Nanomonitors: Electrical Immunoassays towards Clinical Diagnostics

M. G. Bothara*, R. K. Reddy*, T. Barrett**, J. Carruthers*** and S. Prasad*

*Electrical and Computer Engineering Department, Portland State University,
160-11 Fourth Avenue Building, 1900 SW Fourth Avenue, Portland, OR, USA, sprasad@pdx.edu
**Department of Veteran Affairs, Oregon Health Sciences University Portland OR, barretth@ohsu.edu
***Department of Physics, Portland State University, Portland, OR, USA,

ABSTRACT

Proteomics research has elucidated many new biomarkers that have the potential to greatly improve disease diagnosis. Electrical biosensors can be extremely sensitive and can be modified to function as protein detectors, meeting the improved performance parameters requirements. We employ the protein specific capacitance measurement method as the basis for protein biomarker detection. We have demonstrated the performance parameters for individual protein biomarker detection in purified and spiked serum samples to be comparable to ELISA. We have explored the feasibility of concurrent detection of the two protein biomarkers with comparable performance parameters.

Keywords: vulnerable coronary plaque, biosensors, multiplexed detection, C-reactive protein, myeloperoxidase

1 INTRODUCTION

Proteomics research has elucidated many new biomarkers that have the potential to greatly improve disease diagnosis [1-4]. Combination of multiple biomarkers has been determined to provide the information necessary for robust diagnosis of a disease in any person within a population [5-7]. In addition, detection of biomarkers associated with different stages of disease pathogenesis could further facilitate early detection. Widespread use of protein biomarkers in healthcare will ultimately depend upon the development of techniques that allow rapid and multiplexed detection of a wide range of biomarkers with high selectivity and sensitivity. This goal has not been attained with any existing immunoassay method, including ELISA [6-10]. On the other hand electrical biosensors can be extremely sensitive and can be modified to function as immunoassays meeting the improved performance metric requirements.

Electrical detection of protein biomarkers is a relatively new approach that monitors a specific electrical parameter that undergoes change during the detection event. With the advent of nanotechnology in the field of clinical diagnostics, material with nanometer physical dimensions have been utilized for their improved surface area towards developing detectors with enhanced sensitivity and reduced use of reagents. The major classes of nanomaterials that have been used for protein biomarker detection are: nanotubes, nanowires, nanoparticles and nanotemplates. In all the nanomaterial based electrical protein biosensors, detection response is in the order of minutes with very low sample volume (in the order of nanoliters). But, of the four-nanomaterial classes, nanotemplates enable protein isolation and localization that in turn results in the development of robust sensors with improved specificity at lower concentrations. This is the key requirement in achieving aim 1(c). Hence, for this application we have incorporated the use of nanotemplates for the development of the nanomonitors (NM).

Our clinical application for this emerging technology is the identification of the disease state: presence of vulnerable coronary plaque, which is the cause of acute coronary syndromes such as stroke, peripheral vascular limb ischemia, and other end-organ ischemic diseases. Vulnerable coronary plaque is believed to be present in the blood vessels of a patient by the simultaneous detection of at least five proteins: C-reactive protein (CRP), Myeloperoxidase (MPO), CD40 ligand, oxidized-LDL, and tissue factor. The pre-operative identification of the disease state is expected to improve the post surgical outcomes of the patient population.

We have demonstrated that the multiple protein biomarkers of vulnerable coronary plaque in the pre-operative state can be detected by an electrical immunoassay device fabricated from nanoporous alumina membranes operating on the principle of electrochemical signal transduction. These devices will be referred to henceforth as “nanomonitors”(NM).

2 MATERIALS AND METHODS

2.1 Nanomonitor Device

The nanomonitor comprises of two parts. They are the micro-fabricated platform; and the nanoporous membrane implanted on the platform as shown in Figs. 1 and 2 below. The details of fabrication are given in previous work [11].

