‘Lab On A Chip’ Label Free Protein Sensor Systems Based on Polystyrene Bead and Nanofibrous Solid Supports

V. Kunduru*, J. Grosch*, S. Prasad*, P.K. Patra**, S. Sengupta***

*Portland State University, ECE Dept, PO Box 751, OR USA, sprasad@pdx.edu
**Rice University, TX, USA, prabir@rice.edu
*** University of Massachusetts Dartmouth, MA, USA, ssengupta@umassd.edu

ABSTRACT

A demonstration of the study of polystyrene solid supports on platform based technologies for label - free immunoassay based reactions with improved sensor performance is presented here. Nanomaterials based biosensor was developed by coupling sensing elements with sensitive electronic capture equipment. Thus we constituted a ‘Lab on a chip’ device. The prototype sensor was evaluated for an inflammatory biomarker protein – C reactive protein (CRP). Polymer composites were used to provide both surface area for protein functionalization and efficient means of electron transduction. Important biosensor features such as the dynamic range for linear response to the analyte of interest and rapid response time are highlighted.

Keywords: *polymer, polypyrrole, protein, sensor, ‘lab on a chip’*

1 INTRODUCTION

Current commercially available immuno - assay type detection techniques involve high throughput detection of the analyte using the corresponding enzyme labeled antibody [1]. Most quantitative immuno assays use fluorescence/luminescence detection schemes that display the quantity of analyte present as a function of the intensity of light emitted. Expensive and bulky optical imaging equipment is required for observing the labeled immunological detection results. Most of these technologies face several challenges like assay miniaturization, system robustness, microtiter plate logistics and other fluid handling complications [2-4].

Our research involves the study of polystyrene solid supports on platform based technologies for label - free immunoassay based reactions with improved sensor performance. Appropriate combination of suitable sensing materials with efficient sensing techniques is the focus of this research for the development of protein sensors. We compare the efficiency of polystyrene beads vs electrospun polystyrene nanofibers in terms of analyte detection using different functionalization schemes. A silicon based, photolithographically fabricated microelectrode array [5] (MEA) coated with biofunctionalized polystyrene provides the platform for accessing the electrical signature of the analyte of interest. The presence of nanosensing sites on the polystyrene surface enhances anchorage of the capture antibodies relevant to the experimental antigen of interest owing to their very large specific surface area. A comparative study of the sensitivity of polystyrene bead vs. polystyrene nanofibrous web is conducted to determine the efficiency of the protein sensor with respect to surface area of the sensing material. The surface chemistries of the synthetic polystyrene beads and nanofibers are modified for biomolecule reception by use of various functionalization schemes. In order to enhance the sensitivity we incorporated a layer of conductive polymer that acts as the electron transducer between the protein monolayer and the electronic capture system. We have evaluated the system performance in both the nanostructures using the inflammatory protein CRP as a model system. Important biosensor features like linear dose response to the analyte of interest and rapid response time are highlighted. Issues of repeatability, sensitivity and scalability are addressed in terms of making the prototype biochip commercially viable. The polystyrene based protein prototype sensor chip is encapsulated in a biocompatible flow cell system to constitute a hand held, portable, ‘lab on a chip’ protein sensor device [6].

1.1 ‘Lab on a chip’

Well established fabrication techniques that are adapted from the semiconductor industry such as micromachining, injection and replica molding,

soft lithography, wet etching and photolithography in an effort to miniaturize fluid handling systems to palm held 'Micrototal Analysis Systems' or 'Lab on a chip' devices [7] which can perform a myriad of tasks associated with a standard laboratory. Microfabricated 'Lab on a chip' microfluidic devices are significantly smaller in size than conventional fluid manipulation systems, rendering them portable and extremely useful in the areas of nanobiotechnology [8, 9], bioanalysis [10] and pharmaceutical [11], medicine and diagnostics. Advances in nanotechnology have impacted research in biotechnology with the development of 'smart devices' capable of molecular manipulation [8]. Microfabricated bioanalytical devices offer highly efficient platforms for genomic, proteomic and metabolic studies. Polymer based microfluidic devices have emerged based on the principles employed in silicon and glass based chips for various applications. Microfluidic devices using biocompatible and cost effective polymer materials for their construction are replacing their more expensive micromachined silicon and glass counterparts as disposable and effective alternatives [7]. In the current research work, we have developed devices that have micro and/or nanostructured active sensing surfaces on to which the biomolecules of interest are adsorbed to yield biomolecule specific electrical perturbations that can be measured in a consistent manner by utilizing the advantages afforded by micro fluidics.

1.2 Analyte of Interest

Measuring the amount of this CRP in the bloodstream can help doctors predict the risk of a heart attack. Globally conducted studies have showed that higher the concentrations of CRP in the blood, greater are the risks of suffering a heart attack. Thus, CRP can be well perceived as a disease biomarker for inflammatory coronary disease. In the current application we are investigating the development of a miniaturized model test system that exploits the electro-activity of CRP to identify low concentrations of CRP in a rapid manner from test samples. The existence of the fundamental coupling between the electronic supports and biological entities in this biosensor system, gives rise to the electrical signatures required for identification of the analyte of interest. Such an electrical contact between biomolecules and electrodes is essential for the functioning of most bio-electronic systems.

