Chemically Modified Poly(Acrylic Acid) Monolayers in Alumina Nanoporous Membranes for Cancer Detection

R. Kishton[1], N. A. Monfared[2], M. Bothara[3], S. Varadarajan[1], S. Prasad[3], and S.V. Atre[2]

[1]University of North Carolina, Wilmington, NC, varadarajans@uncw.edu
[2]Oregon State University, Corvallis, OR, sundar.atre@oregonstate.edu
[3]Portland State University, Portland, OR, sprasad@pdx.edu

ABSTRACT

In this paper we have developed one of the key building blocks of the clinical proteomics test technology. This building block is a nanostructure that enables the confinement of protein biomolecules based on size-matched trapping. The prototype system is a nanoporous alumina membrane that is fabricated using a standard two-step electrochemical anodization process. We have chemically modified these membranes with poly(acrylic acid) to understand the effects of this chemical modification on the protein detection sensitivity. We plan on evaluating this prototype system in identifying cancer markers for early breast cancer detection to experimentally evaluate the effect of nanoscale-confined spaces on protein biomarker detection.

Keywords: nanoporous alumina, nanomembrane sensors, chemical functionalization, electrical measurements

1. INTRODUCTION

Table 1 summarizes the current state of technology in the building blocks of the manufactured systems comprising of nanostructured materials for biosensing [1]. There are three basic building blocks for such systems (i) nanostructures for binding organic species from solution, (ii) device architecture, and (iii) measurement techniques. The focus of this paper is in identifying an appropriate device architecture to enhance protein detection capabilities. With respect to nanomaterial assembly towards the development of functional systems for interrogating organic species a wide range of device architectures have been investigated, from planar configuration and three-dimensional structures to stacked devices. We report a multi-scale device configuration to identify the effects of nanoscale-confined spaces in protein detection based on poly(acrylic acid) derivatized nanoporous membranes.

Parameter		Key Attributes
Techniques	Optical	Measures amplitude, energy, polarization, decay time and/or phase
	Surface Plasmon	Measures resonant oscillation of surface electrons
	Piezoelectric	Oscillation frequency of crystal varies with its mass
	Acoustic	Measures acoustic resonance of quartz
	Electrical	Amperometric or potentiometric devices
Nanostructures	Particles	Metal and metal semiconductor isolable particles coated for preventing agglomeration, Semiconductor quantum dots, colloidal quantum dots, nanodiamonds
	Tubes	Conductance perturbations due to biomolecule binding in carbon nanotubes
	Wires	High surface-to-volume ratio increases interaction with the biomoleules leading to variations in electronic properties
	Pores	Nanoporous membranes with uniform, rigid, open-pore structure enhance biomolecule entrapment
Device Design	Microarrays	Parallel single analyte biosensors for multi analyte sensing arrays
	3D	3D microarrays for applications like gene expression and clinical diagnostics involving high diversity biomolecules
	Stacks	Vertical arrangement of microarrays leads to formation of stacks

Table 1: Examples of Biological Sensor Platforms

2. EXPERIMENTAL

2.1. Alumina Membrane Fabrication

The prototype system comprised of a nanoporous alumina membrane that was fabricated using anodized alumina, since it's electrically insulating, can be very chemically stable and can be easily incorporated into the CMOS fabrication technique. Anodized alumina is biocompatible [2]. Purified aluminum is used as the positive electrode or anode in an acid electrolytic bath comprising of 0.1 M Oxalic acid. Anodization results in the formation of porous alumina. The dimensions of the pores are controlled by varying the temperature of the bath, applied DC voltage and the time of anodization. The porous membrane fabricated for the electrical detection application comprised of pores 200 nm in diameter and 250 nm in depth as shown in Figure 2. The porous membrane is embedded in anisotropically etched silicon/silicon dioxide substrate. These pore arrays behave like a miniaturized micro-titer well plate with picoliter volume and can be thought of as nanowells. Protein binding is expected to occur within these nanowells. In order to improve

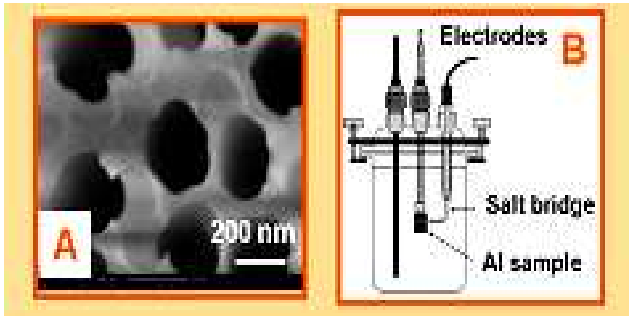

Figure 2: A nanoporous alumina membrane (A) produced by us in a 2-step anodization process (B). Pores of 200 nm diameter were produced.

