Encapsulation of Single Cells in a Droplet-Based Microfluidic Device

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Abstract:

Microfluidic devices have great potential in cell analysis due to their small scale, high-throughput operation, and adaptability to a variety of analytical methods. The applications of microfluidics include droplet-based microfluidic devices, which would allow for more robust single-cell measurements than conventional electrochemical detection in Petri dishes, thus better revealing cellular functions and dynamics. For this idea to be beneficial, high-throughput encapsulation of single cells in a droplet-based microfluidic device is necessary. Cells must be individually encapsulated and stored in droplets of media dispersed in a continuous carrier phase. In this project, standard soft-lithography techniques were used for polydimethylsiloxane (PDMS)-based chip fabrication. Tris buffer and perfluorinated polymer solution were utilized as the media and carrier phase, respectively. Lastly, polystyrene beads were used to simulate cells. The devices should have a high percentage of one-cell droplets to be useful for single cell measurements. Ultimately, electrodes will be integrated into this device to produce an ideal tool for electrochemical measurements of immune cell exocytosis.

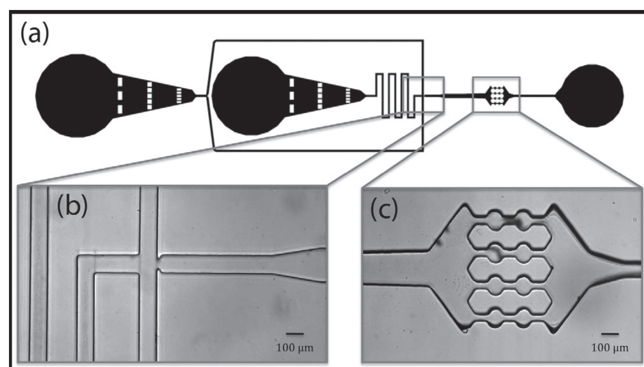

Figure 1: (a) Device design. From left to right: oil inlet and filter, aqueous inlet and filter, aqueous channel, constriction, storage array, outlet. (b) Image of fabricated channel constriction. (c) Image of fabricated storage array, where droplets will be eventually be stored for measurements.

Introduction:

Exocytosis is the critical cellular process by which cells secrete chemical messengers to each other; measuring exocytosis on-a-chip was the ultimate goal of this project. One current method of measuring cell exocytosis is carbon-fiber microelectrode amperometry (CFMA), which measures the excretes of a stimulated cell using electrochemistry. Although this method provides excellent temporal and spatial resolution, its disadvantages include inefficiency and difficulty of electrode fabrication, thus resulting in a low-throughput method.

To overcome the disadvantages of CFMA, a droplet-based microfluidic device was designed to provide a high-throughput method of measuring single cell exocytosis (Figure 1a). In this device, an aqueous cell suspension flowed between an oil carrier phase. When these fluids came to a constriction in the design, droplets of single cells in aqueous media formed.

This approach is advantageous because the droplets are identical, thus allowing for statistically reliable measurements. It also avoids the low level-of-detection issues present in CFMA due to diffusion of excretes. For this design, a high percentage of droplets containing single cells is desired.

Experimental:

Fabrication. Standard soft-lithography with negative photoresist (SU-8) was employed to fabricate the microfluidic devices. Photoresist was spun onto a wafer at 100 μm thickness, following instructions provided by MicroChem. The photoresist was exposed to UV light through a photomask with the desired device designs. After developing the wafer, PDMS was poured over the wafer and cured overnight.

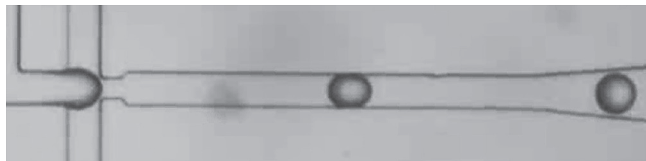

Figure 2: Formation of water droplets in carrier phase.

Two methods of bonding between PDMS and glass substrate were tested. The reversible process, performed by soaking PDMS in HCl and binding to glass over heat, was not strong enough for the fluid pressures exerted on the device. The irreversible process, using silicon-oxide bonds between the PDMS and glass, proved to be reliable for this experiment.

