Lecture 21: Cells in Microfluidics – 02 / PCR

Four Stages of Cell Analysis

- **Cell Sampling**
  - Extracting the sample, microneedles

- **Cell Treatment**
  - Cell lysis
  - Electroporation/gene transfection
  - Cell fusion

- **Cell Trapping and Sorting**
  - Mechanical filtration
  - Hydrostatic pressure
  - Dielectrophoresis: electric field-based manipulation and separation
  - Magnetoophoresis
  - Optical tweezers
  - Fluorescence-activated cell sorting (FACS)
  - Magnetic cell sorting (MACS)
  - Centrifugation

- **Cell Analysis**
  - Cell identification
  - Ion channel study

---

**Cell Treatment**

- **Cell Lysis**
  - Conventional: Enzymes, chemical lytic agents (detergent), mechanical force (sonication), thermal
  - Microfabrication: thermal, chemical, electrical, mechanical

**Thermal Lysis**

- **Thermal Lysis**
  - Start from serum
  - Repeated freezing and thawing
  - Elevated temperature (simultaneous denaturing step in PCR)
  - Remove inhibitors for better PCR
  - Clogging: debris of cell

- **Microwave (US6623945)**
  - System and method for microwave cell lysing of small samples, Motorola

- **Laser Lysis**
  - Laser-Irradiated Magnetic Bead System (LIMBS) designed for the rapid lysis of bacterial cells
  - Debris removal by magnetic beads

---

**Fig. 4. A silicon micromachined device with microjets that homogeneously deflect cells.** [http://www.small.buffalo.edu]
Chemical Lysis

- Most commercial kits
  - Complicated steps and long time
- Combination of Detergent and Lytic Enzymes
- Organic Solvents
  - Create channels through the cell membrane
    - Toluene
    - Ether
    - Phenylethyl alcohol
    - DMSO
    - Benzene
    - Methanol
    - Chloroform
- Detergents
  - SDS
- Enzyme
  - Lysozyme
- Osmotic Pressure
  - Cells exposed to rapid changes in external osmolarity can be mechanically injured

Electrical Lysis

- Electroporation
  - Micropores on the cell membranes due to pulsed high-electric-field
  - Electroporation for gene delivery & therapy
- Electroporation & Electrophoresis
  - Electroporation: 12.5kV/cm, 200 usec pulse
  - Separation: 350V/cm
  - Andreas Manz (uTAS 2002, pp.817)
- Micro Electroporation for Cell Lysis (US6287831)
  - Dr. Yu-Chong Tai (Cal Tech), 1999
  - For better lysis efficiency ➔ increasing lysing area

Electrolysis

- Electrolysis Actuation
  - Anode (+): Acid (pH 1)
    $2H_2O \leftrightarrow 4H^+ + 4e^- + O_2$
  - Cathode (-): Base (pH 1)
    $4H_2O + 4e^- \leftrightarrow 4OH^- + 2H_2$
- On-chip cell lysis by local hydroxide generation
- Electrolysis Actuation
  - CHO cell (animal cell) in 500ul NaCl solution (100 mM)
  - Microscope image capture of cell lysis for 25 sec (X 400)
  - Time: 5 V, 1mA
Mechanical Lysis

- **Sonication (US6100084)**
  - Strong cell lysis method
  - Cepheid, MFSI
  - Titanium metal horn resonating @15-25 kHz
  - Power consumption

- **Bead-Beating**
  - Cell lysis on a microfluidic CD (compact disc)

- **High Pressure (US6120985)**
  - 1. A method of lysing a cell, the method comprising:
    - providing a frozen cell under atmospheric pressure;
    - while maintaining the cell at a subzero temperature, exposing the cell to an elevated pressure in a pressure chamber, the elevated pressure being sufficient to thaw the frozen cell at the subzero temperature;
    - depressurizing the pressure chamber to freeze the cell at the subzero temperature, and
    - repeating the exposing and depressurizing steps until the cell is lysed.

Cell Lysis, DNA purification, and Recovery

### Table 1: Cell lysis, DNA purification, and recovery

<table>
<thead>
<tr>
<th>Method</th>
<th>Technical difficulty</th>
<th>Cost</th>
<th>Efficiency</th>
<th>Ease of miniaturization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Of the mechanical lysis methods reviewed, ultrasound is most desirable.</td>
</tr>
<tr>
<td>Chaotrophic</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Depends strongly on the volume and type of medium, can be incorporated into a membrane, facilitating removal.</td>
</tr>
<tr>
<td>Thermal</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Easy to minimize, but not suitable for all cell types</td>
</tr>
<tr>
<td>Electronic</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Easy to minimize</td>
</tr>
<tr>
<td>Purification method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion exchange</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>Easy to implement on a miniaturized platform</td>
</tr>
<tr>
<td>Resins</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Somewhat more cumbersome</td>
</tr>
<tr>
<td>Molecular weight cut-off (MWCO) membranes</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Easy to implement on a miniaturized platform</td>
</tr>
<tr>
<td>Recovery method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silica column</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>If a flow-through system is used miniaturization of low-volume buffers is easy to accommodate</td>
</tr>
<tr>
<td>Beads column</td>
<td>Low</td>
<td>Low</td>
<td>Medium</td>
<td>More cumbersome</td>
</tr>
<tr>
<td>Membrane column</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Easiest to miniaturize</td>
</tr>
</tbody>
</table>