2.2. Device Fabrication

The fabricated nanotemplates were aligned with the prefabricated silicon substrates with gold micro patterns. These membranes were aligned in such a manner that the gold occludes the nanotemplate in specific regions. The silicon substrates with the gold micro patterns were made using standard photolithography methods. An optical micrograph of the fabricated structure is show in Figure 3. The fabrication process required four steps and used one optical mask. (1) A polished silicon wafer (4 inch diameter and 500μm thick) with a thermally deposited oxide that functioned as the dielectric was used as the base platform. The dielectric provided electrical isolation among sensing sites. (2) Sensing sites were defined using standard mask based photolithography techniques. (3) By thin film deposition techniques a chrome (20 nm) adhesion layer and a gold (150 nm) measurement layer were deposited. The gold layer was laid over the chrome layer. (4) Finally, using wet etching techniques the sensing sites were physically isolated. This formed the base of the nanomonitor. Figure 3 is the representation of the base micro-fabricated platform.

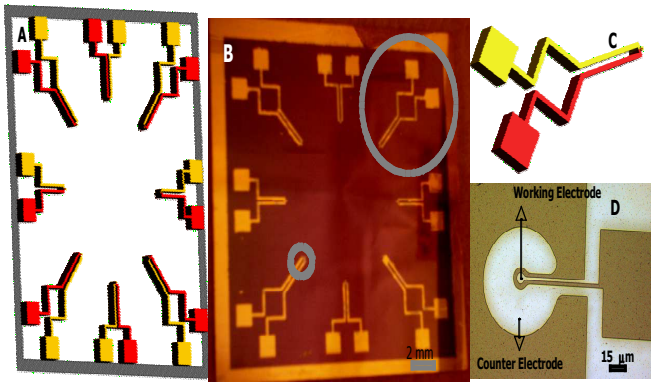

Figure 3: (A) schematic representation of the physical isolation of eight sensing sites (B) Optical micrograph of the micro fabricated platform. (C) Schematic representation of the geometry of the electrode leads from each sensing site. (D) Optical micrograph indicating the working and counter electrode on a single sensing site. The counter electrode is 10 times larger than the working electrode.

2.3. Nanoporous Membrane Functionalizaton

We have previously shown [3] that poly(acrylic acid) (PAA) readily adsorbs from dilute solution (10^{-3} %) on to the intrinsically basic surface of the hydrated native oxide of aluminum to form a robust, uniform monolayer film (0.5-1 nm thick) as represented in Figure 4(A-B). Analysis of the infrared spectrum revealed that both free, undissociated -CO_2H groups and surface-bound -CO_2^- groups were present, in the approximate ratio of 1:2. The free -CO_2H groups could be easily derivatized in quantitative yields as amides and esters, as observed with other approaches to derivatize polymer surfaces with -CO_2H groups to tailor the surface chemistry. The infrared spectra of surfaces of PAA adsorbed on Al evaporated on Si wafers showed the ease of functionalization as stearyl amide and p-nitrobenzyl esters are shown in Figure 4(C). This work indicates a facile approach to create nanowells with controlled chemistry by reacting the free -CO_2H groups with suitable amine-linked molecular recognition sites on the nanoporous alumina membranes. Our objective here was to functionalize the nanowell the estrogen receptor α (ERα) binding ligand, estradiol. ERα is over-expressed in the early stages of breast cancer. We synthesized an estradiol tethered butyl amine using published procedures (Figure 5) [4]. The amine was subsequently covalently linked to the PAA-detivatized membranes (Figure 6).

