Enhancing monoclonal antibody production with picodroplet technology.

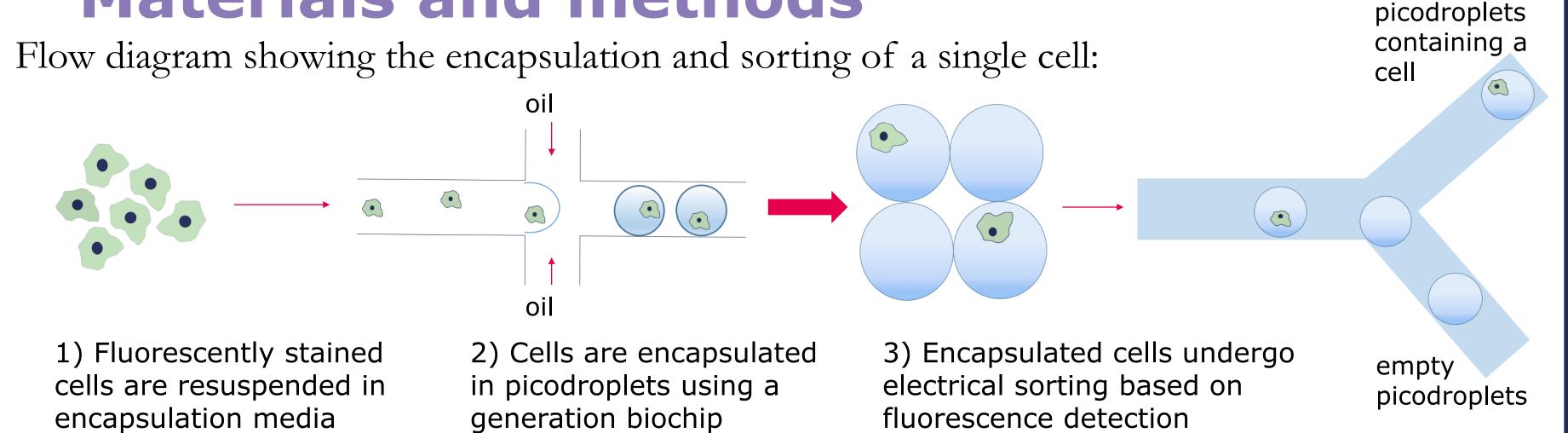
Tara-Jane Bloomfield-Gerdes, Giuseppe Benazzi, Karine Enesa Sphere Fluidics Limited, The Jonas Webb Building, Babraham Research Campus, Cambridge, CB22 3AT, UK

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**®: Improves viability of single cells when encapsulated in picodroplets compared to traditional FACS sorting. Allows accurate measurement of antibody secretion

Materials and methods



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.

encapsulation media picodroplet sorted picodrople Cells single cell electrode

To generate picodroplets containing a single cell, Pico-SurfTM 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

