Bio-transport and Design of BioMEMS Devices
(Bios 10 lecture)

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September 30, 2009

I have a hammer.

I can put things together!
I can knock things apart!
I can alter my environment at will and make an incredible din all the while!

Ah, it's great to be an engineer.

Laboratory for Particle Mixing and Self-Organization
What is chemical engineering?

Chemical engineers design processes that perform molecular transformations of raw materials into products.

Intersection of Math, Physics, Chemistry, Biology

but

Math + Physics + Chemistry + Biology ? ChE
Industries relevant to chemical engineering and biology:

- Agriculture
- Chemical
- Cosmetic
- Food/beverage
- Energy/Petroleum/Biofuels
- Pharmaceutical
- Medical devices/drug delivery
- other…
What is chemical engineering? (and why might biologists care)?

Core areas of chemical engineering:

- **Reactor engineering:**
  Most biological processes are highly efficient and selective.

- **Transport processes:**
  Organelles, cells, and organs are structured to control transport rate of chemicals.

- All of chemical engineering is rooted in **Material and Energy Balances**

Cells that do not transport material and energy cannot survive.

Images from wiki
Transport processes across scales (distance and time)

Two primary modes of material transport:

• Diffusive
  • molecular ? cellular scale
  • random motion of macromolecules discovered by botanist Robert Brown in 1827
    – explained by Albert Einstein in 1905
  • collisional diffusion

• Convective (flow)
  • cellular ? system scale

Péclet number (Pe): diffusion time-scale / flow time-scale

    Pe << 1 – processes depend on diffusion
    Pe >> 1 – processes depend on convection
Examples of diffusion:

A single blood cell in a droplet of water will take over 2 years to interact with a 40x40 micron sensor by diffusion alone.

Most molecules within a cell diffuse in microseconds.
Example: blood transport

Increasing scale

Laminar to turbulent flow

Echocardiogram of flow in heart
Developing Labs on a chip: why BioMEMS?
(MEMS – micro-electro-mechanical systems)

Why miniaturize?
“Better, faster, cheaper”
• Smaller scales optimize transport properties related to macromolecular and cellular processes
• Faster and more accurate/reproducible analyses

Also
• Smaller sample size
• Less waste of reagents and lower fabrication costs
Developing Labs on a chip: why BioMEMS? (MEMS – micro-electro-mechanical systems)

Two considerations:

- How do suspensions of macromolecules or cells flow, mix, and segregate in these systems?
- How can we design microscale capture-and-release platforms for disease detection?
Blood flow in microchannels – Fahraeus-Lindqvist effect

In flow in a microchannel, the flow is fastest near the center and slowest near the wall.

Water and other ‘normal’ or Newtonian fluids develop a parabolic velocity profile.

Blood has shear-dependent viscosity (Fahraeus-Lindqvist effect):
- Less shear – higher viscosity
- More shear – lower viscosity

‘Separates’ into cell-rich and cell-lean streams
Mixing at small scales

- Smaller scales produce both quantitative and qualitative differences.
- No turbulence.
- Pe >> 1, diffusion is slow.
- Surface forces are significant.
- Incorporation of conventional approaches to manipulation difficult.

- Only recently has design of the channels begun to overcome limitations in mixing by introducing chaotic advection via breaking symmetries.
  - Geometric modulation
  - Time-periodic forcing

- Even with chaotic advection, invariant manifolds often exist, inhibiting mixing.
Straight Channel (1D Flow), ~30 mm from entrance

<table>
<thead>
<tr>
<th>φ</th>
<th>Re</th>
<th>Pe</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>~0.04</td>
<td>~600</td>
</tr>
<tr>
<td>0.2</td>
<td>~0.22</td>
<td>~3000</td>
</tr>
<tr>
<td>0.3</td>
<td>~0.44</td>
<td>~6000</td>
</tr>
</tbody>
</table>

