Devising a Successful Microfluidic Platform for ELISA

UNIVERSITY OF WYOMING

Elizabeth Brown

With the Support of Dr. Debashis Dutta & his Laboratory

Chemistry Department, University of Wyoming, Laramie, WY

Abstract

- Enzyme-Linked-ImmunoSorbent-Assay (ELISA)
  - Used to detect the presence of an antibody or antigen in a sample
- Two existing problems
  - Need a substantial amount of antibody/antigen
  - Requires a substantial amount of time to detect the antibody/antigen in a sample
- Combining microfluidic technology and ELISA can solve these problems
  - Limited research has been done before (1)
  - Our lab was one of the first to use Na-Silicate as a membrane interface within the microfluidic device

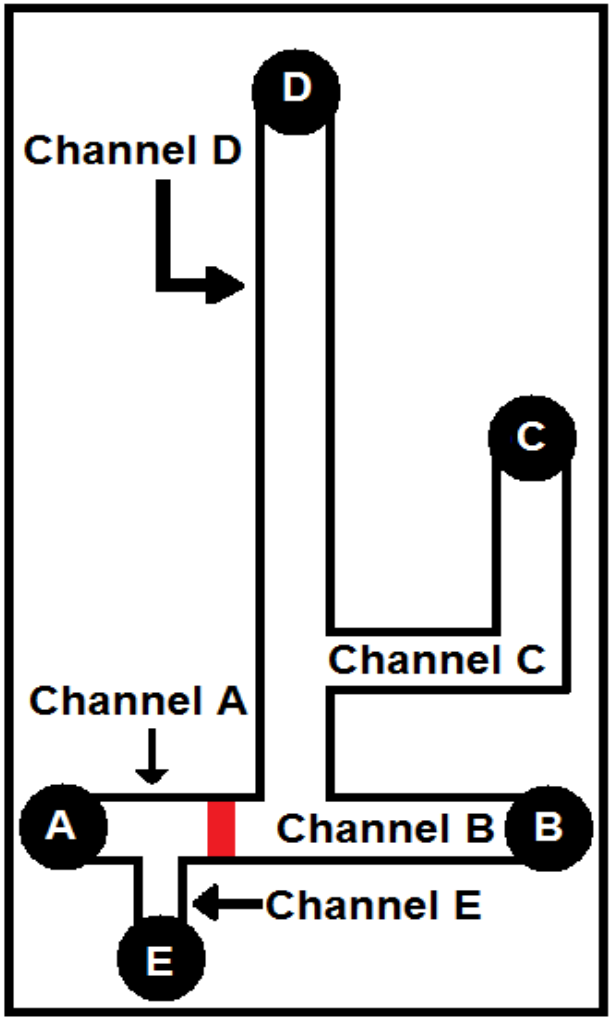
Introduction

- In order to successfully demonstrate ELISA on a microfluidic platform, two aims must first be completed
  - Construct a microfluidic platform with a membrane interface
  - Pre-concentrate small concentrations of dye against the membrane

- My 2009 summer fellowship had two objectives
  - Successfully create a microfluidic platform with a membrane interface
  - Quickly trap very small concentrations of fluorescently dyed molecules against the membrane by using an electrical charge

- Both objectives were successfully completed, which allowed for further research in demonstrating ELISA on a microfluidic platform with a membrane interface

Microchip Fabrication



Microfluidic Platform Design

- 5 channels, 5 reservoirs

- Red area: membrane interface

- Width and length of all channels was controlled by an etching mask.

- Depth of channel was controlled by the amount of time the chip was exposed to the HF etching solution.

Microchip Fabrication

1. Using standard photolithographic techniques, design is etched onto the glass plate (2), which contained the glass, a Cr layer, and a layer of photoresist.
2. The additional channel, channel E, was drawn onto the glass plate with acetone.
3. Microchip was immersed in Cr etching solution until channel E shone through on the opposing side of the chip.
4. Backside of chip was coated in photoresist, dried in oven.
5. The channels were all etched to .5µm, and the progress of the etching was monitored by an instrument called a profilometer.
6. Membrane region was covered in photoresist.
7. Remainder of all channels were etched to 20µm

Microchip Fabrication

8. Both sides of the chip were coated in photoresist.
9. Five access holes were blasted into the chip by using the sandblaster.
10. All photoresist was cleaned from the chip by rinsing the chip with acetone.
11. Chip was completely immersed in the Cr solution until all Cr was removed from the chip.
12. The chip was completely cleaned with soapy water, and thoroughly rinsed with de-ionized water.
13. An additional blank glass cover chip was also cleaned with soapy water, and thoroughly rinsed with de-ionized water.
14. The cover chip was then bonded to the microchip by using only water and extreme pressure (2). The chip was left to rest overnight.

