Iridium Oxide Nanomonitors for Protein Detection

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ABSTRACT

This research focuses on the development of a "point-of-care" device based on iridium oxide nanowires for physiological state identification to monitor human health. This device is based on electrical detection of proteins that are biomarkers for the physiological state. The key innovations of this technology include increasing the sensitivity and selectivity of detection, the linear dynamic range of detection and reducing the response time.

Highly sensitive and rapid detection of two such protein biomarkers C-Reactive Protein (CRP) and Myeloperoxidase (MPO) in pure form and in serum/clinical samples was achieved with a detection limit in the order of high pg/ml and a response time in seconds. Iridium oxide has very good conductivity and charge storing capacity, and nanowires have an ideal morphology to crowd protein molecules and highly increase the surface area of interaction. Hence, IrOx nanowires help bring forth highly sensitive electrical detection of proteins.

Keywords: iridium oxide, protein, nanowires, electrical detection, point-of-care

1 INTRODUCTION

Monitoring human health in order to prevent the occurrence of diseases and organ failures is one of the major concerns prevailing in this world. Detecting multiple protein biomarkers in the system help to indicate the susceptibility of an organ to fail or a disease to occur. Traditional methodologies incorporate huge infrastructure and highly skilled personnel and hence limit the access to preventive action [3].

Electrical detection methodologies have always used prominent techniques like Electrochemical Impedance Spectroscopy (EIS) with large electrodes when compared to the size of the biomolecules. EIS is used typically to find the impedance change at particular frequencies in order to quantitatively determine the concentration of biomolecules from the biochemical reaction. Protein binding events produce very small changes in impedance and have hence never been possible to detect with large electrodes and electrochemical cells. Impedimetric biosensors have found a new definition since the pioneering work of Arwin et al where they measure enzyme activity through the adsorption of proteins on to the electrode surface [11]. However, due to very small signal changes, high sensitivity of detection was not possible. The advent of nanotechnology with the rapid development of nano fabrication techniques, high sensitivity label-free electrical detection is now possible.

Nanomaterials effectively increase the surface area to volume ratio, hence providing much more surface area for interaction of the biomolecules along with making the device much smaller [10]. Nanowires have an ideal morphology to crowd the protein biomolecules and confine them to smaller spaces, hence retaining their original conformations for a much longer time [1]. Iridium oxide has very good conductivity and charge storing capacity, and hence has an ability to detect very small changes to the surface charge. Hence, the nanowires along with the physical and electrical properties of iridium oxide (IrOx), help bring forth highly sensitive electrical detection of proteins and also forms the basis of the key innovations of this technology.

Highly sensitive and rapid detection of two study protein biomarkers C-Reactive protein (CRP) and Myeloperoxidase (MPO) in pure form and in serum/clinical samples was achieved with a detection limit in the order of high pg/ml and a response time in seconds. CRP and MPO are biomarkers for cardiovascular diseases, whose early detection would help prevent cardiac arrests and enable early disease diagnosis [4]. The methodology that has been adopted is based on measuring capacitance and calibrating its change in magnitude with concentration of proteins. High selectivity is achieved by incorporating monoclonal antibodies (protein receptors) on the nanowires, which bind only to specific antigens (proteins). The following sections describe the device fabrication, principles of detection, performance metrics achieved by the device and future improvements.

2 DEVICE AND FABRICATION

The device constitutes a two-electrode arrangement - a working electrode (WE) and a counter electrode (CE) with an area ratio of WE:CE - 1:50.

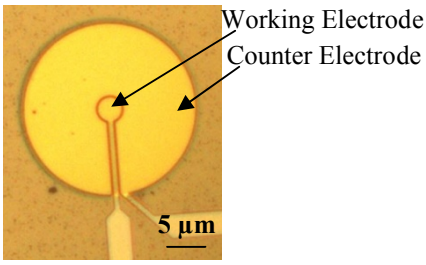

Figure 1: Optical micrograph of the electrode arrangement

The electrodes are circular in shape to in order to maximize the surface area of interaction between the electrodes and avoid possible edge effects. The diameter of the counter electrode is 30 µm and that of the working electrode is 8 µm. These electrodes are separated by a gap of 1 µm as shown in Figure 1. The working electrode is the major sensing site for the proteins while the counter electrode acts as a differential reference. Interconnects from these electrodes lead to I/O pads across which impedances and conductivities were measured and analyzed using Electrochemical Impedance Spectroscopy.