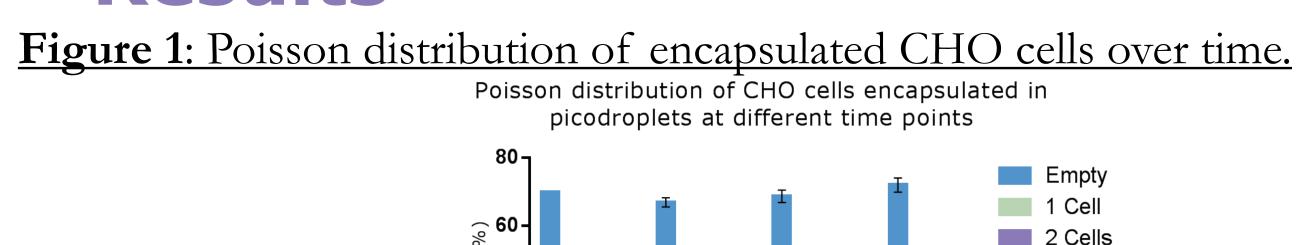
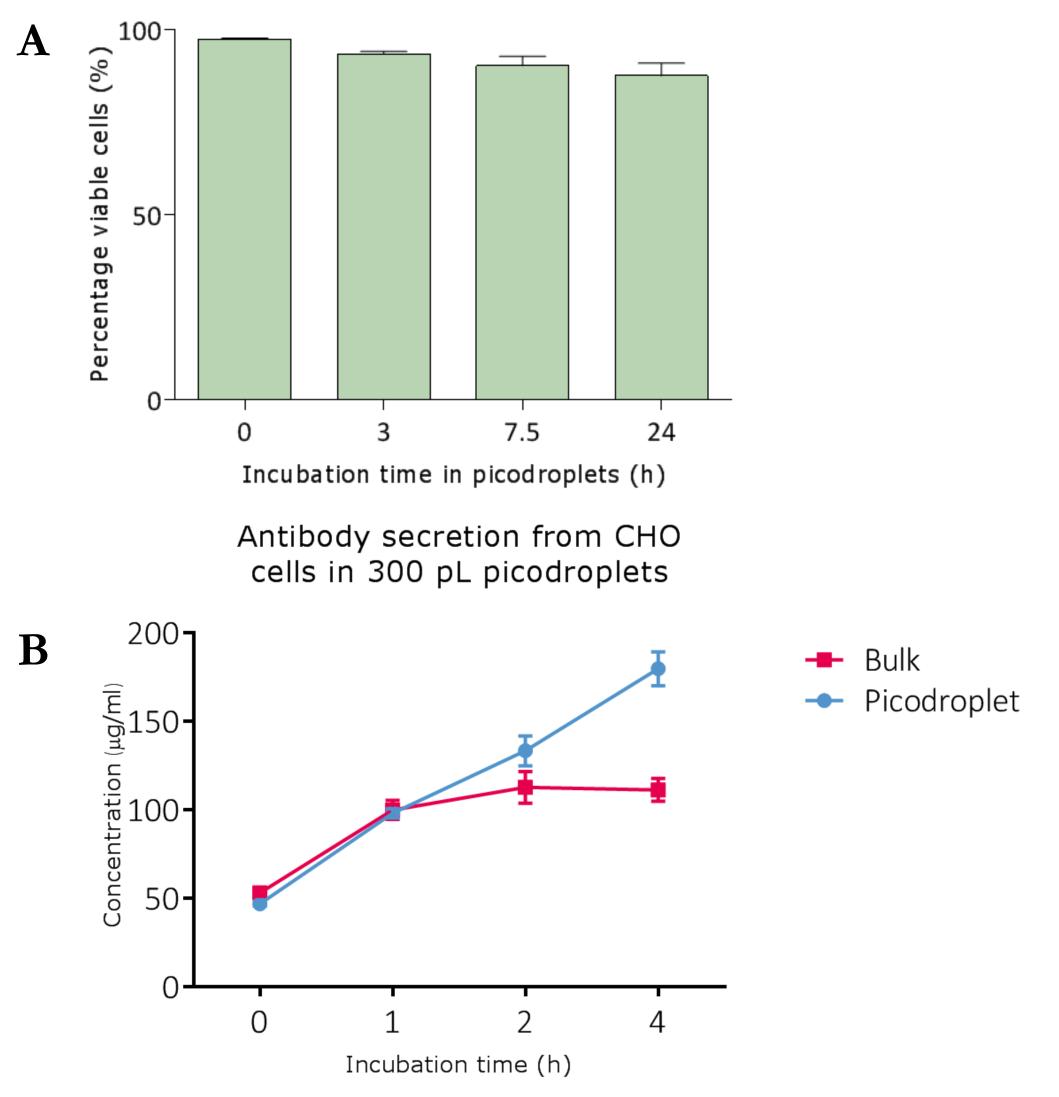
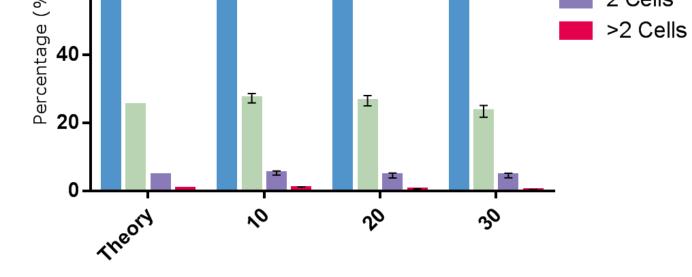


Figure 3: Detection of antibody secretion from CHO cells in picodroplets.







