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

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Introduction:

Early stages of drug screening involve a broad library of drug compounds. Oftentimes the limiting factor in initial screening is the low volumes of candidate drugs. Microliter well assays are the current conventional method for initial screening. However, a well can only efficiently pipette 10 µL while in a droplet of 50 µm diameter the volume is 65 pL, 10^5 times less than a well. The aim of this project was to develop a platform to screen chemotherapeutic drugs at different doses on cancer cell lines at high-throughput using; a) live/dead cell assay, b) fluorescent dye as indicator of drug concentration, and c) photomultiplier tubes (PMT) for readout for both drug concentrations and cell state within each droplet.

The novelty of high-throughput drug screening using droplet microfluidics has the capability of decreasing time for analysis, volumes of reagents, and cost using polydimethylsiloxane (PDMS) microfluidic technology, while increasing the number of quantified interactions in comparison to conventional drug screening. High throughput is describing the fact that in 20 minutes ~ 10^5 cell and drug interaction chambers can be screened with PMT sensor technology. Droplet chambers and PMT allow us to rapidly screen for candidate drugs by quantifying their efficacy at distinct concentrations in killing cancer cells as function of time with high statistical resolution.

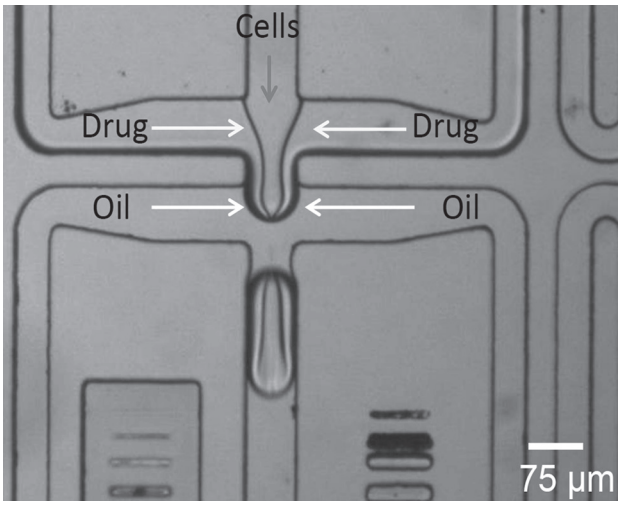

Figure 1: The junction of the co-encapsulation flow device showing formation of water-in-oil emulsions.

Experimental Procedure:

PDMS is favored as a material for microfluidic device fabrication because it is optically transparent, biocompatible, and gas permeable, among other benefits. Device fabrication consisted of standard soft lithography methods. First, SU-8 photoresist was spun onto a silicon wafer and placed under UV. After developing with a photo-mask, it became a master mold to make PDMS devices. Photoresist was poured onto the mold and then bonded to a glass slide by plasma oxidation. Lastly the channels were coated with aquapel to make the micro-channels hydrophobic [1].

The feature size of the co-encapsulation flow device was 50 µm, which determined the size of the droplets. It is these narrow micro-channels diameters that give laminar fluid dynamics, which are governed by low Reynolds numbers.

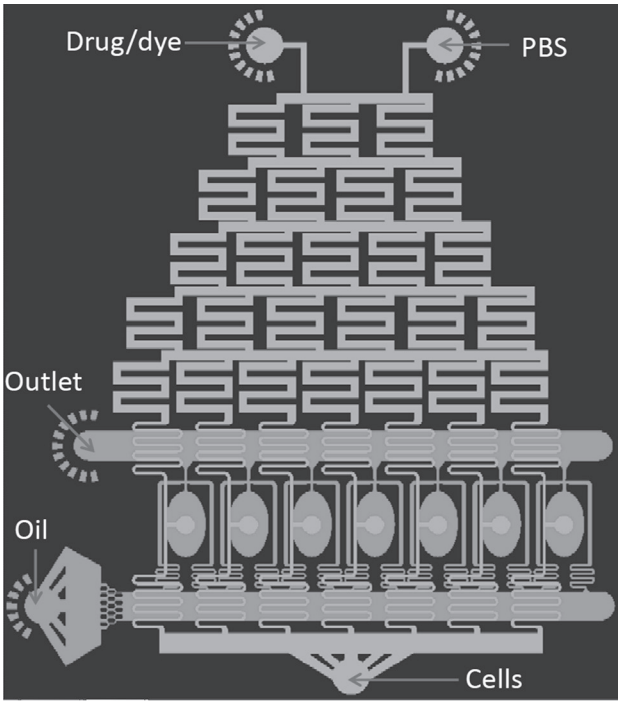

Figure 2: CAD of the bilayer PDMS microfluidic device generating droplets with varying drug concentrations.

