Enhancing monoclonal antibody production with picodroplet technology.

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Introduction

Single cell cloning is a crucial step in **monoclonal antibody production**. It can be achieved through costly, challenging and time consuming processes, *e.g.* FACS or limiting dilution.

We present a novel platform, **Cyto-Mine®**, that enables **ultra-high throughput sorting** of single cells and the **detection of their secreted products** in miniaturised (pL to nL) volumes called **picodroplets**. Using Chinese Hamster Ovary (CHO), we demonstrate that **Cyto-Mine®**:

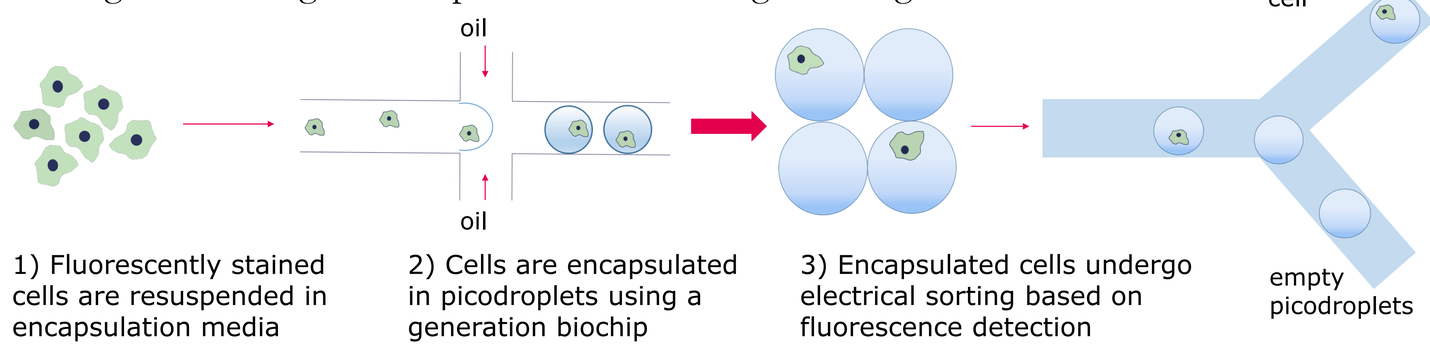
1. Improves viability of single cells when encapsulated in picodroplets compared to traditional FACS sorting.
2. Allows accurate measurement of antibody secretion levels from encapsulated single cells within 1-2 hours.

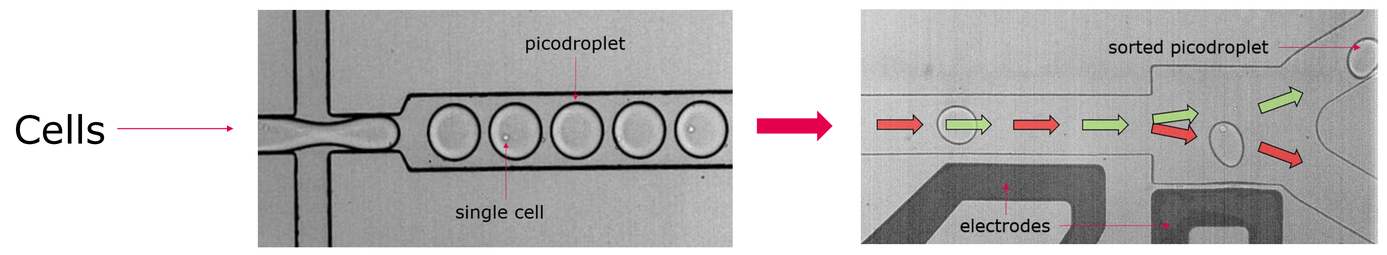
There are many applications for this technology, including:

- **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 antibodies.