2 MATERIALS AND METHODS

2.1 Microfabrication of Base Microelectrode Array (MEA)

The planar microelectrode array consisted of four microelectrodes, each having a narrow edge, which extends to the middle of the surface and spaced 45 degrees from each other. The distended pads are 300μm wide for easy manipulation using the micromanipulators, and the narrow extensions are 50μm each. Two of the four electrodes are selected to generate electric fields around them when a gradient voltage is applied across them. Standard photolithography methods were used to fabricate the microelectrode array [12].

2.2 Polymer Solid Supports

Electrospun polystyrene nanofibers are laid out as an intertwined fiber matrix on the sensing area of the chip. Both functionalized and SO_3H functionalized fibers are utilized to prepare the fiber matrix as the antibody functionalizing surface. The wires are approximately 500nm – 1μm in average diameter and several hundreds microns in length. An electroactive polymer – polypyrrole (doped, 5 wt. % solution in water, *Aldrich*) was used to create a polystyrene – polypyrrole composite to increase the electron transduction to the underlying gold electrodes. The polymer matrices were allowed to incubate in antibody solution for 30 minutes at room temperature before administering it as a mat on the gold sensing area. A baseline signal was recorded and changes in conductance values were noted. Corresponding impedance values were later used for validating the conductance response.

2.3 Polystyrene - Polypyrrole Conductive Matrix

Polypyrrole is used under an applied field to give an additional, and more favorable, conductive pathway. Pyrrole has low energy electrons in the highest occupied molecular orbital (HOMO) attributed to its conjugated aromatic character. The aromaticity of pyrrole is attributed to the planar delocalized pi electrons following the

Hückle rule of 4n+2 pi electrons. Figure 1 describes the ring structure of monomer pyrrole.

A sufficiently powerful electric field can remove an electron from the HOMO resulting in an ionized species. Similarly a conjugated polymer of pyrrole under an electric field will lose HOMO electrons becoming a conducting pathway along its entire length. Lengths of polypyrrole adsorbed onto the CRP/Anti CRP complex may leech electrons from the protein complex resulting in increased conductivity.

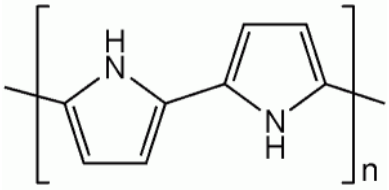

Figure 1: Chemical ring structure of pyrrole monomer *(adapted from www.wikipedia.com)*

2.4 Polystyrene Bead Matrix

Functionalized and unfunctionalized polystyrene bead mats were used spin coated and desiccated on the sensing area of the MEA chip. Plain, unfunctionalized beads were 15µm in diameter and functionalized beads with COOH groups as functional terminations, also of 15µm diameter were used (*PolySciences Inc*) to create a bead bed for functionalization of antibody. Polypyrrole was incorporated into the polystyrene bead system and allowed to coat the beads before the addition of the antigen.

3 DISCUSSION

The polymer matrix was saturated with the protein receptor Anti-CRP and the analyte of interest/antigen is introduced to this bed of antibody. The binding event of Anti - CRP and CRP causes a shift from the baseline signal due to release of electrons [6]. Conductance measurements were executed using a femtoammeter *(Keithley 6430 Subfemtoammeter)* to calculate the extent of electron conductivity through the solution. Impedance spectroscopy was employed to understand the transient behavior of the protein system under non homogenous electric fields. AC oscillating voltage of 0.05V was applied in conjunction with a DC bias voltage of 0.2V together with a frequency sweep from 40 Hz – 100KHz to collect various impedance responses of the layered protein system. Solutions containing protein populations behave as layers capable of developing surface/interfacial charges and increasing electrical double layer gradients. Such potential gradients arising from the interfacial charges give rise to capacitor – like effects, thus contributing to overall system impedance.

4 RESULTS

Impedance spectroscopy studies were conducted first with the control experiment response of the various schemes of polymer layer and that of proteins on a plain chip without underlying polymer matrix as seen in figure 2. Figures 3 and 4 show the impedance responses of functionalized and unfunctionalized polystyrene nanofibers in the presence of polypyrrole. Size matching between the nanoscale spaces in the polystyrene nanofiber mat allows for protein binding. Polypyrrole enhances electron transduction resulting in less significant observable difference between baseline and experimental measurements.

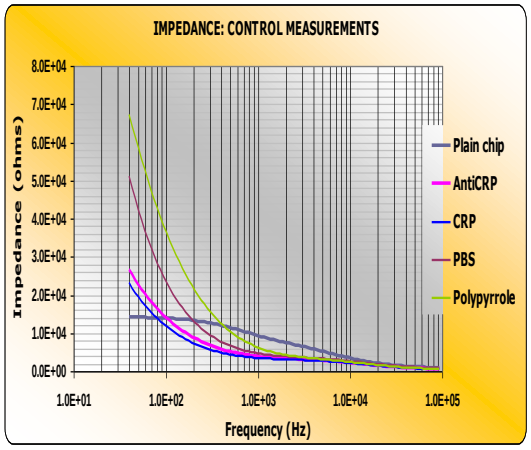

Figure 2: Impedance spectroscopy of control experiments

Similar responses are observed with functionalized and unfunctionalized polystyrene bead mats. Such responses inform the validity and importance of polypyrrole as an efficient electron transporter. However, the absence of clear differences in responses from baseline and experimental steps in the case of functionalized polymer matrices may make the use of polypyrrole meaningless when using as a sensor.