Membrane Creation

1. Na-silicate (1:10 dilution) was entered into the chip through access hole A, while a vacuum was applied to access hole B until all excess moisture was removed from the 20µm channels.
2. The chip was then treated at 90°C under atmospheric conditions to transform the solution into a silica gel membrane.
3. After the creation of the initial membrane, reservoirs were glued to the five access holes of the chip using UV glue.

Membrane Stability

1. Reservoirs and channels B, C, and D were all filled with 1µM Resorufin, prepared with 10µM phosphate buffer (pH~7.45).
2. Reservoirs and channels A and E were filled with 10µM phosphate buffer.
3. With reservoir A as the ground, .5kV were applied to reservoir B.
4. If the dye permeated the membrane and could be seen in channel A, the leak had to be fixed with Na-silicate (1:10 dilution).
    1. Completely fixing the membrane could take several tries, and often a 1:5 or 1:3 dilution of Na-silicate could be used.
5. If there was no flow of dye in the channels, the membrane could be severing the electrical connection between A and B.
    1. Fixing blockage of the membrane could be done by introducing 1M NaOH to all channels and letting it rest 30 minutes-24 hours, pending of the severity of the blocked membrane.
6. A good membrane was one that had no leaking, but still had an electrical connection between A and B.

Coating the Channels

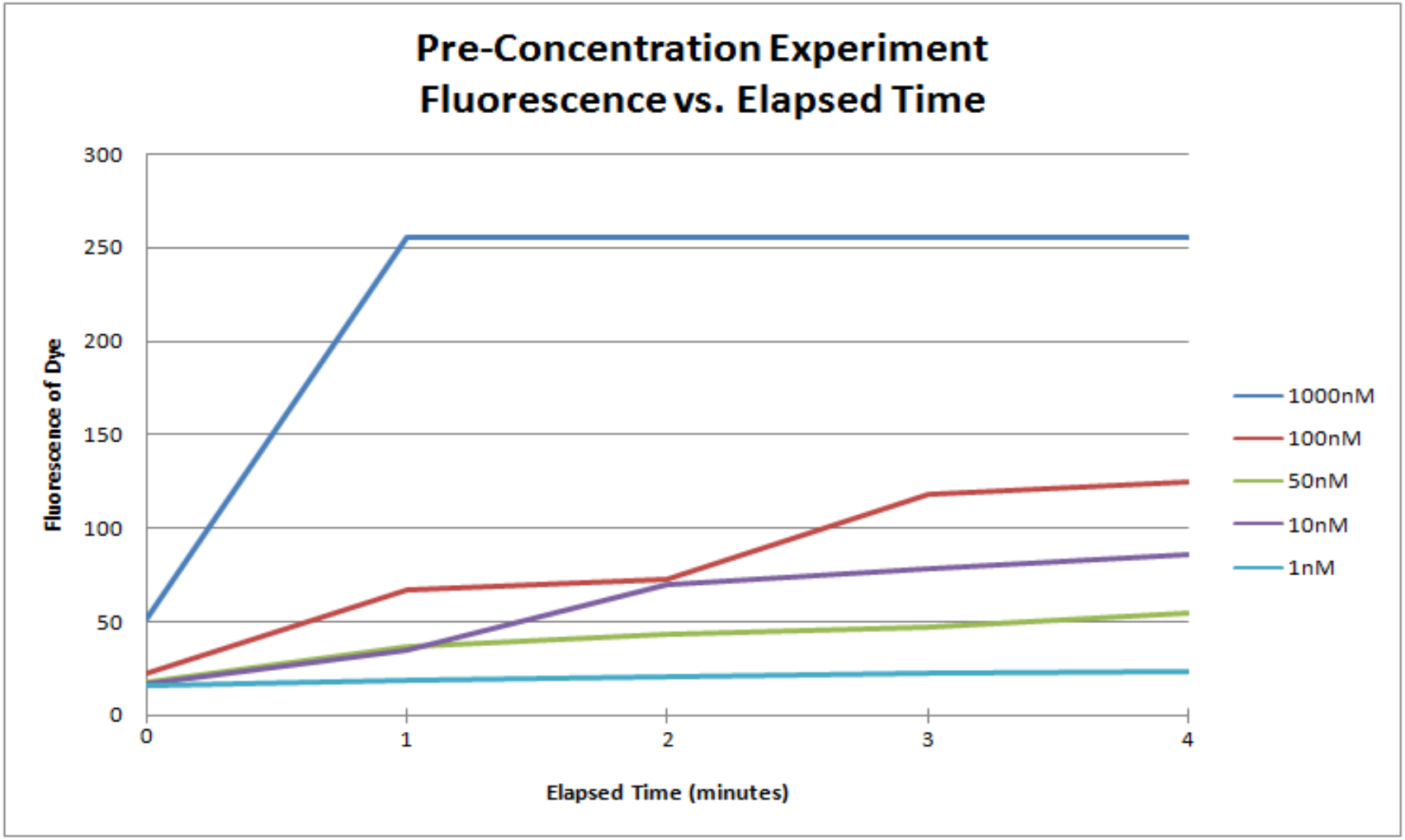
1. In order to cap the naturally occurring negative charges inside the channels, a formamide coating was applied to all side channels of the chip.
2. The coating was prepared by adding 1.8mL ethyl formate to 5.0mL 3-aminopropyltriethoxysilane in a large vial which was then mixed using vortex mixing. The solution was then allowed to react for 48 hours.
3. In order to prepare the chip for coating (3), all channels were rinsed with methanol, then the channels were filled with 1M NaOH for 15 minutes, then flushed out with de-ionized water and acetone, and was finally heated for 30 minutes at 70°C to completely dry.
4. The formamide coating solution was introduced into reservoirs A, B, and E, and methanol into reservoir D, and a vacuum was applied to reservoir C.
5. The chip was then allowed to rest for 3 hours, with reservoir D being refilled about every 15 minutes with methanol.
6. Finally, the chip was thoroughly rinsed out with methanol, while applying the vacuum to reservoir C.