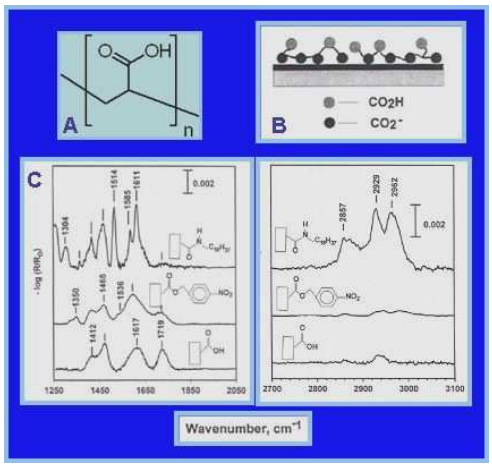

Figure 4**:** Poly (acrylic acid) (**A**) adsorbs on alumina via carboxylate groups (**B**) leaving free carboxylic acid groups for derivatization into esters and amides as monitored by FTIR (**C**).

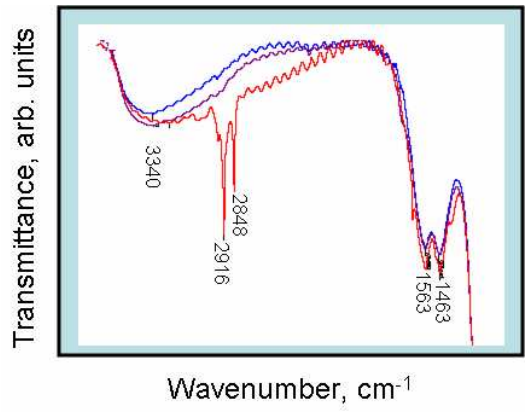

Figure 6**:** Poly (acrylic acid) derivatized with the estradiol-tethered amine by carbodiimide coupling, as monitored by FTIR. The blue line is the underivatized nanomembrane. The purple line is the poly(acrylic acid)-covered nanomembrane. The red line is the estradiol-linked polymer monolayer on the nanomembrane surface.

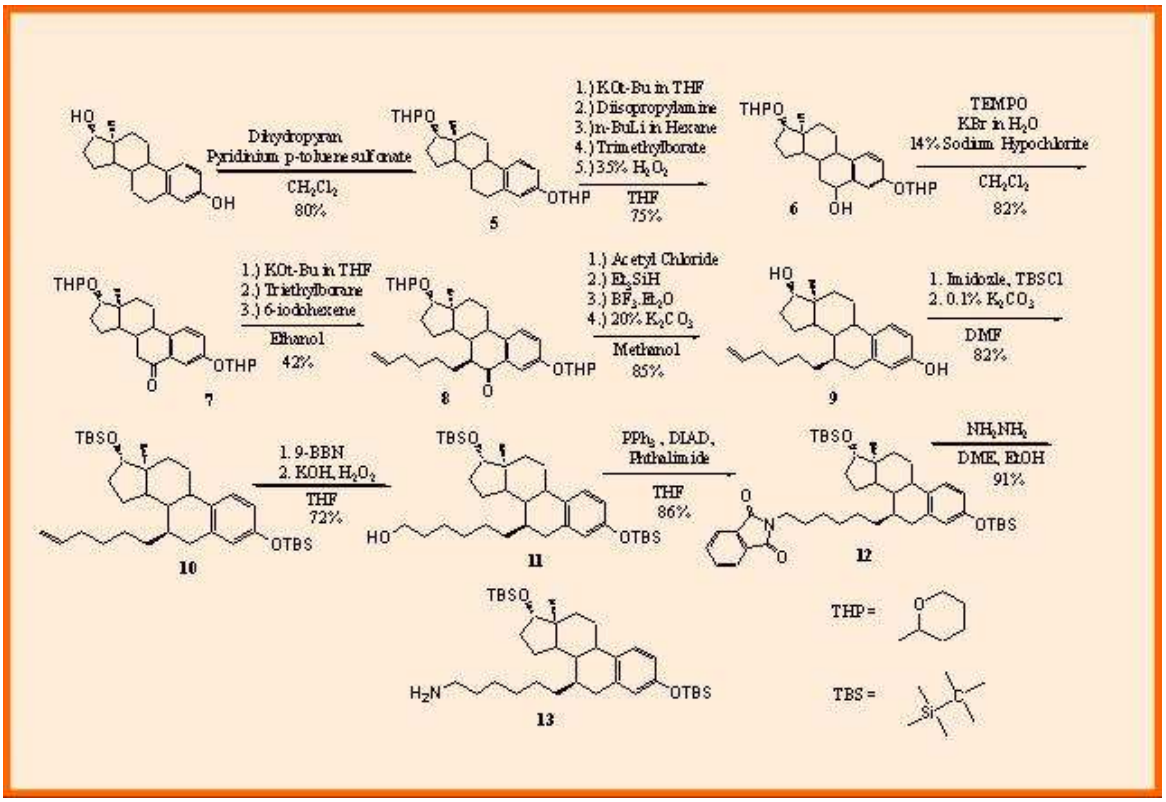

Figure 5: Synthesis sequence for generating estradiol tethered to various substituents. The $-(CH_2)_4-$ tether with amine end group shown in the above sequence has been successfully synthesized by us.

