A Comparative Analysis of Iridium Oxide Nanowires in Electrical Detection of Biochemical Reactions

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ABSTRACT

Pt, Ir, Au and few other precious metals have highly conducive electrical and chemical properties; hence have been widely used in pH sensors and bimolecular sensing applications. The chief objective of this research is to highlight and demonstrate the advantages that Iridium Oxide (IrOx) nanowires offer over these competing metals in improving the performance metrics of biomolecular sensing. Iridium oxide has very good conductivity and very high charge storing capacity, and hence has an ability to detect very small changes in the surface charge. Nanowires have an ideal morphology to crowd protein molecules and highly increase the surface area of interaction. Higher area of interaction along with iridium oxide's high intrinsic physical adsorption rate, strongly enhance the rate of immobilization of biomolecules and hence enabling high sensitivity detection. Inflammatory protein, C-Reactive protein (CRP) that is a biomarker for cardiovascular disease was used as the model biomolecule for this study.

Keywords: iridium oxide, protein, nanowires, electrical detection, capacitance

1 INTRODUCTION

High sensitivity detection of biomolecules is imperative for accurate diagnosis of many diseases [3]. In order to achieve this it is imperative to localize biomolecule sin size matched spaces. Nanomaterial offer this unique ability for size based trapping and localization. The need for nanotechnology has been widely increasing over the years in order to improve the performance metrics of detection along with making the detection devices highly portable and inexpensive. Nanomaterials highly increase the surface area of interaction to bulk volume ratio; hence highly improving the amount of device interaction with the biomolecules leading to higher sensitivity of detection, and making the device highly portable [4].

The key to developing miniaturized biomolecule sensors is by incorporating the concept of label-free detection. The process of biomolecule detection without using fluorescent labels is termed as label free detection. Label-free detection methods have been gaining popularity over the last few decades, out of which, electrical and optical methods have proven to be very sensitive and reliable. The precious metals have been established to have very good electrochemical properties such as high conductivity and charge carrying capacity and hence have been widely used for the electrical detection of biomolecules as well as pH sensing. This research strives to signify the clear edge offered by Iridium Oxide (IrOx) nanowires over other competing precious metals towards electrical biomolecular sensing.

For this study, an electrical arrangement consisting of two electrodes which act as the biomolecule sensing sites is used. Au, Pt, Ir, IrOx, and TiN (Titanium Nitrate) thin films were chosen as the metals of study, which were deposited on these electrodes for analysis using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV). The protein biomarker CRP and its antibody were used as the biomolecules of study. The specific binding event of the protein (antigen) with the protein receptor (antibody) was analyzed and hence used as the biochemical reaction of study. The following sections will explain in detail the fabrication techniques, the rationale behind choosing these metals, the principles of detection involved and data analysis.

2 DEVICE AND FABRICATION

A two-electrode arrangement consisting of a working electrode (WE) and a counter electrode (CE) was setup wherein the area ratio of WE:CE is 1:20. The size of the counter electrode is in the order of 45 µm x 45 µm and that of the working electrode is 20 µm x 5 µm. These electrodes are separated by a gap of 5 µm as shown in Figure 1. The working electrode acts as the major sensing site for biomolecules 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 EIS and CV techniques.

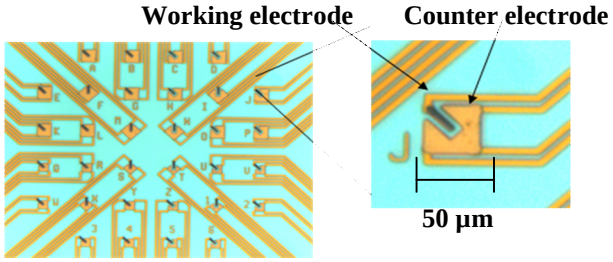

Figure 1: Optical micrograph of the electrode arrangement

The above electrode setup was achieved by the standard process of photolithography on a Si wafer. A thin film of one of the study metals 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. Different such chips with different metals (Pt, Au, Ir, IrOx, TiN) as thin films on the electrodes were fabricated for the comparative study.