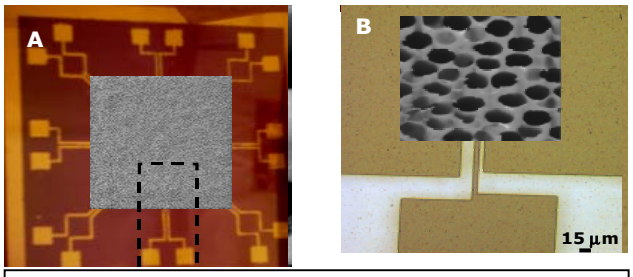

Figure 1: (A) Optical micrograph of NM (B) Combination image of a single sensing site: the base is an optical micrograph and the nanoporous membrane is a SEM.

2.2 Principle of Operation

Nanomonitor works on the principle of double layer capacitive measurement. In each well the following happens: the antibodies are in the size range of 1-10nm.These antibodies when inoculated flow to the bottom of the well due to capillary forces and they fall within the inner Helmholtz layer of the double layer thereby causing a perturbation and cause a change in the capacitance. The charge associated with the antibody modifies the double layer. When the antigen is added to the sensing site, this further modifies the interface and the formation of the immuno-complex changes the charge distribution causing a change in the capacitance measured (Fig. 2).

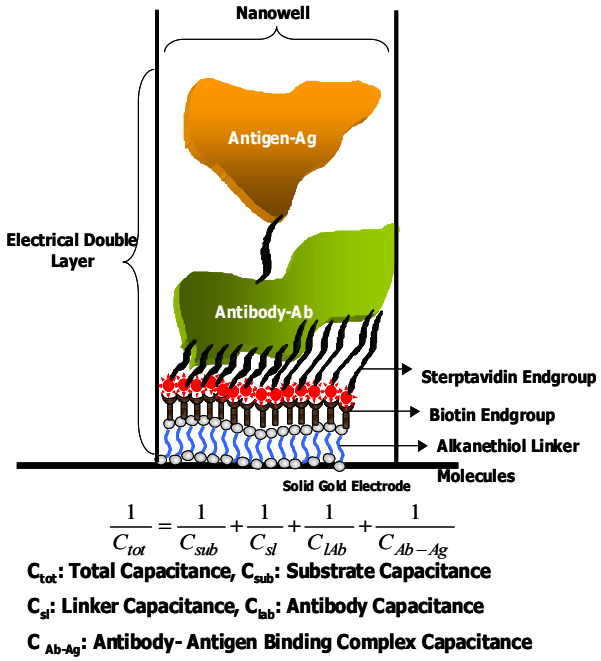

Figure 2: Schematic representation of the principle of electrochemical capacitance measurement from an individual nanowell.

Each nanowell is located on the pre-charged sensing site. Since so many wells are interrogated simultaneously, this would improve the signal to noise ratio. Individual nanowells with trapped biomolecules are electrically equivalent to multiple capacitors connected in parallel. Hence the equivalent capacitance obtained from a single sensing site is the sum of the individual capacitors associated with each nanowell. This results in signal amplification, which is relevant during the detection of lower concentration (less than 10 ng/ml) that in turn improves the limit of detection. In addition as the capacitance is averaged over multiple nanowells this reduces the variability in measurement during the testing of replicates, thus making the NM more robust.

The capacitance is measured from each sensing site. Each site comprises of a counter and working electrode. The capacitance is measured from the working electrode with respect to the counter electrode. The two electrodes are connected to an Impedance analyzer (HP 4194A) that directly measures the capacitance values.

3 RESULTS

The first step was to determine the antibody saturation concentration of the NM. This is similar to the saturation of the micro-titer plate in the ELISA method. As in the case of capture ELISA, where the antibody is saturated at the base of every well in a micro titer plate, the antibody in the NM saturates the nanopore. The saturation concentration is identified as the concentration at which all the nanopores are filled with the antibody. This translates into a negligible change in the capacitance measurement at concentrations greater than saturation (Fig. 3).

