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# A Microfluidic Platform for High-Throughput Screening of Aquaporin Performance

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## 1. Introduction

Aquaporins are responsible for water transport across the cell membrane [1]. They detect osmotic differences and move water to reach homeostasis in the cell. Aquaporins are polar which helps polar water molecules flow through them [2]. Average mammalian cells do not feature a large amount of aquaporins in their cell membrane. The cells used in this study (CHO-GFP and CHO-AQP1) have been modified to promote aquaporin and GFP expression. An inducible gene expression method called Tetracycline controlled transcriptional activation was used that turns on the transcription of aquaporins or GFP in the presence of the antibiotic doxycycline. To develop a high-throughput screening platform for studying aquaporin response to osmotic stress at single cell level, we developed a microfluidic device which encapsulates single mammalian cells in picoliter droplets. Here, we demonstrate encapsulation of single CHO- GFP cells in microdroplets where their distribution is dictated by Poisson statistics.

## 2. Methods

### *2.1 Microfluidic device design and fabrication*

A microfluidic device with two oil inlets, one water/cell solution inlet, and a single outlet was designed on AutoCad (Fig. 1a). It was fabricated by photolithography and soft lithography. Photolithography was performed by using a negative photoresist called SU-8. First, a silicon wafer was cleaned. Then, 15 mL of SU-8 was spin-coated on the wafer at 3000 rpm for 30 seconds. SU-8 was then exposed to UV light at

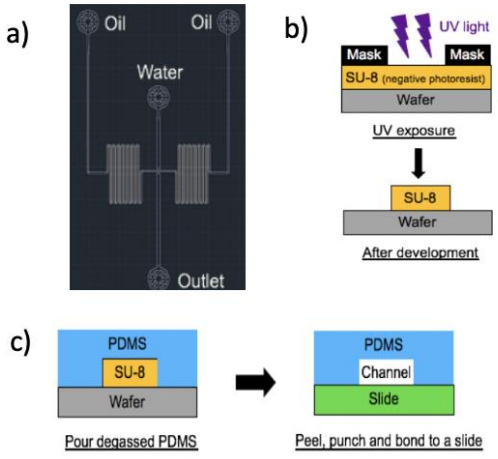


**Figure 1.** Schematics and images of the microfluidic device and fabrication. (**a**) Image of the device used for microdroplet generation. (**b**) Schematic showing the UV exposure and development of SU-8. (**c**) Schematic showing the steps of soft lithography.

215 mJ/cm$^2$ for 9 seconds using an acetate mask and hard-baked. SU-8 was then developed using PGMEA (solvent based developer) (**Fig. 1b**) and we used this mold to fabricate the microfluidic device using soft-lithography. During soft-lithography, the device was silanized for 20 minutes, rendering it hydrophobic. A PDMS mixture is prepared at 10:1 ratio and degassed using a vacuum desiccator. PDMS was poured on the wafer and it was placed in the oven for two hours at 75 ºC. PDMS was then peeled off the wafer and punched to create the inlets and outlets. Lastly, it was bonded on a slide using oxygen plasma (**Fig. 1c**). This device was used to generate droplets to encapsulate CHO-GFP cells.

### *2.2 Cell Culture*

CHO cells were cultured in 25 and 75 cm$^2$ flasks using DMEM supplemented with 10% FBS and Penicillin-Streptomycin (100 U/mL) and doxycycline (1ug/mL). The cells were then harvested by a 5-min Trypsin-EDTA (0.25%) treatment. Cells were transferred to a falcon tube with fresh medium and spun down at 100 xg for 5 minutes. Supernatant was removed and cells were resuspended in 1.2mL of 20% Ficoll+PBS. Ficoll is a non-ionic, high molecular mass polysaccharide which dissolves readily in aqueous solutions and is used to density-match growth medium. 20% Ficoll+PBS solution effectively matches the density of CHO cells and eliminates cell sedimentation, thereby facilitating efficient cell encapsulation. 20 µL of the cell suspension was pipetted into a hemocytometer, which was then imaged using a microscope to determine the cell concentration.

### *2.3 Experimental Setup*

The microfluidic device was mounted on an inverted microscope (Nikon Ti2-E) equipped with a CCD camera (Basler acA1920-155um). Two 1 mL glass syringes were filled with light mineral oil (Fisher 0121-1). A 1mL plastic syringe was filled with the cell

suspension. The glass syringes were set up on a syringe pump (Harvard Apparatus Pump 11 Elite), and the plastic syringe was set up on a separate pump (Harvard Apparatus PHD Ultra). The syringes were connected to the microfluidic device via luer-lock adapters and tygon tubing. Oil phase and cell solution was delivered at 200 and 80 µL/hr, respectively. Formation of droplets were observed through the microscope and ensuing was harvested into an Eppendorf tube for 1-2 hours.