Materials and methods

Flow diagram showing the encapsulation and sorting of a single cell:





To generate picodroplets containing a single cell, **Pico-Surf™ 1, 5% in Novec 7500** (surfactant dissolved in fluorinated oil) and cell suspension are injected into the channels of the picodroplet generation biochip. The flow rates are controlled by syringe pumps. To generate **300 pL** picodroplets, the flow rates for the cell suspension and oil are 1000 µL h^{-1} and 1400 µL h^{-1} respectively. With those conditions, we can generate **3.6 million picodroplets** per hour. The picodroplets are then sorted based on fluorescence, which correlates with the amount of antibody produced by each single cell.

Results

Figure 1: Poisson distribution of encapsulated CHO cells over time.

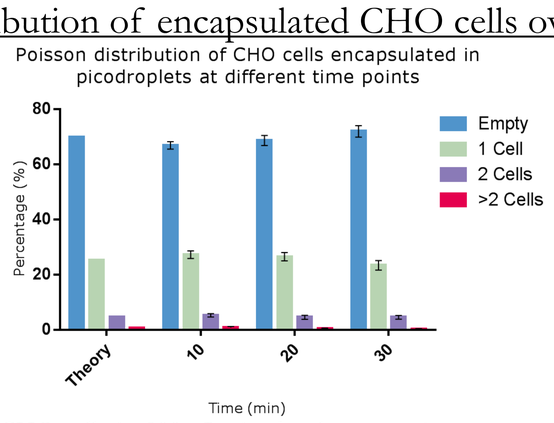


Figure 1: Poisson distribution of CHO cells in 300 pL picodroplets over time compared to the theory. Cells were stained with 0.5 µM Calcein AM, and 300 pL picodroplets were generated with aliquots collected at dedicated time points. Images were taken with a fluorescent microscope and analysed visually.

Figure 2: Viability and recovery of CHO cells after sorting.

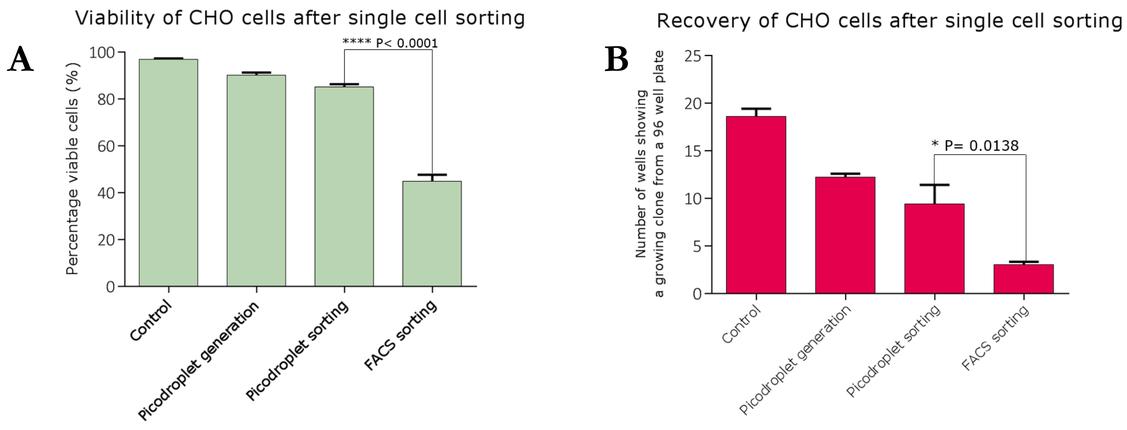


Figure 2: Viability and recovery of CHO cells compared to FACS sorting. **A)** CHO cells were co-stained with 0.2 mM Cell Tracker™ Green CMFDA and 3 mM DRAQ7™. Stained cells underwent single cell sorting (as described in materials and methods), or were sorted using a BD FACSAria™ III cell sorter. Cell viability was assessed by detecting the ratio between green and red cells using fluorescent microscopy. **B)** Cells were not stained, and underwent single cell sorting (as described in materials and methods), or were sorted (and dispensed) using a BD FACSAria™ III cell sorter. The cells were then serial diluted to statistically give 1 cell per well, and plated on a 96 well plate. After approximately 4 weeks, visible clones were counted.