Cell Treatment

- **Gene Delivery**
  - Microinjection
  - Electroporation
  - Sonoporation

Gene Delivery

- Microinjection
- Electroporation
- Sonoporation

EE 428/528 BioMEMS & Lab-on-a-Chip

Lecture 21: PCR

Kwang W. Oh, Ph.D., Assistant Professor
SMALL (nanobioSensors and MicroActuators Learning Lab)
Department of Electrical Engineering
University at Buffalo, The State University of New York
215E Bonner Hall, SUNY-Buffalo, Buffalo, NY 14260-1920
Tel: (716) 645-3115 Ext. 1149, Fax: (716) 645-3656
E-mail: kwangoh@buffalo.edu, http://www.SMALL.buffalo.edu
Sample-to-Answer

1. **Cell Sampling**
   - 1. Whole blood sample

2. **Cell Trapping and Sorting**
   - 2. WBC separation/concentration

3. **Cell Treatment**
   - 3. Cell lysis

4. **DNA Extraction**
   - 4. DNA purification/recovery

5. **PCR (Polymerase Chain Reaction)**
   - 5. DNA amplification

6. **Analysis**
   - 6. DNA detection

---

**Polymerase Chain Reaction (PCR)**

- **PCR = Enzymatic Amplification + Repeated Thermal Cycling**
  - A copying machine for DNA molecules
  - Invented by Kary B. Mullis in 1985
  - One of the most widely used techniques in molecular biology
  - Enzymatic Amplification
    - Primers are short, artificial DNA strands of about 18 to 25 nucleotides that match the beginning and end of the DNA fragment to be amplified. The primers anneal to the single-stranded DNA template at these points. Once the primers bind, the DNA-Polymerase enzyme binds and begins the synthesis of the new DNA strand from free nucleotides.
  - Repeated Thermal Cycling
    - The chain reaction is a three-step process, **denaturation** (@95 °C), **annealing** (@55 °C), and **extension** (@72 °C), that is repeated in several cycles. At each stage of the process, the number of copies is doubled from two to four, to eight, and so on.

---

A sample of chromosomal DNA, also called genomic DNA, can be used as the starting material for the polymerase chain reaction (PCR). With PCR, an investigator can amplify a single copy of a DNA segment into billions of identical copies. The DNA segment of interest, or target DNA, is indicated in red.
In addition to the target DNA, a PCR reaction contains several other ingredients. These include free nucleotides, DNA primers, and the enzyme Taq polymerase. The primers are typically about 20 nucleotides long and are complementary in sequence to the ends of the target DNA. Taq polymerase is derived from thermus aquaticus bacteria and can tolerate the intense heat of a PCR reaction.

A PCR reaction lasts several hours and typically consists of 20 to 35 repeating cycles. A cycle begins by heating the reaction mixture to 95 degrees Celsius. The heat denatures the DNA, breaking the hydrogen bonds that hold the strands together.

After denaturing the DNA, the temperature is reduced to around 60 degrees so that the primers can form hydrogen bonds, or anneal, with their complementary sequences in the target DNA. Note that the primers and the target DNA follow base-pairing rules: An adenine (A) pairs with a thymine (T), and a cytosine (C) pairs with a guanine (G).

In the next phase, the temperature is raised to 72 degrees Celsius. Taq polymerase functions optimally at this temperature and begins polymerization, adding nucleotides to the 3' end of each primer attached to a DNA strand. After one complete cycle, there are two double-stranded copies of the target DNA.
The PCR reaction mixture contains many copies of the primers and an abundant supply of nucleotides to perform many addition cycles. After a second cycle, there are four copies of the target DNA.

After cycle 3 is finished, there are eight copies of the double-stranded target DNA sequence. Note that only two of the double-stranded copies consist of just the target fragment. The others also include flanking DNA regions.

As the number of cycles increases, the products consist of a greater proportion of fragments with just the target DNA. After four cycles, half of the fragments consist of just target DNA, and half of the fragments also contain flanking DNA.

With each additional cycle, the number of copies of our target sequence doubles. At the end of cycle 25 there are more than 33 million copies of this double stranded target region.

\[ 2^2 = 4, \quad 2^3 = 8, \quad 2^4 = 16, \quad 2^5 = 32, \quad 2^6 = 64, \quad 2^{30} = 1,073,741,824 \]
Copies of Target DNA

- **PCR = Enzymatic Amplification + Repeated Thermal Cycling**
  - Amplification of Target DNA (up to ~ 30 cycles)
  - Saturation (after ~ 30 cycles)
  - The copies of target DNA ($C$) with cycle numbers ($n$)
    $$C = f(n) = C_0(1+e)^n$$
    where $C_0 = f(n=0)$ and $e = 1$. 

The graphs depict the relationship between cycle number and the number of copies of target DNA, showing the exponential growth and saturation phases.