**On-Chip Electrophoretic Concentration of Liposomes for Antibody-Based Viral Biosensors**

CNF Project # 1360-05
Principal Investigator(s): Prof. Brian Kirby
User(s): Sowmya Kondapalli

Affiliation(s): Department of Mechanical Engineering, Cornell University
Primary Research Funding: Environmental Protection Agency
Contact: bk88@cornell.edu, sk485@cornell.edu
Web Site: http://www.kirbyresearch.com/index.cfm/page/rp/virus.htm

Abstract:
This work presents a microfluidic device that can be used to detect the presence of viral pathogens in environmental water samples. The device integrates a membrane-based preconcentration unit with an electrochemical biosensor on the same platform. This integration has the potential to increase the sensitivity of detection thereby enabling detection of small concentrations of viruses at environmental levels.

Introduction:
Detecting the presence of viral pathogens in source water is critical for designing the right water treatment methods to prevent disease outbreak. However, conventional techniques, which include concentrating viruses in a ceramic filter followed by off-site laboratory analysis, are inefficient and time-consuming. Our goal is to build an integrated device with membrane-based concentration [1] and microbiosensor-based detection units [2] on the same platform to enable low detection limits and rapid response.

Although existing concentration techniques like solid phase extraction, isotachophoresis, dielectrophoresis, etc., provide high concentration factors, they are limited by fabrication complexities or buffer handling challenges making the integration with lab-on-chip systems difficult. We have developed a novel membrane-based on-chip concentration system that can achieve high concentration factors [1]. These membranes are relatively simple to fabricate and have architectures suitable for integration with microbiosensors.

Materials and Methods:
The nanoporous membrane was fabricated in polyacrylamide at the cross-junction of glass microchannels by an *in-situ* photopolymerization technique. Figure 1 shows the schematic of the optical set-up for photopolymerization. The glass channels were filled with monomer acrylamide/photoinitiator mixture [3] and the shaped laser beam was directed at the junction, resulting in photopolymerization of a nanoporous membrane. The electroosmotic flow in the channels was suppressed by coating them with a
layer of linear polyacrylamide [4]. Fluorescently labeled liposomes prepared by reversed phase evaporation [5] were used for concentration experiments. Liposomes can be functionalized with antibodies and encapsulated with electrochemical markers for detection experiments.

**Results and Discussion:**

We have fabricated nanoporous polyacrylamide membranes at the junctions of glass microchannels as shown in Figure 2a. We then performed concentration experiments using fluorescently labeled liposomes as shown in Figure 2b. We applied a voltage of 140V across the membrane which resulted in the electrophoretic movement of the liposomes towards the membrane and subsequent concentration due to the function of the membrane. We estimated the concentration factor achieved by averaging the pixel intensities in a measurement window around the membrane as shown in Figure 3b. These preliminary concentration experiments show that spatially averaged concentration increased by about two orders of magnitude. Figure 3 shows liposome concentration and elution performed by switching voltages at the four ports. Liposomes are concentrated by applying a high voltage (200V) to port 4 (Figure 3b) and are eluted by applying high voltage to port 3 (Figure 3d). A pinch voltage is applied to minimize diffusion of the sample away from the membrane.

The schematic of the integrated membrane-based concentrator and microbiosensor is shown in Figure 4. The integrated design enables elution of the liposome-virion complexes (formed by incubating virus particles with antibody-coated liposomes) concentrated at the membrane towards the detection unit where virions are captured. The electrochemical markers entrapped by liposomes can then be quantified on the interdigitated ultramicroelectrode array (IDUA).

**Conclusion:**

In conclusion, we have successfully fabricated nanoporous membranes in glass microchannels and preliminary concentration experiments with liposomes show about two orders of magnitude concentration. The sensitivity of electrochemical detection of liposomes combined with the ability of electrophoretic concentration to extract liposome-virion complexes from large sample volumes gives potential for viral detection at environmental levels.

**References:**