Development of Functionalized Semipermeable Membranes for Microfluidic Separations

MELISSA J. GELWICKS
Detection and quantitation of target molecules in a sample is important for research and diagnostics (e.g. viruses or hormones in biological samples).

Size-based separations are appropriate for gross and sophisticated separations:
- **Gross**: "cleaning up" a sample, i.e. blood in order to then perform...
- **Sophisticated**: ability to separate smaller molecules in a way that allows for quantitation.

The goal is the ability to accomplish separations on very small samples over a large range of molecule sizes in aqueous medium.
Separation Techniques

- Gas Chromatography - inappropriate for charged and/or very high molecular weight molecules
- High Performance Liquid Chromatography (HPLC) - slow, expensive, requiring bulky instrumentation
- Silica-gel column chromatography - standard for macroscale, but ineffective on a microscale
- Membranes with controlled pore sized to affect size-based separations
Microfluidics

- General method for accomplishing complex separations rapidly, with small sample sizes on a microscale
- Membranes may be synthesized *in situ*
- Enhance sensitivity of common testing methods—performance of separations tends to improve with reduction in size
- Reduced expense and greater mobility of testing
Spring 2010 Research

- Microchip fabrication
- Membrane formation
- Evaluation of membranes
Microchip Fabrication

- Fascinating, but complex, method to create microchips
- Design used for this project specific to function
- 30µm depth channels for introduction of reagents and solutions, membranes formed in shallower channel ~2µm deep.
Semipermeable Membranes

• Polyacrylamide membranes used to trap biological molecules of MW=5,000-1,000,000
• First goal is to vary permeability of membranes through synthetic means
• Desire ability to trap MW≥200-500 molecules
• Second goal is incorporation of silica and amine derivatives into the membrane, to give a functionalized membrane
Membrane Synthesis

- Polymerization and cross-linking of acrylamide and bisacrylamide

\[
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O}
\]

- Maleic anhydride sub-units provide sites for specific molecular associations

\[
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \\
\text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O} \quad \text{O}_2\text{N}H_2 \text{O}
\]

5% cross-linking agent, photoinitiator

and/or
Membrane Synthesis

- Modified in a two-step synthetic procedure after polymerization to give a hybrid membrane including organic molecules and silica molecules attached to reactive centers in the membrane.

\[ \text{(EtO)}_3\text{Si} - \text{NH}_3 \]

\[ + \text{NH}_3 \]

\[ \text{and/or} \]

sodium silicate
(polymeric SiO\textsubscript{2}/HO\textsuperscript{−})
Functionalized Membranes

- Initial membranes contained high concentration of maleic anhydride subunits in order to achieve minimum porosity.
- Wanted to test for the passage of buffer molecule while retaining small organic molecules.
Evaluation of Membranes

- Electroosmotic flow - neutral molecules carried with bulk solution as it flows from cathode to anode
- Dominant form of flow in microfluidic channels
- Utilized neutral dye molecule, Rhodamine B, MW = 480

![Rhodamine B structure](image_url)
Evaluation of Membranes

- Electrophoretic flow - ions in solution are attracted to corresponding electrode
- In the absence of electroosmotic flow, may be used to selectively move positive and negative ions
- In this way, molecules can be selectively directed to membranes
Evaluation of Membranes

- Glass has inherent surface charge, must neutralize to reduce interference by electroosmotic flow-formamide coating
- Utilized dye molecule that may be easily ionized, Resorufin, MW= 213.

\[
\begin{align*}
\text{resorufin} & \quad \xrightarrow{\text{base}} \quad \text{resorufin anion} \\
\text{(highly fluorescent)} &
\end{align*}
\]
Evaluation Process

- Introduced dye molecules to channels under green light to excite fluorescence
- Applied voltage to ports to enhance flow through microfluidic channels
- Monitored flow of solution to and through membranes in order to evaluate ability of membrane to stop molecules
Results

- Electroosmotic flow
- Control membrane, just acrylamide: bisacrylamide polymer
Results

- Electroosmotic flow
- Hybrid membrane with maleic anhydride
Results

- Electrophoretic flow
- Polymer

Hybrid
Conclusion

- The polyacrylamide membrane was not expected to stop the flow of dye molecules, and it performed as expected.
- The hybrid membrane successfully trapped dye molecules slightly larger than MW = 213.
- Visible concentration of dye at membrane interface.
Future Research

- Synthetically vary concentration of reactive sites (e.g. maleic anhydride) in membrane in order to control permeability of membrane
- Long-term goal is to isolate aliquots containing molecules of certain size, for example MW = 1,000
- Incorporation of functionalized amine groups into membranes in order to perform size- and affinity-based separations
Acknowledgements

- Many thanks to:
- Naoki Yanagisawa
- Dr. Robert C. Corcoran
- Dr. Debasish Dutta
- EPSCoR