Going with the flow: cell line development meets microfluidics

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Abstract

Here, we present a microfluidics-based process for assurance of monoclonality of biopharmaceutical cell lines. Cell line development (CLD) culminates in the production of cell lines which express high yields of recombinant proteins with the required product quality attributes in order to satisfy clinical and commercial needs[1-2]. In addition, to fulfil regulatory and technical requirements, manufacturing cell lines have to be demonstrated to be derived from a single progenitor cell line[1,3]. Cloning methods using limiting dilution or the isolating colonies from semi-solid medium (ClonePix FL) require two rounds of cloning to achieve a high probability of monoclonality. The aim of this collaborative project is to develop and validate an innovative microfluidic device to transform the CLD process to having a single round of cloning whilst maintaining a high probability of monoclonality. The new process will enable production of monoclonal cell lines with greater speed and efficiency.

Introduction

- A series of microfluidic biochips and associated hardware which, when integrated, will enable the user to encapsulate and detect single cells within picodroplets and subsequently dispense these picodroplets into plates. This will be the first commercially available microfluidic instrument for a cell line development application.
- Single cell encapsulation technology has been shown to have a low impact on cell viability and productivity[4-6].
- A microfluidic cell cloning process has the advantage of decreased reagent costs, the use of disposable and sterilisable components, improved efficiency whilst achieving high assurance of monoclonality and the potential to couple with analytical technologies.

Microfluidic Design

Microfluidics-based cloning workflow

Figure 1 shows the workflow for the microfluidic cell cloning process. Cells are stained with a green fluorescent dye and combined with oil (Pico-Surf™1, 3% in Novec 7500) at point A. Encapsulated cells are detected based on fluorescence intensity and subjected to voltage pulses at a sorting junction (point B). Empty picodroplets are sent to waste whilst occupied picodroplets are dispensed into plates at point C.

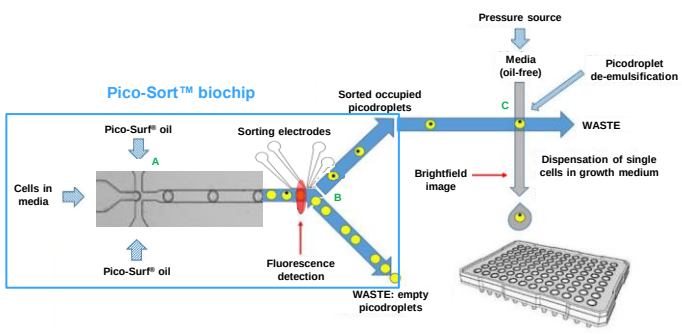


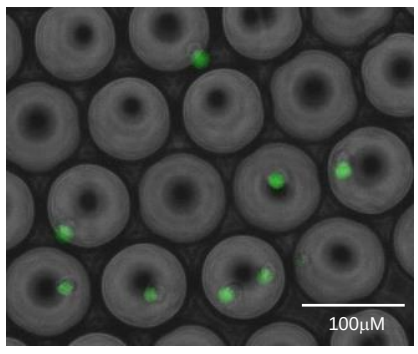
Figure 1: Schematic of the microfluidic process.

A combined generation and sorting polydimethylsiloxane/glass biochip (Pico-Sort™) has been used to generate results for this study.

Results

Cells are successfully encapsulated within picodroplets

Figure 2 shows cellular occupancy within picodroplets, which accurately follows Poisson statistics. Empty picodroplets do not fluoresce and are sent to waste channels upon sorting.



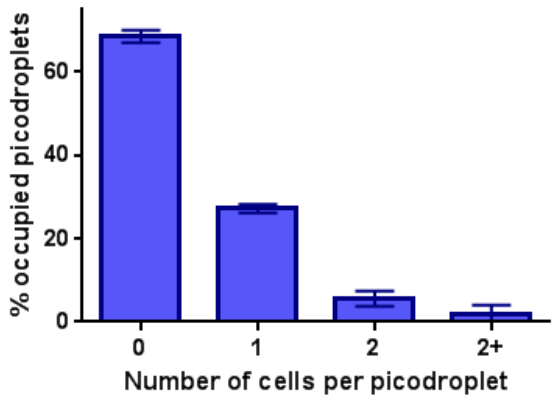


Figure 2: Left: Image showing collected picodroplets 30 minutes after encapsulation. CHO cells expressing a mAb109 monoclonal antibody are stained with 0.2µM calcein-AM. Right: Distribution of cells in picodroplets (error bars signify SEM).