Re ~ 0.04
Pe ~ 600
Re ~ 0.22
Pe ~ 3000
Re ~ 0.44
Pe ~ 6000
Re ~ 1.1
Pe ~ 15,000
Herringbone Channel (2D Flow)
Staggered Herringbone Channel (Chaotic Flow)

$\phi = 0.3$
Re $\approx 0.44$
Pe $\approx 6000$

- Multiple local maxima in concentration profile.
- Center dense region "roping" through channel.
- Final concentration profile is steady
2D Concentration contours across the channels

Re = 8.2x10^-7  Pe = 5,100  C. Gao, B, Xu and J. F. Gilchrist, PRE, 2009
3D Confocal Imaging – 2D channels, instability near entrance
Confocal Imaging – Blood flow in 2D channels
Developing Labs on a chip: why BioMEMS? (MEMS – micro-electro-mechanical systems)

Two considerations:

• How do suspensions of macromolecules or cells flow, mix, and segregate in these systems?

• How can we design microscale capture-and-release platforms for disease detection?
Undergraduate Students:
Colleen Curley, Abbe Lefkowitz, Kristen Mason, and D’Andre Watson
Graduate Students:
Pisist Kumnorkaew, Bu Wang, and Alex Weldon
Professors:
Xuanhong Cheng and James F. Gilchrist
Our Microchips

- We want to build devices that reversibly capture cells
  - There are currently no effective methods for the isolation and release of specific cells
- Releasing cells from the device allows for research on targeted infected cells
Objectives

- Deposit a well-ordered monolayer of SiO$_2$ magnetic beads on a glass substrate
- Functionalize magnetic beads with antibodies and build microfluidic channels over the monolayer
- Capture Jurkat and THP1 cells in the microfluidic channels
- Controllably release captured cells by magnetic force
Reversible Cell Attachment Through Magnetic Bead Layer

Glass modification

Magnetic bead deposition

Antibody immobilization

Cell capture

Cell counting
Molecular analysis
Cell culture

Release with shear and field

Characterization
Convective Deposition

- Process by which a meniscus of suspension is drawn across a substrate to deposit a thin film.
- Used to obtain a well-ordered monolayer of polystyrene nanoparticles and SiO$_2$ magnetic bead microspheres
  - Helps quantify the amount of magnetic beads in a given area
  - Gives high surface coverage which prevents cells from binding to substrate
- Each concentration of suspension ($F_{\text{nano}}$, $F_{\text{micro}}$) has its optimum condition (angle, speed, temperature, humidity)
Magnetic Bead Layers

Depending on deposition and surface conditions, we obtain monolayers of magnetic beads.

- Challenges arise due to non-uniform particle size.
Results

Ideal monolayer deposited with 20% SiO₂, 6% Polystyrene, and 30 µm/s deposition speed.

Optimizing nanoparticle concentration

Optimizing deposition speed
Reversible Cell Attachment Through Magnetic Bead Layer

- Glass modification
- Magnetic bead deposition
- Antibody immobilization
- Cell capture
- Cell counting
- Molecular analysis
- Cell culture
- Release with shear and field
- Characterization
Magnetic Bead Liftoff: Surface Chemistry

- Binding force between beads and substrates must be optimized
  - Must be strong enough for beads to endure fluid flow through the microchannels
  - Must be weak enough so that the beads can lift off from the surface when magnetic force is applied
Surface Chemistry

- When deposited on the untreated substrate, magnetic beads bind too strongly.
- Silane treatment alters substrate surface chemistry to weaken binding between the magnetic beads and the substrate.
  - Silanes change liquid contact angle and particle affinity for the substrate:
    - Hydroxymethyl triethoxysilane (H-treated)
    - 2-[Methoxy(polyethyleneoxy)propyl] trimethoxysilane (P-treated)
    - 2-Cyanoethyl triethoxysilane (C-treated)

<table>
<thead>
<tr>
<th>Suspension Droplet Contact Angles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Substrate: &lt;10°</td>
</tr>
<tr>
<td>H Silane: 47°</td>
</tr>
<tr>
<td>P Silane: 41.5°</td>
</tr>
<tr>
<td>C Silane: 37°</td>
</tr>
</tbody>
</table>
Surface Chemistry

- We measured deposition speed necessary to obtain a monolayer of magnetic beads on slides treated with each silane.

