation, the surface was sonicated with 100% ethanol followed by de-ionized water cleaning and dried under low stream of nitrogen. One other similar chip was also modified with 2 µL of trichlorosilane (TCS) and washed with 90% ethanol followed by air dry at room temperature. The 0.5 µL of 11.4 µM PNA solution was applied on TCS modified and unmodified surface and incubated in a wet chamber for 1 hr. After incubation, the samples were gently washed 5 times with 1X PBS solution and let sit 2-3 min in air dry.

The MUA modified surface was placed in an aqueous solution of 100mM 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDAC) and 25mM *N*-hydroxysuccinimide (NHS) (1:1 v/v) for 1 hr at room temperature to attach the NHS to the COOH terminal of MUA modified surface. After incubation, the surface was washed and 1 µL of specific capture antibody/PNA was applied on the surface and incubated for 1 hr in a wet chamber in order to covalently attach these molecules. The surface was washed gently and immersed in a blocking solution of 0.01% BSA, 1mM polyethylene glycol and 1mM glycine for 1 hr. After the blocking step, the surface was washed with 1XPBS of pH 7.2 and 1 µL of targeted bio-molecules were applied and incubated for one hr and gently washed 5 times. All final surfaces which captured the targeted bio-agents were immerse in a DAPI (4,6-

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diamidino-2-phenylindole) solution and placed on a shaker for 1 hr followed by a thorough wash with DI water and inspected under a microscope with DAPI UV filters.

2.5 Bio-agent Detection Procedure

The E.coli DH5 alpha 16S rRNA gene was chosen as the detection target. Photo cleavable biotin labeled probe-1 with an E.coli signature sequence was the capture probe. Alexa Fluor480 labeled probe-2 with bacterial universal sequence (Bac338) was used as signal probe. Target DNA was captured by streptavidin magnetic beads and released from beads by UV exposure. Target DNA were then captured by anti-AF480 antibody immobilized on the top of nano-biosensor surface.

3 RESULTS AND DISCUSSION

The UV microscopic images (Fig 3) of various DAPI stained DNA/RNA/PNA molecules on modified as well as on unmodified surfaces show a very specific binding of bio-molecules on the surface. DAPI a blue fluorescent nucleic acid stain that preferentially stains DNA produces an approximate 20-fold enhancement of fluorescence, apparently caused by the displacement of water molecules from both DAPI and DNA. DAPI also binds to RNA and PNA, but via a somewhat different mechanism. Because of this property, DAPI is a useful reagent for capture verification of DNA or PNA on the surfaces.

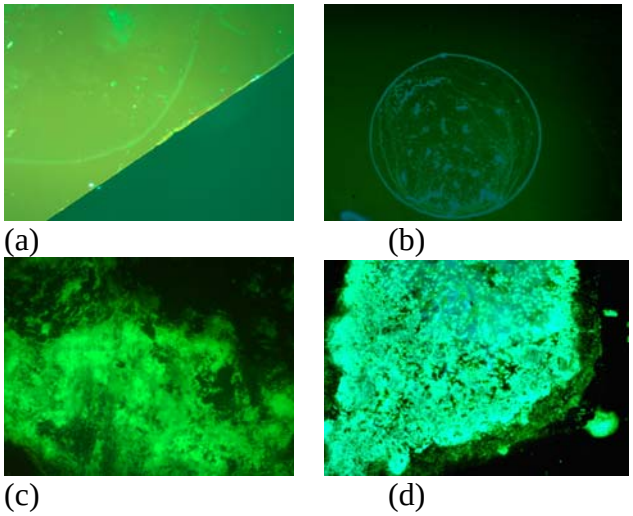

Figure 3: (a) DAPI image of surface have no biomolecules. (b) DAPI stained DNA on unmodified surfaces showing less molecules. (c) DAPI on MUA modified surface showing no binding to surfaces. (d) DAPI stained DNA on modified surface showing dense DNA molecule.

Data gathered from impedance spectra shows that the nano-field effect biosensor is very sensitive to the small varying DC voltage (0.01-0.5mV) through a reference electrode. A significant drop was observed in impedance on low frequency zone while positive DC voltage was increased, demonstrating a field effect behavior of the nano-FET (Fig 4). All sub 30 nm nano-FET were very sensitive to bias voltage as we can see from figure 5, current was changing with very small voltage applied from back gate. The maximum current detection possible through these nanowires was found to be around 75 µA. During characterization of this nano-FET, we did not observe any leakage from the source or drain contacts to the gate electrode through the thin oxide dielectric layer.