Time (min 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.

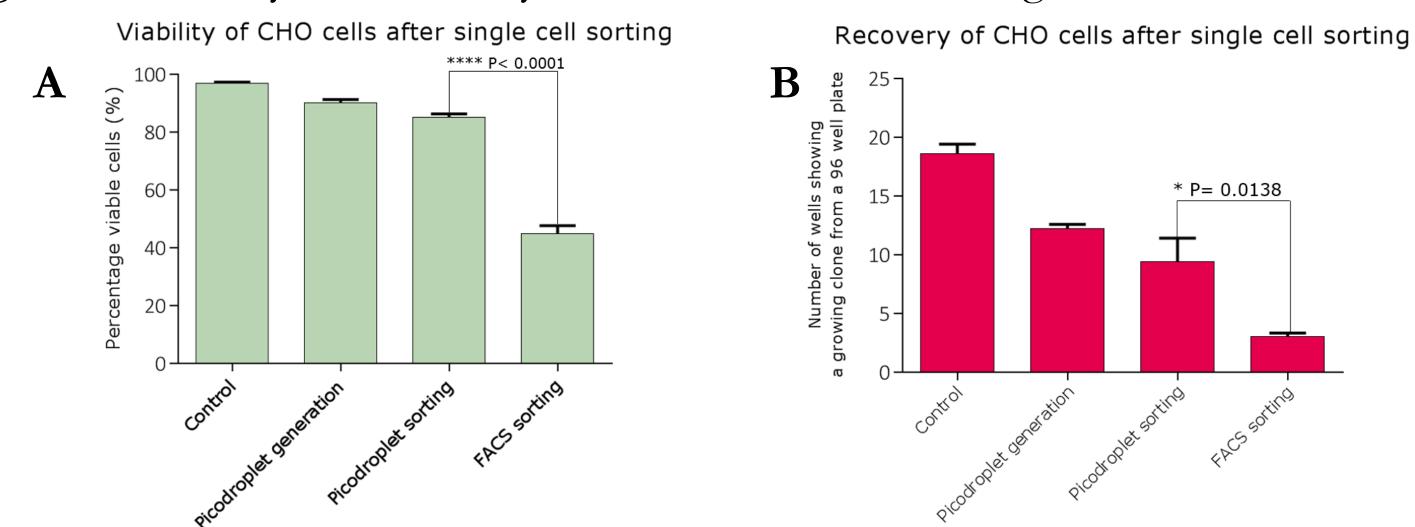


Figure 2: Viability and recovery of CHO cells compared to FACS sorting. A) CHO cells were co-stained with 0.2 mM Cell TrackerTM Green CMFDA and 3 mM DRAQ7TM. Stained cells underwent single cell sorting (as described in materials and methods), or were sorted using a BD FACSAriaTM III cell sorter. Cell 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 FACSAriaTM III cell sorter. The cells were then serial

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 TrackerTM Green CMFDA and 3 mM DRAQ7TM. 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

Chokkalingam *et al.*, 2013, *Lab Chip*, **13**, 4740-4744. Smith et al., 2013, Anal. Chem., 85 (8), 3812–3816. Mazutis et al., 2013, Nature Protocols, 8, 870–891. Debs et al., 2012, PNAS, 109 (29), 11570–11575.

Contact

Dr Karine Enesa (Cell & Molecular Biology): Karine.Enesa@spherefluidics.com

www.spherefluidics.com **Tel:** +44 (0)1223 804200