Figure 3: Detection of antibody secretion from CHO cells in picodroplets.

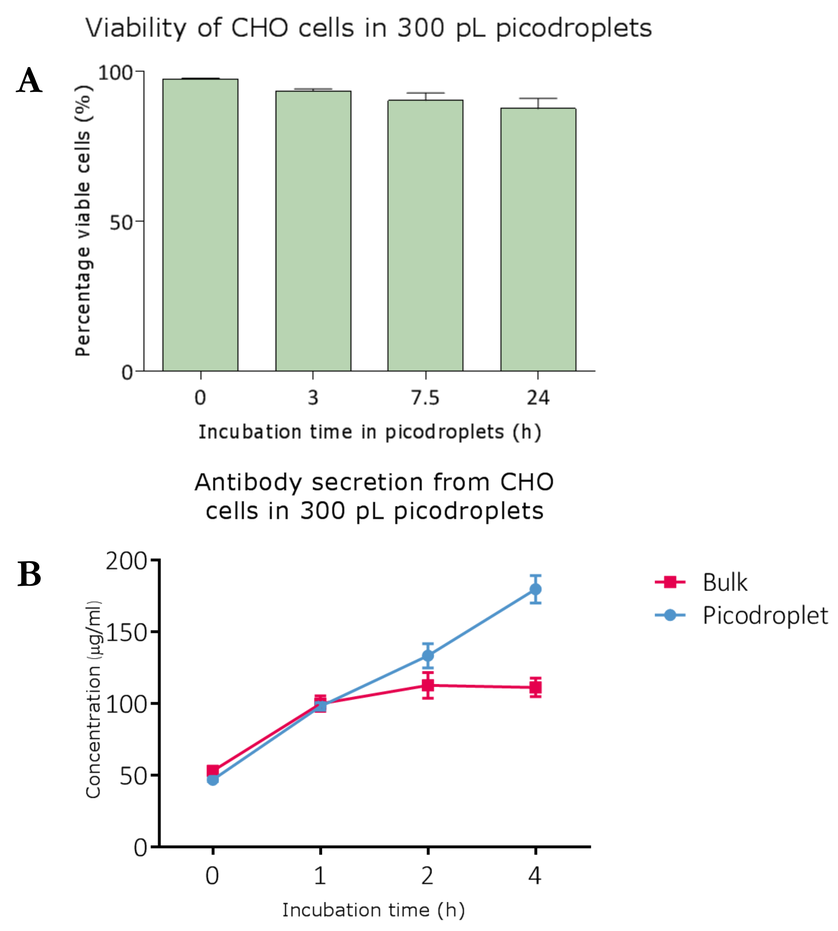


Figure 3: Long term viability of CHO cells in 300 pL picodroplets, and the amount of antibody secretion from CHO cells in 300 pL picodroplets compared to bulk. CHO cells were co-stained with 0.2 mM Cell Tracker™ Green CMFDA and 3 mM DRAQ7™. **A)** After picodroplet generation the encapsulated cells were incubated at 37 °C for the indicated amount of time before viability was assessed. Cell viability was assessed by detecting the ratio between green and red cells using fluorescent microscopy. **B)** CHO cells were encapsulated in the presence of Goat anti-human IgG Fc DyLight 488 and Goat anti human (Fab')2 DyLight 594. After picodroplet generation the encapsulated cells were incubated at 37 °C for the indicated amount of time. Antibody secretion was measured using the SpectraMax® i3 Multi-Mode Detection Platform from Molecular Devices.

Conclusion

We showed that Cyto-Mine® is an alternative technology to perform single cell cloning with statistically significantly higher recovery rates than flow cytometry. It can also be used for high-throughput screening of antibody-secreting cells enabling dramatic savings in consumable costs and improved assay sensitivity.

References

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