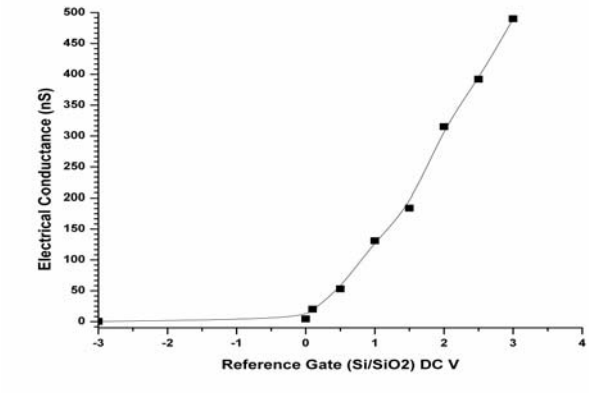

Figure 4: The plot between reference gate voltage and electrical conductance of nano-FET with 75mV AC across nanowires at 1Hz showing changes in conductance.

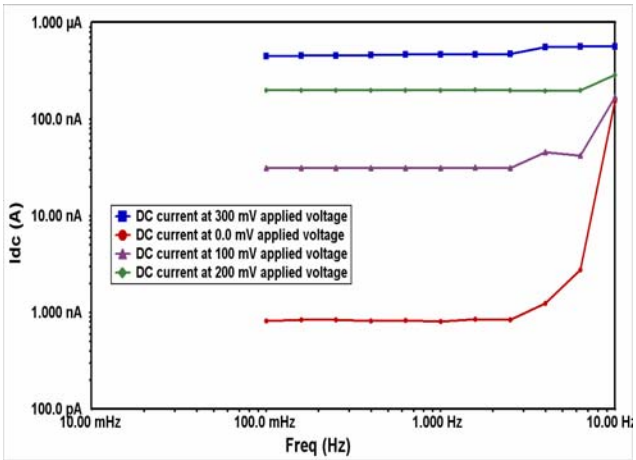

Figure 5: The plot between frequency and DC current of nano-FET at 50 mV AC across nanowires at showing sensitiveness to applied bias voltage from 0.0-300 mV. The current is increasing with small bias voltage applied

Different sizes were tested from 30-500 nm nanowires. Most of them were very sensitive to small charge on the nano-biosensor surface. Some fabricated Nano-FETs had no nanowires between gold pads (separated with 100 nm gap) and behaved as pure resistors and not sensitive to any applied DC voltage or charge on the surface which further proved the effects of nanowires on other device. The nano-FET was also characterized with a specific antibody molecular capture system. The EIS of captured DNA molecules show changes in impedance spectra due to hybridization of a DNA molecule to the sensing agents on the nano-FET (Fig 6).

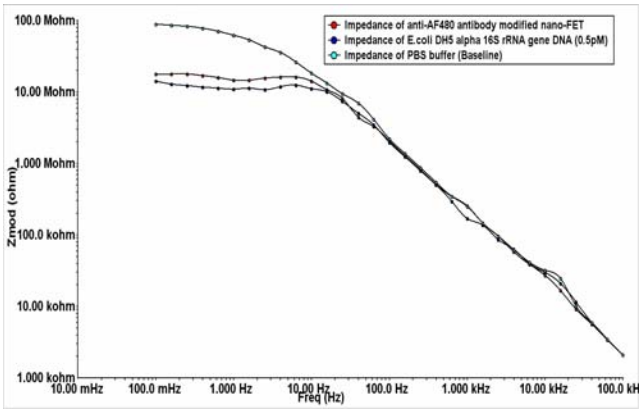

Figure 6: EIS Bode plot of antibody modified nano-FET. The spectra show a clear detection of DNA due to recognition by antibody.

4 CONCLUSIONS

The electrical conductance of the nano-biosensor show significant increase in the positive gate voltage region suggests a transistor type behavior of the devices. The DAPI images suggest that we are able to modify the surface and capture the bio-molecules as compared with unmodified surface where no molecules were captured. The data presented here show that the model for direct electrical detection of E.coli DH5 alpha 16S rRNA using electrical impedance spectroscopic measurements at higher frequencies, we are able to directly detect DNA without damaging the molecular structure. This is due to the overall impedance being influenced by molecular recognition. Overall data indicates that this device can serve as the basis for a robust handheld bio-direction device for harmful bio-agent in the future.

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