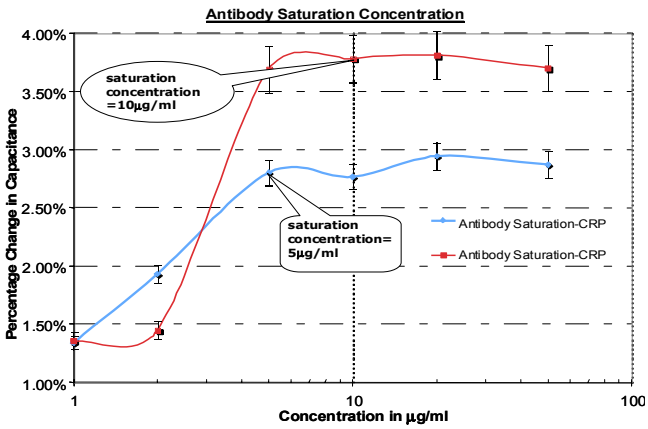

Figure 3: Determination of saturation concentration of both the antibodies (Anti-CRP 10µg/ml and Anti-MPO 5 µg/ml).

The next step was to determine the lowest concentration of the specific antigen that could be detected accurately in replicates. The parameter that was monitored in the measurement technique was the electrochemical

capacitance. Using the NM immunoassay technique we were able to detect CRP and MPO with the present lower limit of detection at 10ng/ml and 20ng/ml respectively. The upper limit of detection for both the antigens was 100µg/ml. The dynamic range for CRP was 100 µg/ml to 10 ng/ml and that for MPO was 100 µg/ml to 20 ng/ml.

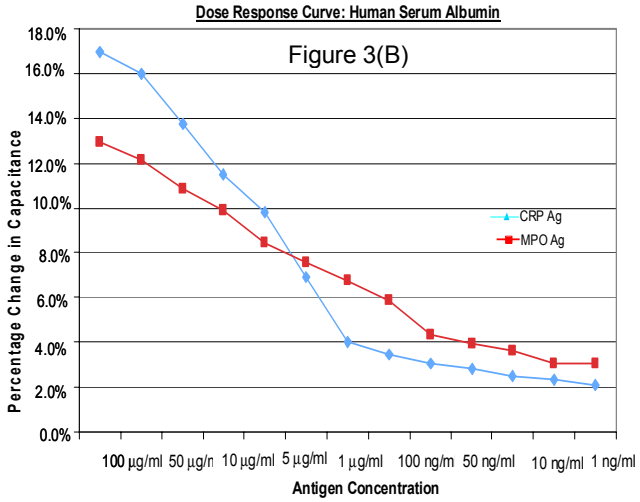

Figure 3: Dynamic range of antigen detection from purified samples for CRP and MPO. The lower limit of detection to be 10 ng/ml for CRP and 20 ng/ml for MPO.

4 DICUSSION

A highly sensitive device for protein detection was designed and fabricated using label-free electrical immunoassay techniques. In addition to being highly portable, it is quite inexpensive and very quick in detection when compared to current predominant labeled immunoassay techniques like ELISA. Table 1 briefly compares the performance metrics of both detection techniques.

From Table 1, we see that Nanomonitors clearly have the edge over ELISA is terms of detection speed, cost, linear dynamic range, sample volume and number of analytes detectable simultaneously. As the detection is label-free, there is no need for any external chemicals as tags, hence reducing the risk of contamination.

In the design of the nanomonitor, nanoporous alumina is embedded on the base metallic electrodes. One of the aspects that this nanoporous structure serves is the spatial confinement of the proteins; to support this there is a compelling argument in the realm of biophysical understanding of the proteins and their structures. There exists a crowded environment inside a cell structure, the cytoplasm, which is typically different from the dilute solutions that are generally used in in-vitro studies of proteins. There exists a school of thought, which states that this may significantly affect the behavior of the proteins [12, 13]. This is called the 'macromolecular crowding' principle and it suggests that confining the proteins within the nanopores of the alumina membrane helps protein to sustain its conformation and also increase the sensitivity in detection.

