Detection of Oligonucleotides Electronically at the Nanoscale

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ABSTRACT

Here we present an ultrasensitive and label-free biosensor referred to as the nanoneedles biosensor. The nanoneedle biosensor shows promise to overcome some of the limitations of existence biosensors. The nanoneedle biosensor has the advantages of being a real-time, label-free and direct electrical detection platform. This novel electrical biosensor is capable of high sensitivity detection, by measuring the change in ionic current and impedance modulation, due to the presence or interaction of biomolecules such as proteins or nucleic acids. In this paper we discuss fabrication of our sensors first and then we talk about the study of electrical properties of DNA at the nanoscale.

Keywords: Nanotechnology, Biosensors, Label free, Impedance Biosensor, DNA.

INTRODUCTION

Sensitivity, detection limit, and multiplexing are the most important issues of all current biosensors. Direct electrical detection of biomolecules without the need for any labeling can play a very important role in realizing the dream of personalized medicine. The dominant performed technique to detect proteins, nucleic acids and cells is optical fluorescence based techniques, which is more costly and timely compared to electrical detection due to the need for expensive and bulky optical equipment and the process of fluorescent tagging.

Out of the different the optical fluorescence based techniques, sandwich ELISA technique is the main and most common technique used for the protein detection. This technique involves several steps. It starts with the incubation of the test sample. Then there is a polyclonal antibody, and finally we have the secondary antibody, which is usually tagged with a fluorescent or luminescent label. There are also several wash steps in between. A label-free technique, which could directly detect the binding of a target protein to the surface antibody would be more desireable.

Various efforts have been made in the field of developing label-free biosensors. Examples include the quartz crystal microbalance, resonant mirrors, planar waveguides and surface plasmon resonance (SPR). One of the advantages of these techniques is their speed. The other advantage of these techniques is simplicity of operation due to lack of need for any labeling. In terms of the mechanisms that SPR and waveguide based approaches use we should refer to the surface-sensitive physical phenomenon called an evanescent wave. It works by utilization of the total internal reflection (TIR) at a surface-electrolyte interface producing an area sensitive to the index of refraction at the sensing surface extending hundreds of nanometers into the solution.

The other class of label free biosensors that have shown very promising results is impedance biosensors. These types of sensors have shown very promising results for point of care and the other applications due to being label free, low cost and their ease of miniaturization. The traditional method for measuring impedance involved use of macro-sized wires immersed in electrolyte [1], [2],[3]. Over the last decades various configurations and geometries of impedance-based techniques have been developed to improve their sensitivity. One of them is the scaling of electrodes down to the microscale. It offers many advantages in terms of sensitivity over macroscale techniques because of the spherical diffusion profile resulting in a greater rate of reactant supply compared to macro-electrodes, which have a semi-infinite linear diffusion profile which results in larger depletion of reactants [4]. Low ohmic drop and increased signal-to-noise ratio has made interdigitated microelectrodes advantageous [5],[6]. Other label-free electrical biosensing techniques include nanowire field effect transistors, nanopore sensing, and nanogap capacitance sensing. Among various electrical detection techniques until today nanowire FETs have reported very low detection limits [8],[9].

FABRICATION

The nanoneedle biosensor is a novel ultra-sensitive, label-free and localized device. A nanoneedle biosensor

structure consists of four thin-film layers. There are two conductive layers with an insulator layer in between. There is a protective oxide layer above of the sensors. This layer is to prevent the exposure of conductive electrodes to the solution. Underneath the bottom electrode, there is another oxide layer, which can be a thermally grown oxide. This layer insulates the first electrode from the substrate. We have fabricated and tested various thicknesses and geometrical designs of nanoneedle biosensors. The thickness of electrodes is 100 nm for the design that we have used for this paper's experimental results. The middle oxide layer thickness is 30 nm. The top protective oxide layer thickness is 20 nm and the bottom oxide layer thickness is 250nm[7],[10]. The width of the sensor is 5 μm. The probe molecule of interest (e.g. DNA molecule or protein) can be immobilized on the tip of the sensor. The binding of target molecules to the probe molecules or the presence of the biomolecule of interest modulates the impedance between the electrodes. The middle oxide layer is the sensitive part of the sensor thus the thickness of that is the critical factor that determines the sensitivity. Figure 1 shows a schematic of a nanoneedle biosensor.