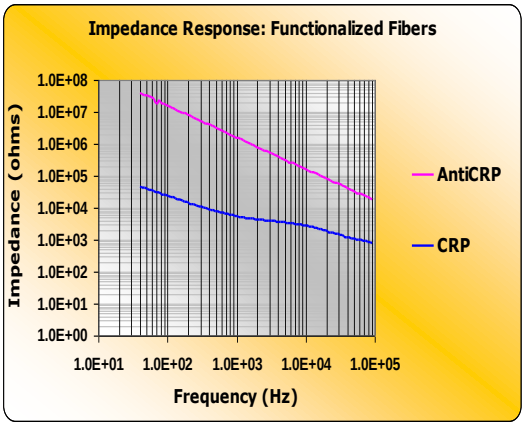

Figure 3: Impedance spectroscopy of Functionalized polystyrene nanofibers with polypyrrole.

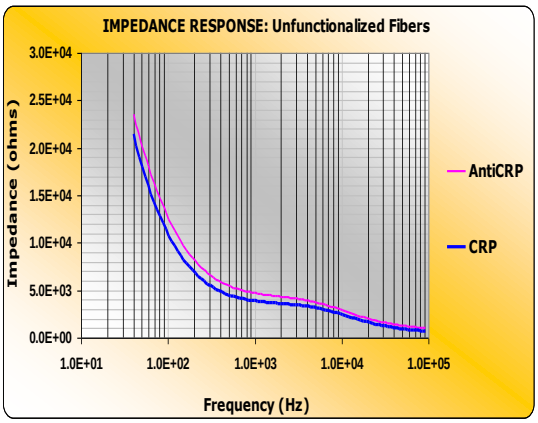

Figure 4: Impedance spectroscopy of Unfunctionalized polystyrene nanofibers with polypyrrole.

5 FUTURE WORK

Further experiments with various types of protein systems with electrical properties different from cardiac disease biomarker CRP are proposed to be studied and analyzed. Such a study would validate the impedance responses obtained from the Anti CRP - CRP immunocomplex functionalization with polystyrene matrices acting as solid supports, thus rendering this method of detection and analyses, universal to all protein immunocomplexes in common.

REFERENCES

[1]. Portsmann T, K.S.T., *Enzyme immunoassay techniques: an overview*. 25 years of immunoenzymatic techniques. Vol.150. 1992, Amsterdam: Elsevier. 5-21.

[2]. *Immunoassay: A Survey of Patents, Patent Applications, and Other Literature 1980 - 1991*, ed. B.S. Judith Sigmond, Marten Terpstra. 1992: Taylor and Francis.

[3]. Craciun, A.M., et al., *Evaluation of a Bead-based Enzyme Immunoassay for the Rapid Detection of Osteocalcin in Human Serum*. 2000. p. 252-257.

[4]. Volkov, S.K., *Immunoassay of alkaloids.* Russian Chemical Reviews, 1993. **62**(8): p. 787-798.

[5]. Vindhya kunduru, S.K.P., Shalini Prasad. *Platform based Detection Technologies from Micro scale to Nanoscale*. in *2006 MRS Spring Meeting*. 2006. San Francisco.

[6]. Vindhya, K. and P. Shalini, *Electrokinetic Formation of 'Microbridges' for Protein Biomarkers as Sensors.* 2007. **12**(5): p. 311-317.

[7]. Chiu, G.S.F.a.D.T., *Disposable microfluidic devices: fabrication, function, and application* BioTechniques, 2005. **38**(3): p. 429-446.

[8]. Lee, S.J. and S.Y. Lee, *Micro total analysis system (μ-TAS) in biotechnology*. Applied Microbiology and Biotechnology, 2004. **64**(3): p. 289-299.

[9]. Ugaz, V. and J. Christensen, *Electrophoresis in Microfluidic Systems*, in *Microfluidic Technologies for Miniaturized Analysis Systems*. 2007. p. 393-438.

[10]. Khandurina, J. and A. Guttman, *Bioanalysis in microfluidic devices.* J Chromatogr A, 2002. **943**(2): p. 159-83.

[11]. Huikko, K., R. Kostiainen, and T. Kotiaho, *Introduction to micro-analytical systems: bioanalytical and pharmaceutical applications.* Eur J Pharm Sci, 2003. **20**(2): p. 149-71.

[12]. Vindhya Kunduru, S.P. *Electrokinetic Alignment of Polymer Microspheres for Biomedical Applications*. in *Materials Research Society (MRS) Spring 2007* 2007. San Francisco, CA.