High cell viability is maintained after microfluidic sorting

Cell viability was measured by staining cells with 0.5µM calcein-AM and 3µM DRAQ7. Green (ex 495nm) and red (ex 633nm) fluorescent images were captured and live/dead cells counted using ImagePro software. Cell viability is not affected by the microfluidic process (Figure 3). FACS sorting does have a small effect on post-sort viability.

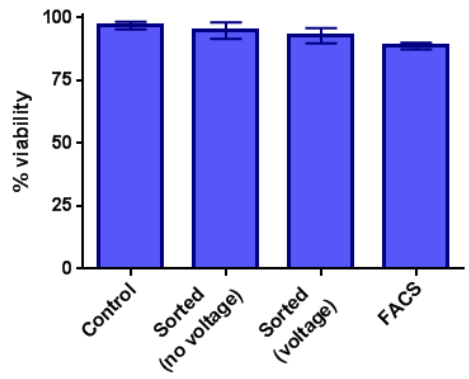


Figure 3: Cell viability post-sort. Cells subjected to microfluidic manipulation were extracted from picodroplets and stained with calcein-AM (0.5µM) and DRAQ7 (3µM). FACS samples were stained as above and sorted using FACSAria III (BD). Control cells were prepared as per microfluidic samples but not microfluidically processed. Error bars signify SEM.

Cell recovery is reduced but sufficient following microfluidic sorting

Recovery of microfluidic-sorted cells (outgrowth in wells of a plate) is reduced compared with the non-microfluidic control and is better than recovery following FACS sorting (Figure 4). Microfluidic sorting using the current voltage parameters results in outgrowth from approximately 4.8% of wells (±2%) when cells are seeded at 0.9 cells per well. Although the sorted cells show a lower recovery rate than control cells, the numbers of recovered cells were still sufficient for further manipulation.

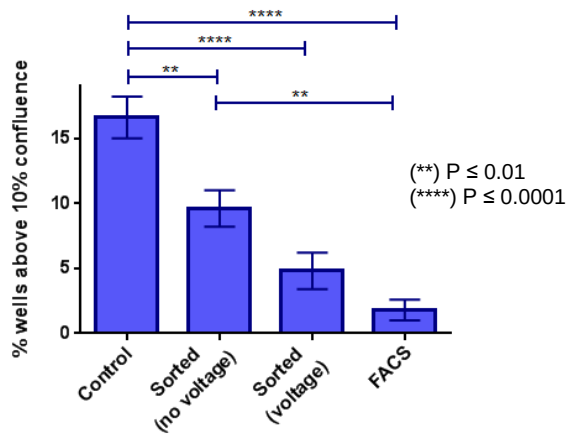


Figure 4: Colony outgrowth in 96-well plates, 29 days post-sort for non-sorted (control), microfluidic and FACS processes. Error bars signify SEM and data was analysed using 1-way ANOVA.

Product titre is not significantly affected by the microfluidic process

Supernatants were harvested from post-sort confluent wells and subjected to octet analysis to determine product titre. As demonstrated in Figure 5, passage through microfluidic channels does not impact cellular productivity.

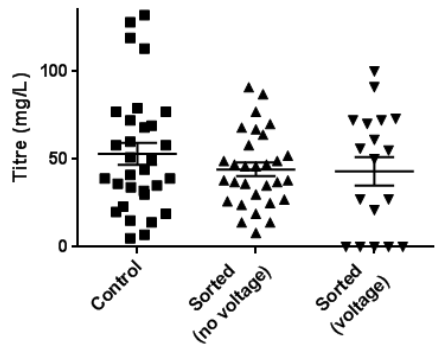


Figure 5: Post-sort titre analysis - days 29-31 (with group mean and SEM). Control cells were prepared as per microfluidic samples but not microfluidically processed.

Conclusions and Future Work

- A system has been designed for encapsulation, sorting and delivery of single cells within oil-based picodroplets for application in biotherapeutic cell line development.
- Cells sorted using this microfluidic system maintain high viability and consistent product titre and only require a single round of cloning.
- Plate recovery rates for sorted cells could be improved through modulation of voltage parameters and further optimisation of growth media.

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