On a new set of chips, IrOx nanowires were grown on different thin films 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. IrOx nanowires require non-continuous surfaces for appropriate growth from the seeds on a Si substrate. This was achievable using all the study metals (TiN, Pt, Ir, IrOx and Au). 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].

The nanowire grown chips were fabricated for the second phase of experiments which constitutes the comparative study of IrOx nanowires with thin films.

3 PRINCIPLE OF OPERATION

The study 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 the biomolecule 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. 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 [5].

Phosphate Buffered Saline (PBS), an isotonic buffer, helps proteins retain and sustain their conformation. All proteins were 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).

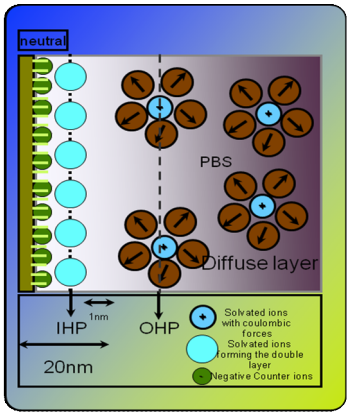

Figure 2: Charge distribution across the liquid/electrode interface forming the double layer

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.

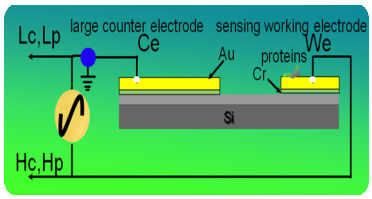

Figure 3: Impedance measured across the working and counter electrodes

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).

Gold (Au) has widely been used in electrical detection of biomolecules due to its high conductivity and biocompatibility. Platinum and Iridium have been extensively used in pH sensing and electrochemical detection as they have more than one stable oxidation states and are highly sensitive to charge transport [7]. Titanium Nitrate has been used in many cases where a non-continuous surface is required. It offers more surface area for interaction of biomolecules. Iridium oxide has been found to have high conductivity and charge storing capacity, hence making it highly suitable for

electrochemical detection of biomolecules. As much as it is stable with the +3 and +4 oxidation states, it also easily shifts interchangeably between the two when there is a change in charge associated. It is also very good with physical adsorption and creates more surface area with waters of hydration. Hence, we perform a comparative study between this set of metals using EIS to find the optimal metal for electrical detection of biochemical reactions.

4 RESULTS

Three series of experiments were conducted towards finding the electrical sensitivity of the metals for biomolecular sensing. Open circuit potential analysis suggested that the capacitance was very less (~ 200 fF) for all the metals, hence concluding that there is negligible background capacitive effects.

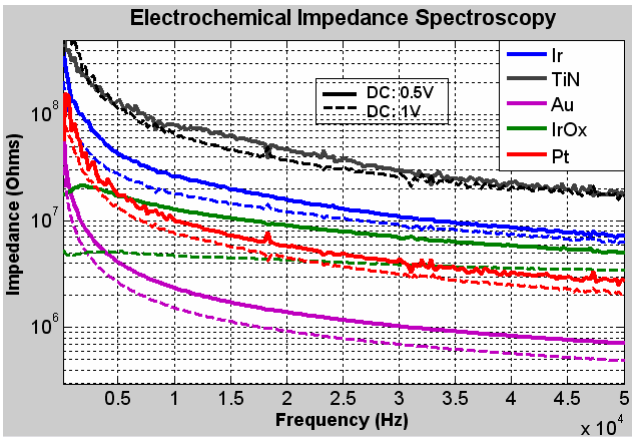

Figure 4: EIS analysis with change in DC bias

Figure 4 shows the EIS analysis with just the ionic buffer present on and between the metal thin film electrodes. As mentioned in the previous section, at lower frequencies (100 Hz – few tens of kHz) the capacitive double layer dominates and is followed by the resistance of the ionic buffer solution at higher frequencies. From Figure 4, we see that TiN has the highest baseline impedance with Au and IrOx the lowest with about two orders lesser at lower frequencies. With change in DC bias applied from 0.5V to 1V, we see that TiN undergoes minimal change. We see a good change in capacitance in Ir compared to Pt and Au. The uniform change in Au suggests that the resistance of the solution had a more dominant change than the capacitance associated with it. However, we see that there is a drastic drop in impedance in IrOx thin film. This shows the huge change in the double layer capacitance as we see a practically flat curve at 1V. Even though it limits the operable voltage with IrOx to few Volts, it shows the high electrical sensitivity associated with the change in charge.