3. RESULTS

The derivatized nanomembranes described in Section 2 will be tested for binding to the cancer biomarker, ERα, by electrical impedance spectroscopy (EIS). The prototype system as shown in Figure 7 works on the principle of double layer capacitive measurement. The other electrochemical measurement techniques namely: trans-conductance and conductance measurements, are limited by the need of redox reactions at the surface for optimal charge transfer. This makes the reactions hard to control and regulate. The non-Faradaic impedance due to the capacitance of the electrical double-layer formed at the electrode surface is sensitive to reactions and is the basis of nanomonitors. This technique is advantageous since it does not require addition of any redox probes. Furthermore, capacitance measurements at different bias voltages and frequencies can reveal much information about dielectric and charge environment at the interface.

The nanomonitors comprised of multiple sensing sites with each sensing site containing approximately quarter million nanowells. The physical dimensions of the nanowell (200nm wide and 250nm in depth) were controlled during the fabrication process such that a single antibody is trapped in an individual nanowell. So, theoretically at antibody saturation, a quarter million antibodies were estimated to be trapped on a single sensing site. In each well the following phenomena are postulated to happen: 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. 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 improving the robustness of the nanomonitor technology.

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

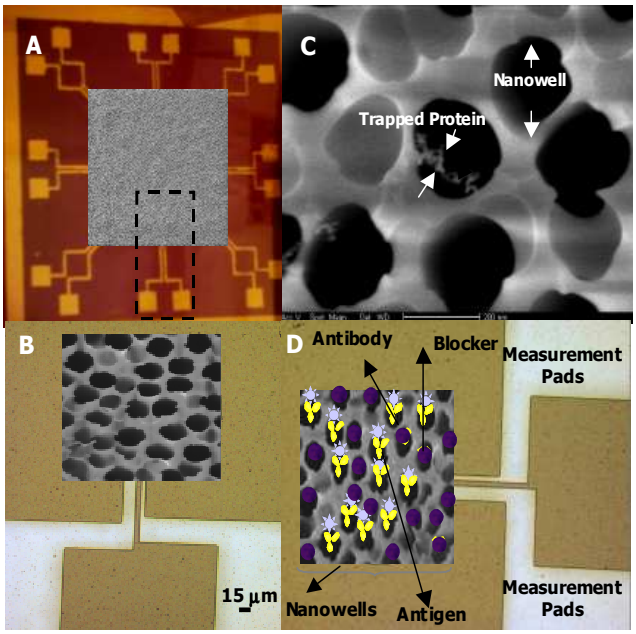

Figure 7: (A) Optical micrograph of the nanomonitor (B) Combination image of a single sensing site: the base is an optical micrograph and the nanoporous membrane is a scanning electron micrograph (SEM). (C) SEM image of the trapped protein within a nanopore (D) Schematic representation of the immuno-complex formation.

In summary, nanoporous membranes were successfully derivatized with poly(acrylic acid) monolayers and estradiol to probe the binding of cancer biomarker, ERα, on a nanomonitor platform.

REFERENCES

[1] J.J. Gooding. Nanoscale Biosensors: Significant Advantages over Larger Devices. *Small*, 2(3):313-315, 2006.

[2] R.K. Reddy. Nanomonitors: Electrical immunoassay for clinical diagnostics implementation of a microfabricated biosensor for the detection of proteins. M.S. Thesis, Portland State University, 2007.

[3] D.L. Allara, S.V. Atre, and A.N. Parikh, "Self-Assembled Monolayers as Polymer Surface Models," *Polymer Surfaces and Interfaces II*, Eds.: W.J. Feast, H.S. Monro, R.W. Richards, John Wiley and Sons Ltd., pp.27-40, 1993.

[4] S.D. Kuduk, F.F. Zheng, L. Sepp-Lorenzino, N. Rosen, and S.J. Danishefsky "Synthesis and Evaluation of Geldanamycin-Estradiol Hybrids" *Bioorg. Med. Chem. Let.* 1999, 9, 1233-1238.