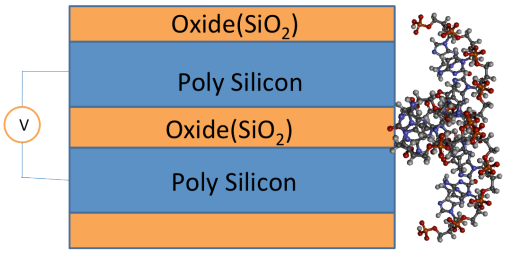

Figure1: Schematic of a nanoneedle biosensor with two DNA molecules located at the sensitive part of the sensor.

The nanoneedle device shows a high sensitivity feature for in-vitro detection, due its integrated structure. Since the sensing area (middle oxide layer) is the nanometer-sized section, which is in the same range as the size of bio-molecules of interest for detection; therefore the nanoneedle device detects the modulated signal generated in the binding or presence of just few molecules at the sensing area.

To demonstrate a proof-of-principle, we first arrayed 600 nanoneedles, 15 bundles each containing 40 nanoneedles. There is a SiO_2 layer underneath of the devices to insulate the electrodes from the silicon substrate. The thickness of this thermally grown oxide layer is 250nm. Then we have a low pressure vapor deposition (LPCVD) to deposit 100 nm polysilicon to make the first conductive electrode. Then this polysilicon film was doped with phosphorus to increase the conductivity of the electrode. Then two nanoscale electrodes were separated out from each other by thermally growing of a 30 nm layer of SiO_2. Then the second electrode was deposited using low-pressure vapor deposition (LPCVD) technique to deposit 100 nm polysilicon followed by phosphorus doping. Then we deposited the second layer of SiO_2, which is a protective oxide layer. It was deposited using PECVD. Then multiple dry etching steps to form a sharp edge on the nanoneedle sensor-side followed the lithography process. Then we opened the channel underneath the nanoneedle devices by a wet etched step. The final step was to etch the wire bonding pads area to enable contact access for measurements. Figures 2 shows a TEM image of a fabricated nanoneedle Biosensor.

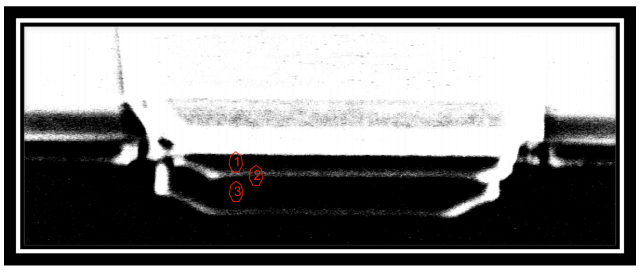

Figure 2: SEM image of a fabricated nanoneedle biosensor. 1& 3 are the electrodes; 2 is the oxide in between the electrodes.

EXPERIMENTAL RESULTS AND DISCUSSION

In order to study the electrical properties of DNA at the nanoscale we have used nanoelectronic probe that we have developed. To do the impedance measurements we used a Versa STAT3 potentiostat. A sinusoidal voltage signal was applied to the top polysilicon electrode and the current entering the bottom electrode was measured. This measured current was used to calculate the impedance. This measured impedance consists of different elements such as fringing capacitance, double layer capacitance, bulk capacitance, bulk resistance, double layer resistance and solution resistance. The ratio of the applied voltage to the passing current gives the value of the impedance. Our measurement shows that the optimal frequency for the bio-sensing measurements is at 15 KHz. For all the measurements, a 100mV RMS AC signal was applied. The measurement occurs in real-time during all the events. We prepared our oligonucleotide samples as discussed below. First we desalted our single stranded oligonucleotide, which was 20 base pairs long. Then we diluted our DNA sample in DI water to achieve our desired concentrations. After every dilution step we used a vortex for 30 seconds to fully mix the contents of the epindorph tube to ensure uniformity. Each sample was prepared 3~5 minutes before than the injection to the well.