<table>
<thead>
<tr>
<th>Silane Treatment</th>
<th>Optimum Speed for a Monolayer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean Glass</td>
<td>12.5 µm/s</td>
</tr>
<tr>
<td>H</td>
<td>12.5 µm/s</td>
</tr>
<tr>
<td>P</td>
<td>13.3 µm/s</td>
</tr>
<tr>
<td>C</td>
<td>11.7 µm/s</td>
</tr>
</tbody>
</table>

All other parameters held constant.
Reversible Cell Attachment Through Magnetic Bead Layer

1. Glass modification
2. Magnetic bead deposition
3. Antibody immobilization
4. Cell capture
5. Release with shear and field
6. Characterization
7. Cell counting
8. Molecular analysis
9. Cell culture
Lift-off Experiments

- We aim to quantify when beads irreversibly bind to the substrate and when they can be controllably released under flow and magnetic forces

- Experimental conditions:
  - Silane treatment, polystyrene removal in a toluene bath, and drying under nitrogen flow and in air
  - Solvent replacement and DI water wash experiments to remove unknown stock solution

- Treated slides rinsed under various flow rates to simulate microchannel conditions
Results

- Toluene baths preceding DI water baths promoted uncontrollable liftoff (in DI water baths)
  - Samples dried between toluene and DI water baths showed no liftoff
- Increased time between deposition and rinse strengthens binding between beads and substrate
- Liftoff on P-treated substrates was difficult to tune
- Solvent-replaced suspensions deposited on H-treated substrates left at room temperature to equilibrate showed most controllable release
  - Optimum silane equilibration time is 1 hour
Results

Liftoff

No Liftoff
Reversible Cell Attachment Through Magnetic Bead Layer

- Glass modification
- Magnetic bead deposition
- Antibody immobilization
- Cell capture

Cell counting
Molecular analysis
Cell culture

Characterization
Release with shear and field
Cell Capture and Culture

Objective
- Our eventual goal is to capture infected cells using a microfluidic channel and keep them viable for analysis

Preliminary Model
- Used cells that simulate white blood cells
- Fabricated and flowed cells through microchannels
- Analyzed cell capture using fluorescence microscopy
Deposition vs. Plain Glass

- Microchannels made on slides with monolayer depositions
- Cells pumped through channels at 2-5 µl/minute for 5 minutes
- Cells (~7 µm diameter) fixed and stained to fluoresce green
- Devices analyzed using fluorescence microscopy
- Plain glass and defect areas captured more cells than areas of monolayer deposition
Geometric Factor in Cell Capture

- The idea: smaller beads will result in more contact area. Therefore will make cell capture more efficient.

  - Small beads: more contact area
  - Big beads: less contact area

- The test:

<table>
<thead>
<tr>
<th></th>
<th>0.5 um silica beads</th>
<th>1um silica beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cells</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>Captured on Deposition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Image: Diagram showing small and big beads with contact areas and cell capture efficiency]
Maintaining Cell Viability

- Devices containing cells must have fresh media
- Difference in pressure causes media to flow constantly through device
  - Easier than injecting media every 12 h
- Flow rate increases as height difference increases
- Stain cells and count to determine optimal flow rate
Ongoing Work

- Refine and optimize deposition, surface treatment, and cell capture
- Continue parametric studies on magnetic bead liftoff and cell capture
- Optimize cell release in microfluidic channels
  - Modeling forces involved for release
- Proof of Concept
  - Transition to capturing rare instead of model cells
Acknowledgements

We gratefully acknowledge funding from the Howard Hughes Medical Institute as well as those in connection with the Biosystems Dynamics Summer Institute.
Acknowledgements