The above electrode setup was achieved by the standard process of photolithography on a Si wafer. A thin film of TiN was deposited on the electrodes leaving an insulating gap between them. The remaining part of the chip constituting the silicon dioxide layer and the interconnects were suitably passivated to prohibit noise from the external environment.

IrOx nanowires were grown on the TiN thin film with non-continuous surfaces on a Si substrate through the Metal Organic Chemical Vapor Deposition (MOCVD) process. Oxygen and (methylcyclopentadienyl) (1,5-cyclooctadiene) iridium(I) were used as precursors in the MOCVD process to promote the growth of IrOx nanowires on the growth promotion film surfaces. The IrOx nanowires have a diameter of about 0.5 nm and lengths in the range of 300 nm - 500 nm, an aspect ratio (length to width) of greater than 50:1 [1, 2].

3 PRINCIPLES OF OPERATION

The device works on the principle of formation and perturbation of the double layer. An ionic buffer solution is always present between the electrodes and is used as the basic platform and the medium for protein detection. 1X concentration of the isotonic Phosphate Buffered Saline (PBS) solution is used as the ionic buffer in this study. An electrical double layer occurs whenever an array of charged particles and oriented dipoles are present near the liquid/metal interface. When an electrode is charged, it attracts oppositely charged species and forms a neutral region around the electrode as shown in Figure 2.

This neutral layer creates other solvent ions in solution. The inner layer, which is closest to the electrode is called inner Helmholtz plane (iHp) and it contains solvent molecules, specifically adsorbed ions. The next layer is called outer Helmholtz plane (oHp) and the layer after this is called the diffuse layer [6, 8]. When the protein binds at the metal/liquid interface, it perturbs the surface charge distribution at the inner Helmholtz layer as the protein is electrically charged [9]. With more proteins binding at the interface, the associated surface charges also changes significantly. Hence, in this setup, the measurement of the protein biomolecule binding occurs by measuring the surface charge perturbations at the electrical double layer resulting in a measurable electrochemical capacitance change.

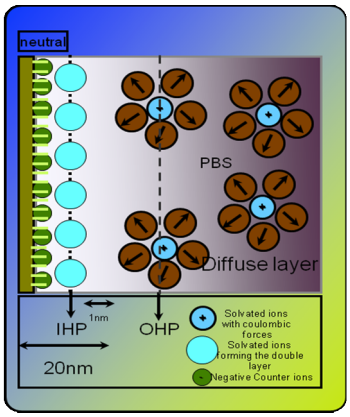

Figure 2: Charge distribution across the liquid/electrode interface forming the double layer

All proteins are prepared and aliquoted using this PBS buffer and all measurements are taken with the presence of this control buffer between the electrodes. By applying a small DC bias (~200 mV) between the electrodes, the ions in solution are attracted to the electrode/liquid interface to form two layers of opposite charges in the iHp, acting analogously to a parallel plate capacitor (Figure 2). This capacitance is called the double-layer capacitance (C_{dl}), which changes when the surface charge distribution is perturbed at the iHp due to protein binding.

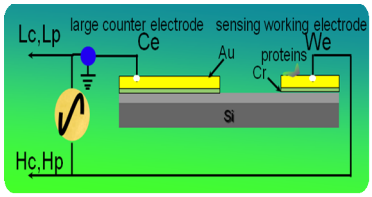

Figure 3: Impedance measured across the working and counter electrodes

C_{dl} is dominant at the lower frequencies (up to 10 kHz) and it changes significantly with the increase in concentration of proteins binding at the double layer. This change in capacitance was analyzed using Electrochemical Impedance Spectroscopy (EIS) technique for the quantification of protein concentration. The impedance analyzer HP 4194A was used for the analysis, sweeping the frequencies between 100 Hz – 10 kHz, to measure impedances across the working and counter electrodes (Figure 3).

The electrical interface can be considered as the series connection of two parallel plate capacitors of the thickness of the compact layer and diffuse layer respectively with an ion rich water dielectric [5]. This is the metal electrode-solution interfacial capacitance of the electrical double layer, C_{dl} as seen from the equivalent circuit representation in Figure 4.