Holes were punched at the inlet and outlet of each device, allowing Teflon® tubes to connect the device to syringe pumps. Each device was also coated with an Aquapel solution, making the channels highly hydrophobic, thus forming smaller aqueous droplets in the carrier phase.

Operation. To test the droplet formation of the devices without cells, MilliQ water (aqueous phase) was flowed between a perfluorinated polymer solution (carrier phase). Rapid formation of water droplets was observed (Figure 2). Droplet size could be decreased by increasing carrier phase flow-rate or decreasing aqueous phase flow-rate.

To test encapsulation, 10 μm polystyrene beads were used to simulate cells. The beads were suspended in Tris buffer at a concentration of 1.8×10^6 beads/mL. This suspension was flowed between a perfluorinated polymer solution.

Results and Discussion:

At a bead concentration of 1.8×10^6 beads/mL, oil/carrier flow-rate of 300 μL/hr, and aqueous flow-rate of 100 μL/hr, the observed percentage of droplets containing a single cell was 31.4% (Figure 3).

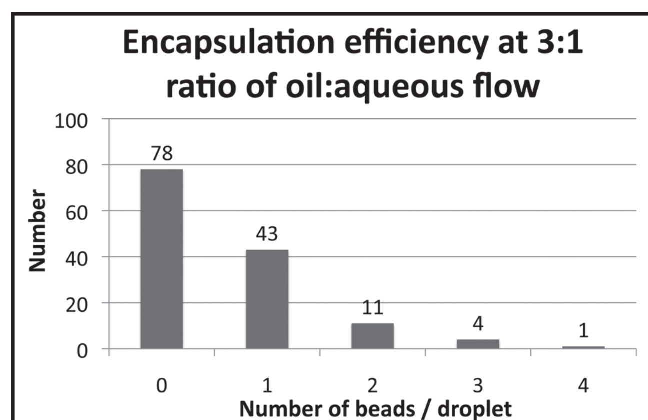

Figure 3: Encapsulation efficiency results at carrier flow-rate of 300 μL/hr and aqueous flow-rate of 100 μL/hr.

Although encapsulation of single beads was seen (Figure 4), many problems were encountered during operation. First, stray PDMS pieces occasionally collected inside the channels and flowed to the channel constriction. This caused beads to also collect at the constriction, thereby restricting regular flow. PDMS parts were sonicated before bonding to glass, which diminished but did not solve the problem. Next, beads aggregated in the channels and settled in the Teflon tubing, preventing regular formation of single-bead droplets. Different pH of Tris buffer were tested to minimize aggregation and settling. Testing showed that pH 7 buffer worked best, but did not eliminate the problem. Finally, the bead concentration used was not ideal for forming single-bead droplets. A curved aqueous channel can be tested to focus the location of beads in the channel.

Future Work:

The percentage of droplets containing single beads can be improved upon. After a maximum efficiency is achieved, the encapsulation efficiency of cells (e.g. mast cells) will be tested in place of polystyrene beads. Ultimately, microelectrodes will be integrated into this device to produce an ideal tool for electrochemical measurements of immune cell exocytosis.

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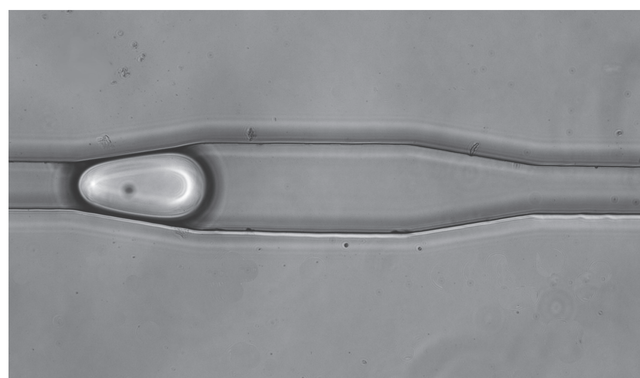

Figure 4: Aqueous droplet containing a single bead.