Enhancing Cell Line Development with Picodroplet Technology

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Introduction

- **Single cell analysis** is a critical component of **monoclonal antibody (MAb) production.**
- It can be achieved through costly, challenging and time consuming processes, *e.g.* FACS, limiting dilution or automated clone-picking devices.
- We present a novel platform, **Cyto-Mine™**, that enables **ultra-high throughput analysis and sorting** of single cells and the detection of their secreted products in miniature (pL to nL) volumes called **picodroplets**.
- Using Chinese Hamster Ovary (CHO) and hybridoma cells, we demonstrate that **Cyto-Mine™**:

(1) improves viability of single cells encapsulated in picodroplets over that of traditional FACS sorting.

(2) allows accurate measurement of compartmentalised Abs secreted from single encapsulated cells within 1- 2 hours.

Examples of Applications:

Biopharmaceutical discovery:

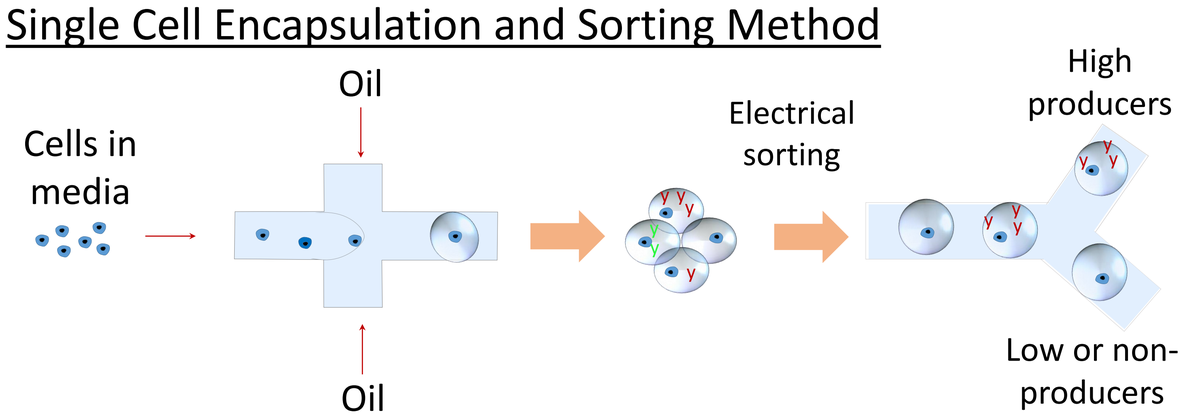
- Antibody (transcript) discovery from primary plasma cells, B-cells or hybridomas.

Bioprocessing:

- Enables measurement and isolation of clones that are high expressors of MAbs.

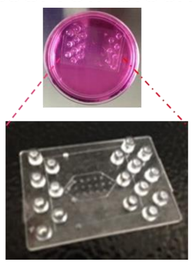
Principle and Methods

Single Cell Encapsulation and Sorting Method

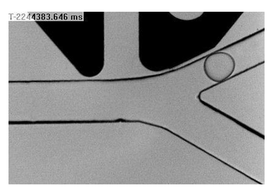


Picodroplet generation biochip

Microfluidic reservoir biochip



Microfluidic sorting biochip

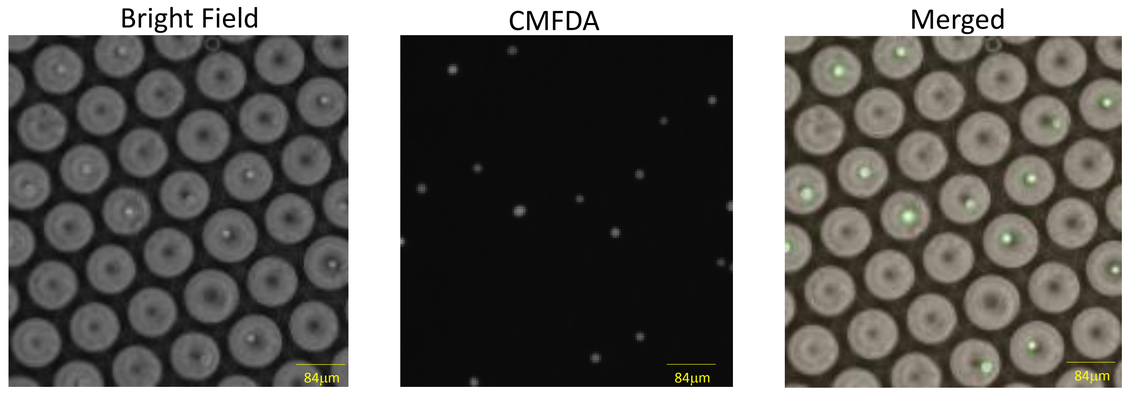


Experimental Procedures

To generate single cell containing picodroplets, liquids such as fluorinated oil Novec-7500 containing 5% (v/v) Pico-Surf™ 1 surfactant and cell suspension are injected into the channels of the picodroplet generation biochip. The flow rates of the liquids are controlled by syringe pumps. For example, to generate 300 pL picodroplets, the flow rates for the oil and the cell suspension are 1000 µL/h and 1400 µL/h respectively. With these conditions, we can generate **3.6 million picodroplets** per hour. Picodroplets are then sorted based on fluorescence, which correlates with the amount and type of antibody produced by each single cell.

