High-Throughput Drug Screening *in vivo* Using Droplet Microfluidics

BIOL

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Introduction:

High-throughput cell-based drug screenings conducted through various technologies, such as in microtiter plates, have significantly advanced drug development. However, the costs and time associated with such technologies are exorbitant. Polydimethylsiloxane (PDMS)-based microfluidic devices provide a popular *lab-on-a-chip* technique where reagents may be combined in sub-nanoliter volumes in a fast and controlled manner. PDMS is a cheap, transparent, and bio-compatible substrate that affords rapid prototyping and an efficient platform for drug screening. We used such devices to generate *water-in-oil* emulsion droplets at high throughput (~ 1000 drops per second) that efficiently encapsulated cells in the presence of drugs. Reducing the size of the reaction compartments to sub-nanoliter volumes allowed us to be parsimonious with reagents while high number of droplets (~ 10^6) provided superior statistical resolution.

In this project, we designed, fabricated and used microfluidic devices to test the efficacy of cancer drugs on a human cancer cell line where the drug concentrations were systematically varied.

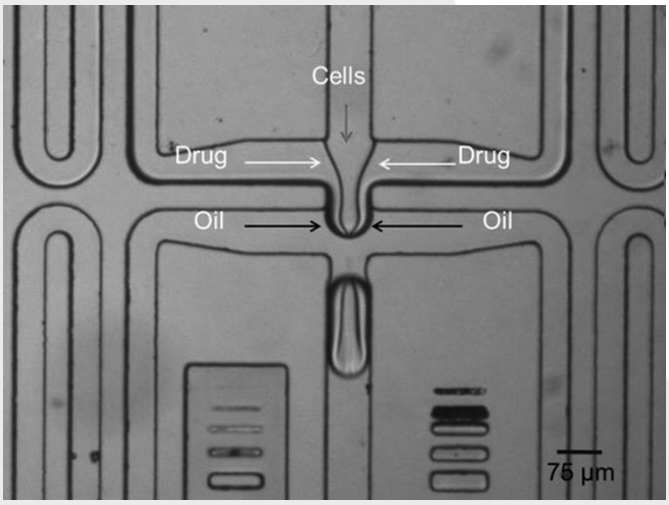

Figure 1: Generation of emulsion droplets in microfluidic co-encapsulation device.

Experimental Procedure:

CAD Designs. We used AutoCAD (Autodesk, USA) software to design microfluidic devices. One of the designs we used was a simple co-encapsulation scheme where two reagent channels met at a junction, which were then encased in oil, generating reverse emulsion droplets (Figure 1). In order to change the drug concentration in each droplet, the respective flow rates of the reagents were changed using syringe pumps. Another design used was a double-layer device that generated a gradient of seven different drug concentrations that were then simultaneously co-encapsulated with cells in oil for a faster droplet generation without changing flow rates.

Soft Lithography. We shone UV light through a CAD mask and exposed SU-8 photoresist-covered silicon wafers to crosslink exposed areas. After development and subsequent washes, this served as a master mold to create PDMS-based microfluidic devices. Uncured PDMS was poured on this master, followed by baking. The solid PDMS layer was peeled off and covalently fused to a glass slide using plasma treatment. Finally, we coated the microchannels with Aquapel (Pittsburgh Glass Works, USA) to render them hydrophobic.

Microfluidic Emulsions. We used cancer cells from a human lymphoblast cell line that were stained using a live-dead fluorescence reporter kit (Invitrogen, USA). We used three syringe pumps (New Era Pump Systems, USA), disposable syringes and needles (BD Biosciences, USA), and polyethylene tubing to flow in drug and a fluorescent dye mix on one, cells in phosphate buffer saline on the second and an oil/surfactant mix [4] on the third channel in our microfluidic devices creating water-in-oil emulsion droplets. We used Geneticin, an anti-cancer drug, mixed with fluorescein (Sigma-Aldrich, USA), to estimate drug concentrations in droplets. Uniform-sized droplets with 75 μm diameter were collected in microcentrifuge tubes. The reagent flow rates were changed to generate droplets with different drug concentrations. The droplets were incubated and tested at different time points.