Droplets were water-in-oil emulsions that were stabilized by a fluorinated surfactant. The aqueous media contained phosphate-buffered saline (PBS) and a hydrophilic fluorescent dye. The emulsions were formed by a continuous flow of the oil; droplet fission occurred when the continuous oil phase exerted sufficient viscous stress that caused an imbalance in the surface tension [2].

The water-in-oil emulsion occurred in a flow-focused geometry as seen in Figure 1. The manipulation of the flow rates of the PBS and dye with respect to each other determined the concentration of the dye within the droplet.

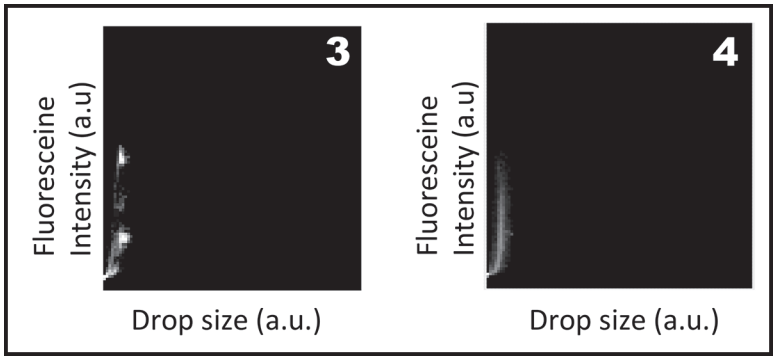

***Figure 3, left**: PMT heat map representative of same size droplets that have diffused during droplet incubation. **Figure 4, right:** PMT heat map demonstrating suppressed diffusion during droplet incubation.*

If 800 μL/s of oil was pumped with 300 μL/s of PBS and 100 μL/s of dye, then you would expect the droplets to have a relative dye concentration of 25%. We strived to generate drug concentrations that would be of interest. After emulsification, there was a serpentine region in the device that promoted mixing of the droplet contents before the mixture exited the device through the outlet and was subsequently incubated.

Figure 2 shows a computer aided design (CAD) for a bilayer PDMS microfluidic device that generates a drug gradient by serial mixing in micro-channels within one layer, and another layer for creating picoliter-sized water-in-oil emulsion where the drug at different concentrations and lymphoblast cancer cells are co-encapsulated in a droplet in parallel. In the drug concentration gradient generating device, mixing is induced by the changes in velocity created by the changes in channel widths (Bernoulli's principle) and tree branching pattern. The advantage of this device is to reliably produce same size droplets with varying concentrations all at once rather than generating varying concentration droplets independently using a co-flow device.

For developmental and experimental purposes chemotherapeutic drugs and cancer lymphoma cells were not used, because the concept has already been proven. More so the focus was to improve the reproducibility of dye concentration within droplets, size of droplets, and design of the serpentine drug concentration gradient generating device.

Results and Conclusions:

The data acquisition was driven by the PMT to identify cell state (live/dead), drug concentration in droplets, relative sizes of droplets, and presence of cell in droplet at high-throughput. A PMT heat map showed the distribution of different concentration clusters and relative sizes.

In Figure 3, there is a vertical smear indicating diffusion between the droplets. After changing surfactant and adding a greater concentration of "empty" drops, diffusion was suppressed.

A horizontal smear would be indicative of droplets lacking size uniformity. As seen in Figure 4, the diffusion was suppressed successfully by having independent clusters of varying dye concentrations. Fluorescence imaging was also used to observe and quantify dye concentration in the drops, however it is labor intensive and impractical for high throughput and the reason for using PMT.

Future Work:

Moving forward we would like to optimize flows and tree design for best mixing and reproducible concentration gradients. Using the PMT, we would like to perform time plots to observe diffusion during droplet incubation periods. Summation effects of drugs would also be interesting to observe drug synergy attacking different cell mechanisms leading to apoptosis. In addition, we would like to evaluate toxicity in healthy cells, not only drug efficacy.

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References:

[1] YC Tan, et al. Design of microfluidic channel geometries for the control of droplet volume, chemical concentration, and sorting. Lab on a chip, 4,. 292-298, 2004.

[2] Whitesides, G., et al. Basic Microfluidic and Soft Lithographic Techniques. Optofluidics: Fundamentals, Devices, and Applications, McGraw-Hill Biophotonics, 2009.