## 3. Results

We encapsulated CHO-GFP and CHO AQP1 cells in picoliter-sized droplets. The aqueous medium was comprised of 20% Ficoll+PBS which density-matched the CHO cells, minimizing their sedimentation. Ficoll contains high molecular weight (400 kDa) polysaccharides which have low membrane permeability and low osmolality, therefore increasing the medium density with negligible change in cell size while not compromising our microfluidic osmotic stress experiments [3]. Upon encapsulation, we observed the presence of single cells inside the droplets. In order to encapsulate single cells within droplets, we adjust the initial cell concentration such that, the quantity of cells per encapsulated volume is determined by Poisson statistics [4]. This discrete probability distribution was used to calculate the probability of having *k* cells in one droplet assuming that cells are randomly dispersed in the cell solution and the flow velocity is constant. The Poisson distribution is given by the following formula:

$$P(k\,,\lambda) \;=\; \frac{\lambda^k e^{-\lambda}}{k!} \qquad (1)$$

Where $\lambda$ is the average number of cells per droplet,

$$\lambda = C * V_D \qquad (2)$$

C is the concentration of cells (cells/mL) in the solution, and $V_D$ is the volume of each droplet. By substituting $\lambda$ from **Equation (2)** into **Equation (1)**, the probability of having *k* cells in a

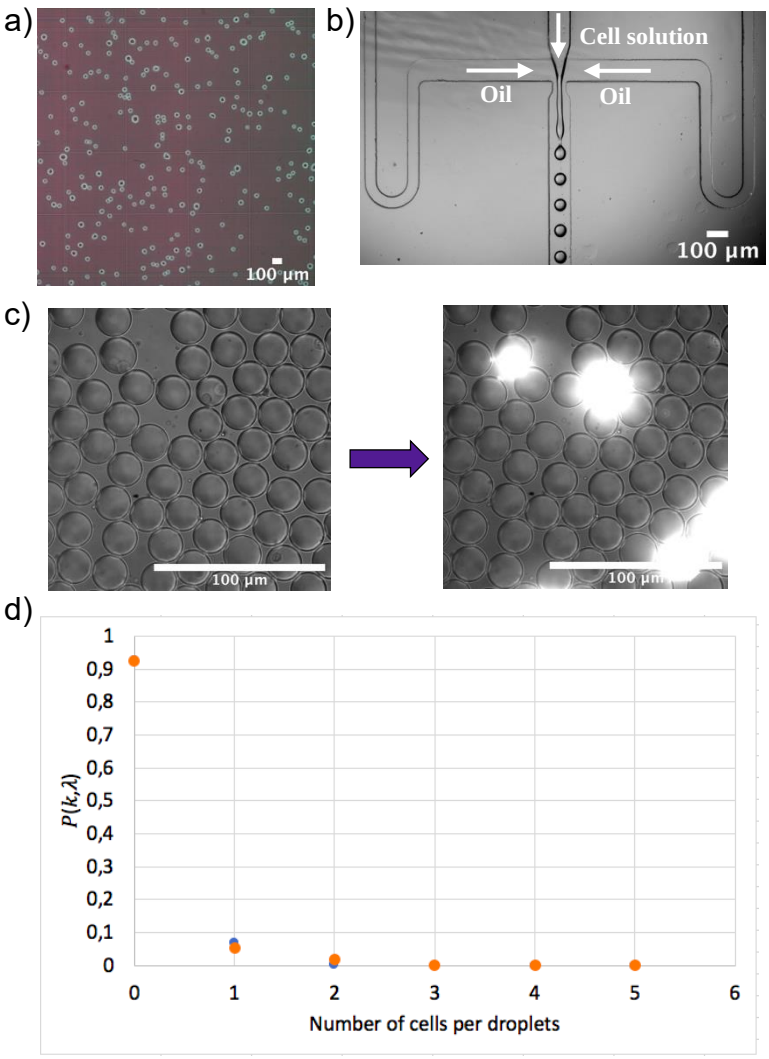


**Figure 2:** Microscopic images of the experiment and resultant graph. (**a**) Microscopic image of fluorescent cells in the hemocytometer ready for counting. (**b**) Microscopic image of the droplet generation in the microfluidic device. (**c**) Image of the microdroplets before and after fluorescence. (**d**) A plot of the probability of getting *k* number of cells per droplet based on $\lambda$.

droplet for a specific droplet size was theoretically determined. Using a hematocytometer count (**Fig.2a**), we determined the initial cell concentration as $1.8175 \times 10^6$ cells per mL. The droplets generated through the microfluidic device had a volume of 0.416 picoliters. Therefore, the average number of cells per droplet was $\lambda = 0.076$ cells per droplet. In addition, we examined a subset of the droplets generated with the device, and counted the droplets containing 0, 1, and 2 or more cells. This experimental result was facilitated by a combination of brightfield and fluorescence images from single cells encapsulated within the droplets. The droplets with CHO-GFP cells were easily identified due to the expression of a fluorescent protein (GFP) (**Fig. 2c**). The theoretical Poisson distribution (blue dots) based on initial cell concentration, and the experimental results (orange dots) obtained by imaging single droplets with encapsulated cells were combined and plotted on a graph (**Fig. 2d**). We observed that

around 91% of the droplets had no cells, around 7% of the droplets had 1 cell and 2% of the droplets had 2 or more cells. Fig.2d shows excellent agreement between theoretical and experimental results, demonstrating effective single cell encapsulation using droplet microfluidics. If the initial cell concentration is increased by a factor of 10, the probability of getting 1 cell per droplet increases to 37%. Thus, the initial cell concentration will determine the distribution of the number of cells per droplet and which will be manipulated in order to optimize single cell encapsulation.

## 4. Conclusion

Here, we successfully demonstrated encapsulation of single CHO cells towards developing a high throughput screening method for aquaporin performance. Our ultimate goal is to study aquaporins performance in single cells subjected to osmotic stress. Single cells can easily be compartmentalized into picoliter-sized droplets where cellular response to osmotic stress can be studied at the single cell level. We encapsulated single cells within picoliter-sized droplets and demonstrate that the encapsulation process follows a Poisson distribution.

This novel platform allows for studying aquaporin performance and cell response to osmotic shocks in a higher throughput and high-resolution format. Our research will focus on combining this method with microfluidic sorting techniques to isolate single cells with the desired osmotic response. Our platform can also be used to provide insights into single cell responses in many other bioengineering applications.

## 5. References

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