Data Acquisition. We used fluorescence imaging and photo-multiplier tube (PMT)-based detection to interrogate the co-encapsulated droplets. We used a fluorescence microscope (IX83, Olympus, USA) for imaging and ImageJ software (NIH, USA) to analyze acquired images. A custom-built

FPGA-based (National Instruments, USA) PMT detector setup was used to detect live/dead cell state and dye concentrations in drops at high-throughput (~ 500 drops/sec).

Results and Conclusions:

We analyzed fluorescence images of cell and drug emulsions. Droplets at different light intensities (Figure 3) indicated different drug concentrations, while bright points inside the droplets marked apoptotic cells. Using ImageJ, we tracked five different fluorescein concentrations (Figure 2). Different concentrations of drug/dye detected after long period of incubation (~ five hours) attested the absence of drug diffusion among droplets. Although fluorescence imaging was informative, it was time-consuming and difficult to analyze large amounts of data using imaging alone.

PMT-based droplet detection provided time-trace plots (Figure 4) that displayed a plateau indicating the drug concentration, superimposed with a spike that marked an apoptotic cell. This manner of detection allowed us to screen thousands of droplets in seconds.

Future Work:

The double-layer microfluidic device is still under development. Currently, only a fraction of the channels were able to generate droplets reliably. We will need to adjust the fluid-flow scheme, which require meticulous fabrication and several iterations of testing. The highest drug concentration of Geneticin used in this project (~ 60 mg/ml) was not high enough to induce appreciable levels of apoptosis in cells. We will need to increase the dosage and test several physiologically relevant concentrations. We will employ the double layer design to provide a wide array of concentrations simultaneously and will continue using the PMT detector setup to test at high throughput.

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References:

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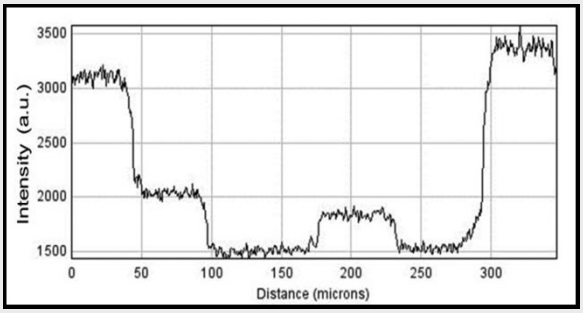

Figure 2: Plot of droplet intensities at different fluorescein concentrations.

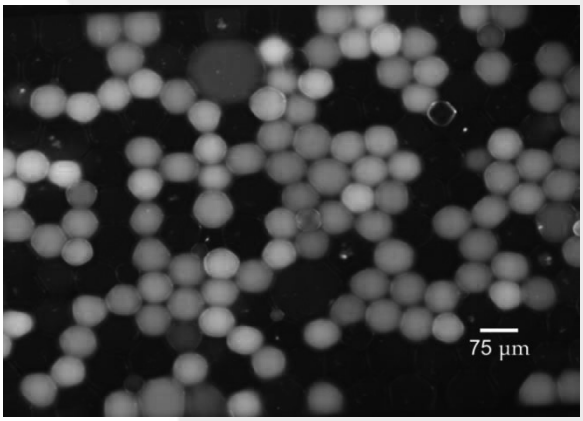

Figure 3: Image of cells and drug encased in droplets taken after five hours from co-encapsulation.

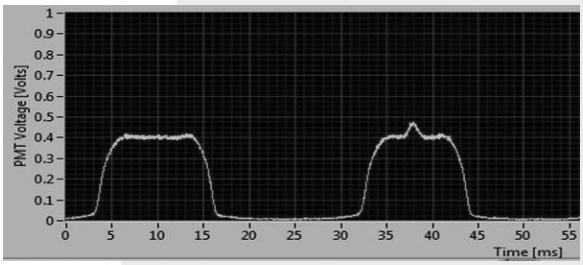

Figure 4: Time-trace snapshot of droplets detected using PMT. The plateaus indicate the fluorescein concentration in droplets, while the peak atop the second plateau indicates an apoptotic cell.