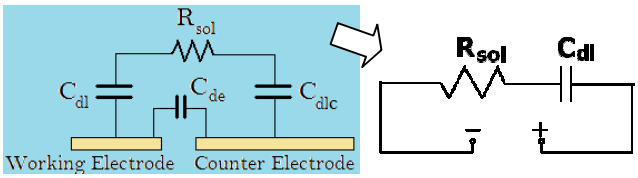

Figure 4: Electrical equivalent circuit

Besides C_{dl}, the electrode-solution interface has faradic impedance towards current flow between the electrode and the solution, including the finite rate of mass transport and electron transfer at the electrode surface. When protein binding perturbs the electrical interface, all other electrical components change negligibly when compared to the change in C_{dl} at low frequencies [7]. This is because the bulk or sample volume is kept constant for all the measurements; hence the order of change in the resistances is very less. The perturbation also affects only the compact layer component significantly. Hence, the change in impedance measured between the electrodes can be directly attributed to the change in the double layer capacitance as suggested in Figure 4.

4 METHODOLOGY

The first step towards protein detection and quantification is to identify background effects. Open and short circuit measurements were noted for appropriate electrical compensation. The first reference impedance curve was measured with the basic ionic buffer 1X PBS on and between the electrodes. All proteins were prepared and aliquoted using this buffer. A Biotin/Streptavidin linker was used to bind the antibody on to the electrodes.

After coating the electrodes with a layer of Streptavidin and appropriate incubation, biotinylated antibodies were dropped on to the electrodes till saturation. Due to the strong avidin linking, the antibodies stay strongly crosslinked to the electrodes. All protein additions were followed by a 15 minute incubation at room temperature. The impedance measured at this point provided the final reference for the measurements. The change in impedance with respect to this reference after exposure to protein samples (antigens) was used for calibration for quantification of the protein. This change in impedance, as discussed in the previous section, denotes the change in the double layer capacitance induced by protein binding at low frequencies. The results section discusses the performance metrics achieved from the impedance curves achieved from detecting CRP and MPO.

5 RESULTS

Figure 5 shows the impedance curve attained from measuring pure samples of protein CRP. As observed from the figure, the impedance is relatively flat at higher frequencies and very steep in the lower frequencies. The steep rise is impedance marks the double layer capacitance and the flat portion is dominated by the resistance due to the solution.

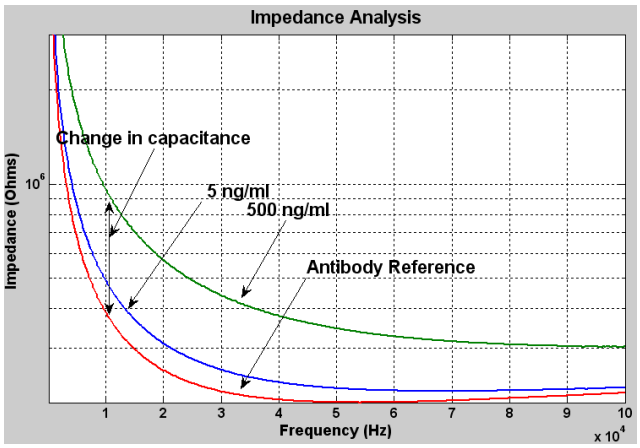

Figure 5: Impedance response for protein CRP

The change in impedance from reference with protein addition at lower frequencies is used for its quantification. As seen from Figure 5, there is a increase in impedance with protein binding. This in turn means that there is an increase in capacitive reactance and hence decrease in the double layer capacitance.