Experiment Procedures

Pre-Concentration Experiment

1. Reservoirs and channels B, C, and D were filled with 1000nM Resorufin, prepared by 10µM phosphate buffer.
2. Reservoirs and channels A and E were filled with 10µM phosphate buffer.
3. With reservoir B as the ground, .3kV were applied to reservoir A.
4. At zero time, a background luminosity signal was captured.
5. Each minute after voltage was applied, an additional luminosity signal was captured.
6. Additional concentrations (100nM, 50nM, 10nM, and 1nM) of Resorufin were also tested.

Results

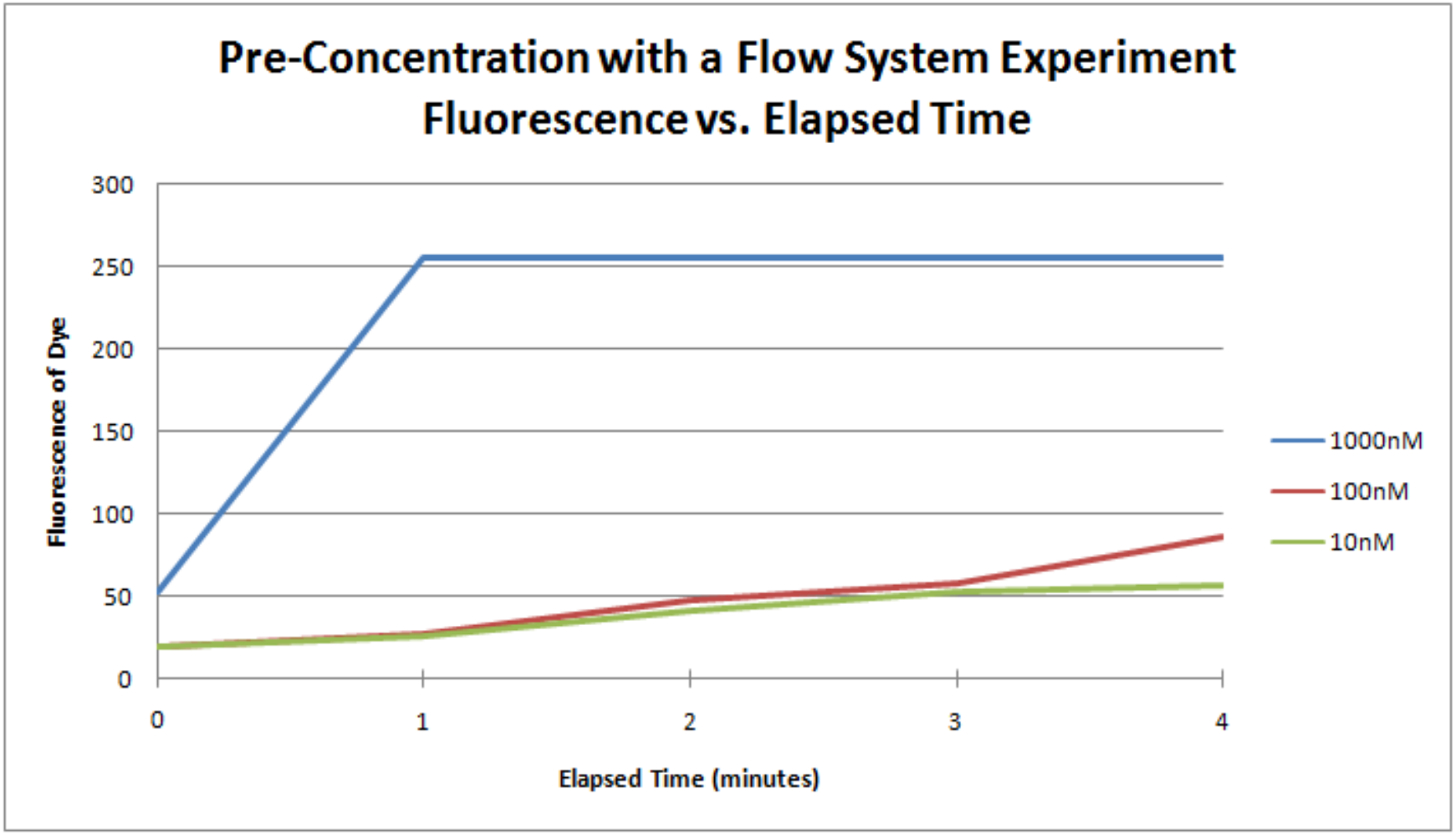

Pre-Concentration Experiment
Fluorescence vs. Elapsed Time
Fluorescence of Dye
300
250
200
150
100
50
0
0
1
2
3
4
Elapsed Time (minutes)
1000nM
100nM
50nM
10nM
1nM

Experiment Procedures

Pre-Concentration with a Flow System Experiment

1. Tall reservoirs were attached for access holes C and D.
2. Reservoir C was filled with 10µM Na tetra-borate buffer (pH~9.2) and reservoir D was filled with 10nM Resorufin prepared by using 10µM phosphate buffer. The height of liquid in reservoir C was kept slightly higher than liquid in reservoir D to prevent pressure-driven flow.
3. A vacuum was applied to reservoir B to draw the dye to membrane, and then reservoir B was also filled with 10nM Resorufin.
4. With reservoir B as the ground, .3kV were applied to reservoir A.
5. At zero time, a background luminosity signal was captured.
6. Each minute after voltage was applied, an additional luminosity signal was captured.
7. Additional concentrations (100nM and 1000nM) of Resorufin were also tested.

Results


Pre-Concentration with a Flow System Experiment
Fluorescence vs. Elapsed Time
Fluorescence of Dye
300
250
200
150
100
50
0
0
1
2
3
4
Elapsed Time (minutes)
1000nM
100nM
10nM

Conclusion

- I completed my 2009 summer fellowship's two objectives:
  - Created a microfluidic platform with a membrane interface
  - Quickly trapped very small concentrations of fluorescently dyed molecules against the membrane by using an electrical charge

- These successes allowed for more research to continue toward the positive goal of actually implementing ELISA onto a microfluidic platform with a membrane interface

- Could lead to earlier, more rapid detection of specific food allergens, West Nile Virus, and HIV

- Overall, I am thankful to have had the opportunity to participate in this project and I feel that it greatly opened my eyes to the world of research.

References

1. *Microfluidics: Applications for analytical.* **Ken-ichi Ohno, Kaoru Tachikawa, Andreas Manz.** 2008, Electrophoresis, pp. 4443-4453.

2. *Capillary Electrophoresis on microchip.* **Dolnik, Vladislov, Liu, Shaorong and Jovanovich, Stevan.** 2000, Electrophoresis, pp. 41-54.

3. *Multiple enzyme linked immunosorbent assay system on a capillary-assembled microchip integrating valving and immuno-reaction functions.* **Henares, Terence G., et al.** 2007, Elsevier Science, pp. 173-179.

4. *Low temerature bonding for microfabrication of chemical analysis devices.* **Wang, H.Y., et al.** 1997, Elsevier Science, pp. 199-207.

5. *Preconcentration of Proteins on Microfluidic Devices Using Porour Silica Membranes.* **Foote, Robert S., et al.** 2005, Analytical Chemistry , pp. 57-63.

6. *Multiple enzyme linked immunosorbent assay system on a capillary-assembled microchip integrating valving and immuno-reaction functions.* **Henares, T.G. et al.** 2007, Analytica Chimica Acta, pp. 173-179.

Acknowledgements

Funding for this research was provided by Wyoming NSF EPSCoR.

Thank you to Dr. Dutta & his lab group for allowing me to work with their group during the 2009 Summer.

Thank You For Attending

Questions?