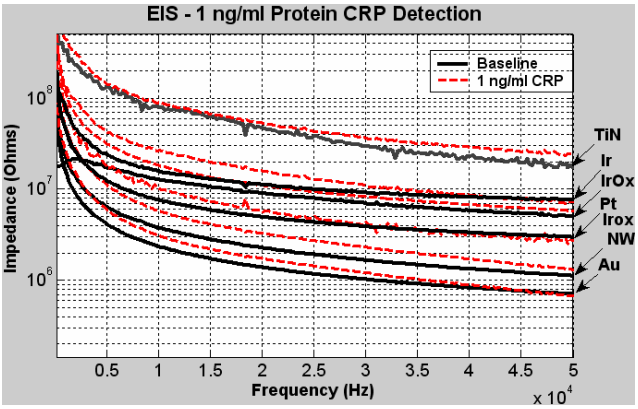

Figure 5: EIS plot showing impedance change with the detection of 1 ng/ml of protein CRP

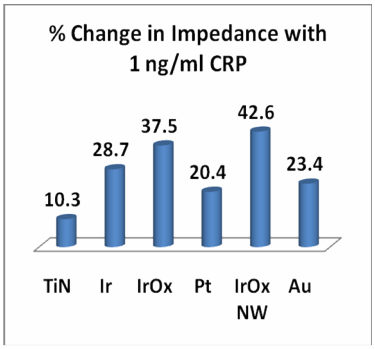

Figure 6: Percentage change in impedance with different metals from protein binding

For the second phase of experiments, the different metal thin films were first saturated with CRP antibodies in order to bind to CRP when exposed [9]. After saturation, 1 ng/ml concentration of the protein CRP which was aliquoted in 1X PBS, was dropped on to the electrodes or sensing sites. Figure 5 shows the EIS plot of this dose response, which in turn shows the amount of change, induced by the protein-binding event from the reference. Baseline or reference corresponds to the reference impedance created after saturating the electrodes with antibodies. Hence, more the change in impedance more is the sensitivity. Figure 6 plots the percentage change in impedance for a direct comparative analysis.

As we observe from Figure 5 & 6, there is negligible change observed with TiN, as the baseline impedance is already much higher than the order of change in impedance induced by protein binding. Au, Ir and Pt show considerable change but IrOx thin film and IrOx nanowires on Ir thin film show significantly higher change hence suggesting higher sensitivity. This is mainly attributed due to the high charge storing capacity and surface charge density of IrOx than the others, hence detecting the change in double layer capacitance with high precision.

5 DISCUSSION AND CONCLUSIONS

IrOx nanowires highly improve the performance metrics of electrical detection of proteins. Nanowires have much larger surface area for interaction hence offering significantly higher sites for biomolecule interaction. They also help in crowding the protein molecules, hence retaining protein conformation for a much longer time. IrOx, as seen from the graphs, has much higher electrical sensitivity at metal-liquid interfaces. The change in charge is quickly detected due to the high charge storing capacity an conductivity of IrOx. IrOx is also bio-compatible, hence enabling it to be used for health and other applications. Use of nanomaterials helps devices to become highly portable and inexpensive. Along with these advantages, IrOx nanowires offer improved performance metrics with rapid detection. These factors make IrOx nanowires ideal for electrical biomolecule sensing.

Other competing metals and morphologies include nanotubes, Au and Pt nanowires and other nanostructured materials. Au nanowires have been found to lack capillary action, hence resulting to act more like a thin film. Nanotubes might offer much better stability for biomolecules due to their enclosing nature. Future work entails researching on new nanostructured materials and methodologies towards improved biomolecule sensing.

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