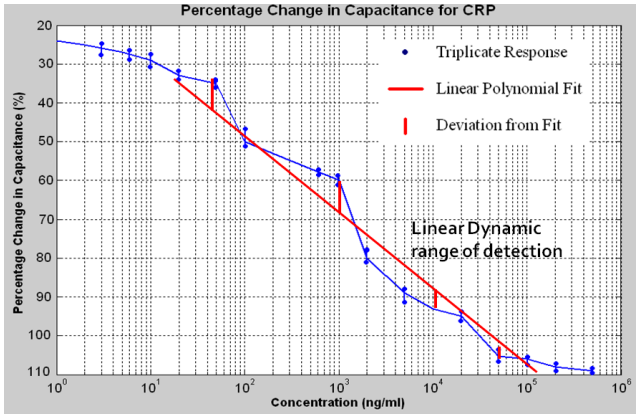

Figure 6: Percentage change in capacitance for CRP

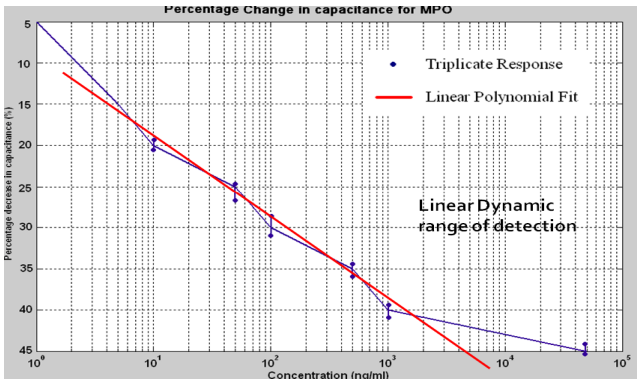

Figure 7: Percentage change in capacitance for MPO

Figure 6 shows the percentage change or decrease in capacitance with increase in concentration of proteins. A polynomial fit suggests that the decrease is linear with the dynamic range extending to many orders of magnitude.

Figure 7 shows the percentage change in capacitance for MPO when MPO is exposed to the electrodes saturated with the antibodies of MPO. Figure 8 illustrates the amount of selectivity achieved by this device. This is shown by dropping protein CRP and MPO separately on electrodes saturated with antibodies of CRP.

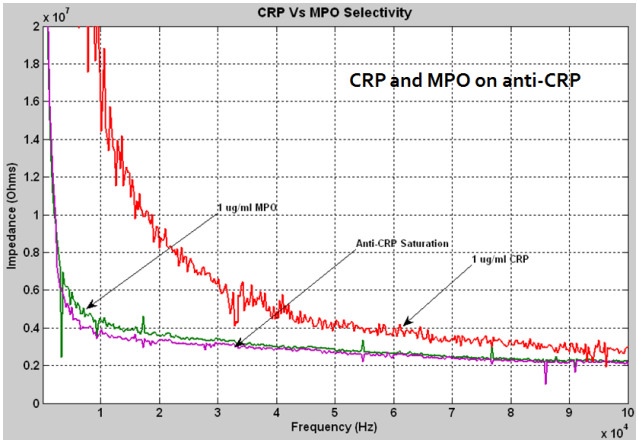

Figure 8: Selectivity analysis with CRP and MPO

Figure 9 shows the detection of protein CRP in Human Serum Albumin (HSA), a serum sample. Protein CRP was spiked with the serum HSA, which is a mixture a number of competing proteins. We can see from the graph that the change in impedance nearly corresponds to the change in impedance observed in pure sample detection. This confirms that the device is operable in real-life conditions to detect proteins in clinically relevant concentrations. The limit of detection was found to be a little lower than 1 ng/ml for CRP and around 500 pg/ml for MPO.

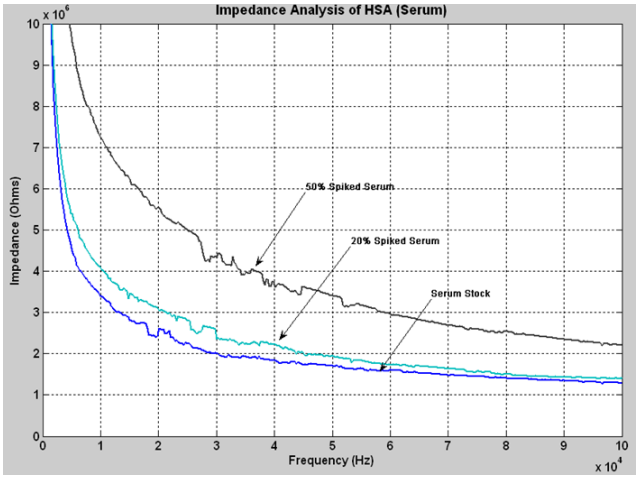

Figure 9: CRP detection in Serum samples

The linear dynamic range however extends to more than three orders of magnitude and the detection time is around few minutes.

6 DISCUSSION AND CONCLUSIONS

As seen from the results section, the detection time is in hundreds of seconds and the infrastructure required for this device is very low. This detection technique offers many advantages over the currently predominant commercial technique, ELISA. ELISA requires a lot of infrastructure and highly skilled personnel resulting in high costs per assay. The detection time is also in the order of hours and the dynamic range of detection is very low. Hence, along with being highly portable, this nanomonitor offers highly improved performance metrics over ELISA.

There are few limitations, including the most prevalent limitation of non-specific binding and cross-reactivity. As can be seen from the selectivity graphs, we see an small impedance change with the presence of CRP on antibodies of MPO. This is due to the cross-reactivity happening between competing proteins. This non-specific binding has constrained the detection limit to higher pg/ml. Higher sensitivities could be achieved by reducing or optimizing this phenomenon.

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