Results

Encapsulation of CHO-S cells



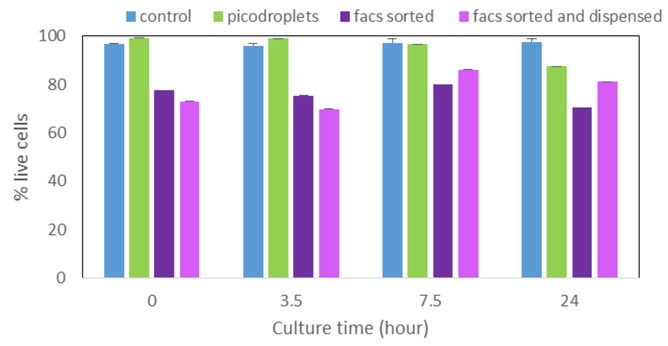
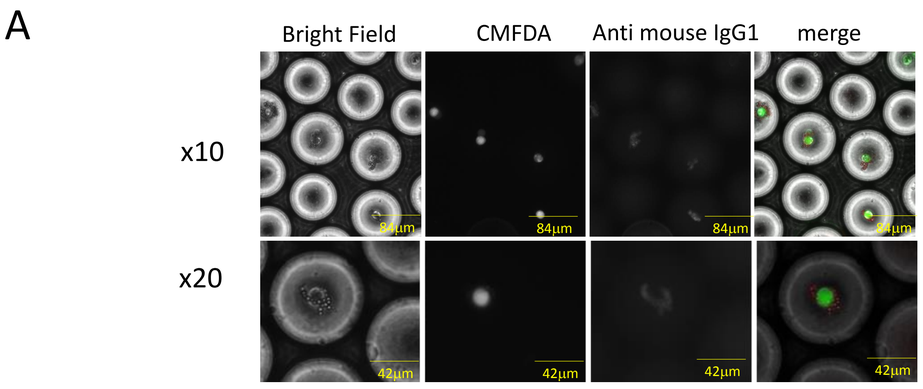
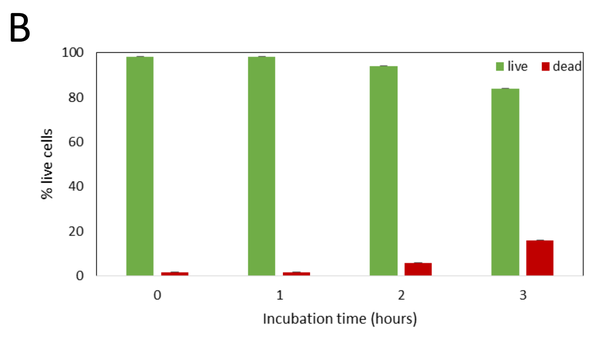


Figure 1: CHO-S were co-stained with 0.2 µM Cell Tracker™ (CMFDA, green cells) and 3 µM DRAQ7™. Stained cells were introduced to a dedicated microfluidic biochip to generate 300 pL picodroplets or were sorted using a BD FACSAria™ III cell sorter. The picodroplets or the sorted cells were then incubated at 37°C for the indicated time. Viability was assessed by detecting the ratio between green and red cells using fluorescence microscopy.

Detection of Ab secretion in picodroplets





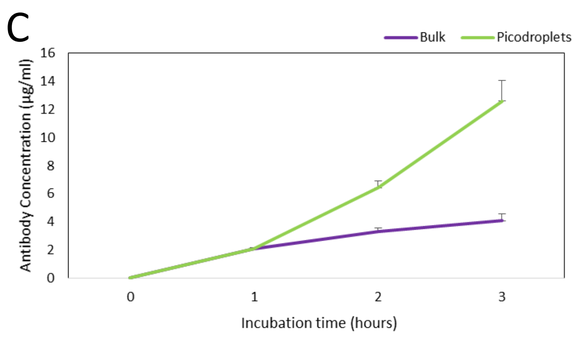


Figure 2: A) Hybridoma cells were stained with 0.2 µM Cell Tracker (CMFDA, green cells). After staining, the cells are mixed with anti-IgG kappa chain antibody conjugated to carboxyl latex beads and Alexa Fluor®680 Goat anti- Mouse IgG1 (γ1). Stained cells were introduced to a dedicated microfluidic biochip to generate 700pL picodroplets.
B) Same as A, except cells were also stained with 3 µM DRAQ7™. The picodroplets were then incubated at 37°C for the indicated time. Images were obtained by fluorescence microscopy.
C) Antibody secretion was assessed via Easy-Titer Mouse IgG Assay Kit from Pierce.

Conclusions

Cyto-Mine™ is an alternative technology, to isolate single cells with higher recovery rates than flow cytometry, perform assays on trapped, secreted Abs and sort single cells. The throughput of 3.6 million picodroplets per hour (~180,000 cells per hour) is superior to automated clone pickers (cited as processing 10,000 clones over a 3 week period). A fully automated, integrated Cyto-Mine™ with novel assay functionality (*e.g.* cell-cell co-incubation) is now being developed.

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References

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