NANOWELL ARRAY IMPEDANCE SENSOR FOR LABEL-FREE QUANTIFICATION OF CYTOKINES IN SERUM AT FEMTOMOLAR LEVEL DETECTION LIMITS

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ABSTRACT

We present a novel method for label-free quantification of cytokines in serum within ten minutes at femtoMolar concentrations. The layout and fabrication process of a novel sensor designed for this work is described. A comprehensive study of sensor response to antibodies and proteins, and the titration curve ranging from 60 pM down to 6 fM is presented.

KEYWORDS: Label-free, Protein detection, Impedance measurement, Nanowell array

INTRODUCTION

Detection of proteins in blood using label-free impedance based techniques is difficult due to high salt concentration of the matrix, which results in screening of the charge of the target proteins. We describe a novel sensing configuration where sensitivity benefits from the high salt concentration of the matrix, and demonstrate robust performance through testing in rat serum. Fig. 1 shows a schematic of the basic device. It consists of a pair of conducting electrodes that are separated by a nanometer-sized gap. An array of micro-wells is formed on each sensor surface. Antibodies are immobilized inside the wells. Binding of target antigen will modulate the impedance between the electrodes, resulting in a rise in impedance due to partial occlusion of ions passing between the two electrodes inside the nanowell. Higher salt concentration results in larger current, thus higher signal power corresponding to larger changes in current due to protein binding.

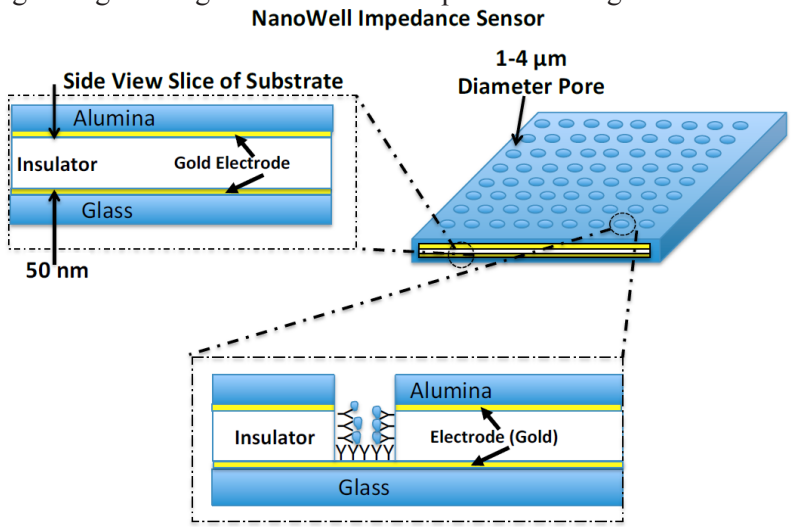

Figure 1. Nanowell Array Label-free Impedance sensor schematic. Electrodes in the array monitor impedance in the well. As protein binds to antibodies functionalized in the well, a rise in impedance is observed between electrodes.

EXPERIMENTAL

The sensor was fabricated using gold electrodes on a glass substrate. Bonding pads are fabricated on opposing sides of the micro-chip with traces leading to the center of the chip. The two electrodes overlap with each other and are separated by a thin (40 nm) aluminum oxide layer. The top electrode is also covered with a protective oxide layer. The overlapping region of the two electrodes are micro-patterned with holes that expose the bottom electrode to solution. A lock-in-amplifier is used to monitor the impedance across the electrodes continuously in real-time.

RESULTS AND DISCUSSION

We repeatedly tested the ability of the sensor to detect target cytokine in both Phosphate Buffer Saline (PBS) and rat serum at low concentrations. We include representative data of physical adsorption of the probe antibody (antileptin IgG) for functionalization of the base electrode, binding of leptin to anti-leptin IgG. (Fig. 3).

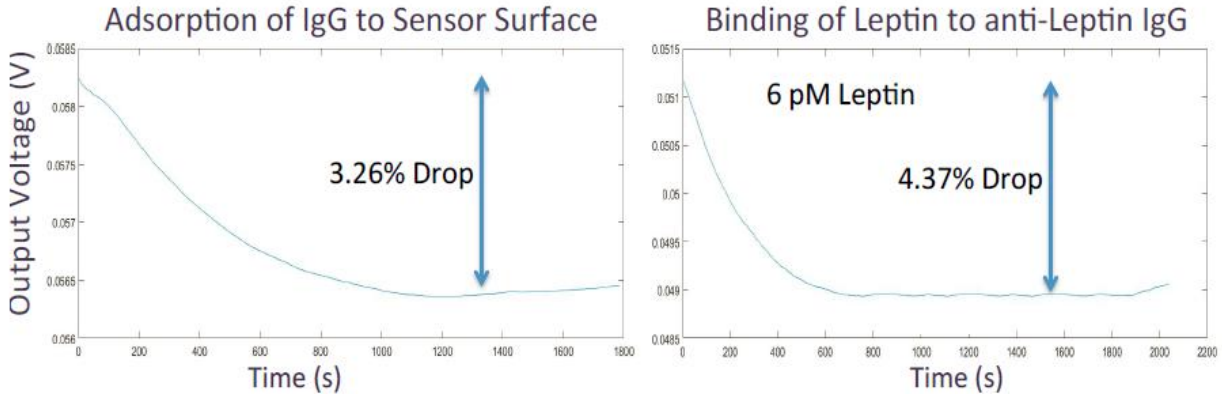

Figure 3. (Top Left) Sensor response with respect to time as monoclonal antibody physically adsorbs in channel resulting in exponential drop in baseline. (Top Right) Sensor response with respect to time as leptin in solution binds to electrode surface functionalized with antibodies.

The titration curve for testing 2 μm diameter nanowell sensors is shown below in Fig. 4. All experiments were performed by spiking leptin into rat serum, and performed in triplicate. All negative control measurements were performed using non-spiked rat serum to ensure that sensor fouling did not result in false positive signals.

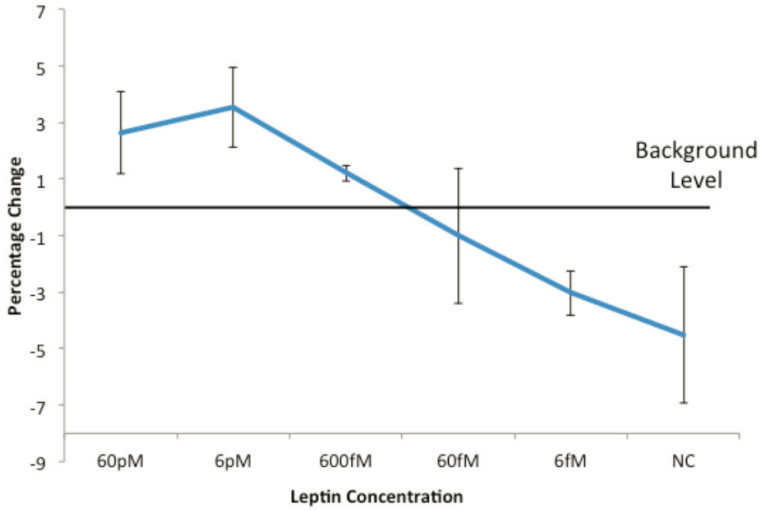

Figure 4. Titration curve for concentrations ranging from 60 pM down to 6 fM. Error bars are standard deviation over 3 points. Negative control (NC) is also included. Background level is defined where change in impedance across electrodes is no longer positive. 600 fM is clearly above background

CONCLUSION

We present a novel method for label-free quantification of cytokines in serum within ten minutes at femtoMolar concentrations.

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