For many diseases, multiplexed detection of protein biomarkers from small volumes in a rapid manner is important for cost effective disease diagnosis. The device technology has potential for "point-of-care" disease diagnostics, as it is highly portable and inexpensive. There is a great promise for this technology in the areas of laboratory clinical diagnostics, healthcare and in pharmaceutical sectors.

The lower limit of detection achieved by ELISA is higher than what is achieved by the NM device. This is majorly attributed to the presence of some non-specific binding or cross-reactivity. Even though specific antibodies bind to their respective antigens, some non-specific binding happens when competing antigens are present. Competing antigens are those whose structures and chemical properties are very similar in nature and hence they create a noise margin which currently inhibits the limit of detection to 200 pg/ml. The second reason would be because of the background noise present from the substrate, the external circuitry and the environment. As all the signals are electrical in nature and the currents are very small, the background impedances tend to become non-negligible, hence creating a noise margin similar to the non-specific binding activity.

	ELISA	**Nanomonitors**
Method of detection	Labeled optical detection	Label-free electrical detection
Detection time	Many hours	Few minutes
Sample volume	High (milli-liters)	Low (micro- liters)
Mode of detection	Single biomolecule only	Multiple biomolecules
Detection limit	~ 3 pg/ml	~ 200 pg/ml
Cost per assay	Hundreds of $	Tens of $
Dynamic range	3 orders of magnitude	~ 6 orders of magnitude

Table 1: A performance metrics comparison between ELISA and nanomonitors

5 FUTURE PERSPECTIVE

Essentially the nanomonitors are protein immunoassays working on the principle of electrical

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2

detection as opposed to optical detection that is employed by ELISA. Hence this technology can be implemented for detection of any protein biomarker operating on the principle of antibody and antigen binding. We intend to broaden the scope of the technology by addressing other diseases in the clinical environment.

REFERENCES

[1] F. Darain, D. S. Park, J. S. Park and Y. B. Shim, *Biosensors & Bioelectronics,* Vol. 19, pp.1245-52, 2004.

[2] J. Hahm, and C. M. Lieber, *Nano Letters,* Vol. 4, No. 1, pp. 51-4, 2004.

[3] J. M. Nam., C. S. Thaxton, and C. A. Mirkin, *Science*, Vol. 301, No. 5641, pp.1884−86, 2004.

[4] O. Niwa, M. Morita, and H. Takei, *Anal.Chem.,* Vol. 62, pp. 447-56, 1990.

[5] M. D. Abeloff, J. O. Armitage, A. S. Lichter and J. E. Niederbuber, *Clinical Oncology*, Churchill Livingstone, New York, 2000.

[6] S. F. Chou, W. L. Hsu, J. M. Hwang and C. Y. Chen, *Biosens. Bioelectron.*, Vol. 19, pp.999−1005, 2004.

[7] P. Arenkov, et al., *Anal. Biochem*., vol. 278, pp.123−31, 2000.

[8] Prasad, S., Zhang, X., Ozkan, C.S., Ozkan, M. Neuron-based microarray sensors for environmental sensing. Electrophoresis. 2004. 25(21-22): p.3746-60.

[9] Prasad, S and Quiano, J., Biosens. Bioelectron. 2004. 21(7): p.1219-29.

[10] Cui, Y., Wei, Q.Q., Park, H.K. and Lieber, C.M. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. Science. 2001. 293: p.1289−92.

[11] R. K. K. Reddy, M. G. Bothara, T. Barrett, J. Carruthers and S. P. Prasad, IEEE Sensors, accepted for publication.

[12] S. B. Zimmerman and A. P. Minton. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu Rev Biophys Biomol Struct*, 22:27-65, 1993

[13] S. B. Zimmerman and S. O. Trach. Estimation of macromolecule concentrations and excluded volume e_ects for the cytoplasm of Escherichia coli. *J Mol Biol*, 222(3):599-620, 1991.