Presence of DNA molecules near the tip of the sensor results in an increase in current across the sensing electrodes. As observed in figure 3 the presence of single

stranded DNA modulates the measured impedance. We tested the device with various concentrations of DNA. Every time a new concentration of DNA was injected onto the sensor surface sequentially. Between each step of DNA injection we dried out the measurement well.

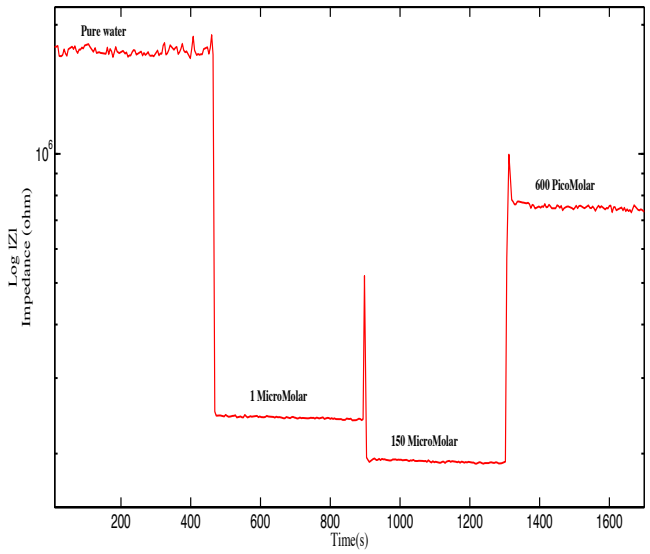

Figure 3: Presence of single stranded DNA modulates the measured impedance. Various concentrations of DNA were injected onto our sensor surface. Between each injection step the measurement well was dried out. As the concentration of DNA in the solution decreases, the measured impedance increases and gets closer and closer to the baseline value.

As seen in figure 3 by deceasing the concentration of DNA in the solution, the measured impedance increases and gets closer and closer to the baseline value. Since the DNA is in free solution and not adsorbed to the surface, this effect of increase in conductivity with higher DNA concentration is due to a higher number of counter-ions being attracted by the backbone charge. We propose that this effect of increase in conductivity with higher DNA concentration is most likely due to two basic mechanisms. One of the mechanisms behind the electrical response of DNA in solution under an applied alternating electrical field comes from the formation and relaxation of the induced dipole moment. The mobile charges in and around the DNA allows for a dipole to be induced in the DNA when undergoing an AC field. The second mechanism is likely the tunneling of electrons through the biomolecules. Presence of DNA molecules near the surface of the electrodes modulates a tunneling current between the two electrodes. The DNA molecule is acting as a charge conductor resulting in an increase in current. As the result of both mechanisms we observe an increase in current with an increase in DNA concentration [11],[12].

Conclusion

In this paper we discussed fabrication and characterization of our sensors. We also discuss our study of the electrical properties of DNA at the nanoscale using a nanoelectronic probe that we have developed. As we observed in the experiments, presence of DNA molecules in the well and close to the tip of the sensor results in a decrease in impedance. This decrease of impedance is due to two main mechanisms. The first mechanism is the increase in tunneling current passing between the electrodes. The tunneling current between the two electrodes is likely being modulated due to the presence of DNA molecules near the surface of the electrodes. The second is an increase in AC coupling current. The increased number of mobile counter-ions results in increasing the conductivity of the solution. This increase of AC coupling is due to an increase in induced dipole moments. The work performed in this study can be extended to various biological applications such as real-time PCR for point of care diagnostics.

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