Introduction:
T-cell immunotherapies show considerable promise in treating various challenging diseases such as blood cancers. These therapies utilise the body’s immune system to destroy tumour cells by collecting a patient’s own T-cells and modifying them to target cancer cells, by transferring DNA into the cells using viral vectors. The vector transfer efficiency and its consistency are critical to the final (cell) product quality and cost.

We have engineered new microfluidic-based technologies to improve the efficiency of transferring DNA into cells.

Picodroplet technology:
The aim of this study was to evaluate the potential of Sphere Fluidics’ picodroplet technology in a model workflow for cell therapy. Encapsulating cells and viral vectors in microfluidic picodroplets allows a high degree of control over cell and virus numbers for transduction. This potentially provides an advantage over conventional bulk transduction in terms of efficiency and viral copy integration number.

Results 1: Jurkat cells are efficiently transduced in picodroplets

1) Cells and a GFP-encoding lentivirus are encapsulated together in picodroplets.
2) The resulting emulsion is collected and stored off-chip at 37°C for 2-4h, during which transduction occurs.
3) Encapsulated cells are released from picodroplets and incubated in cell culture plates for 7 days.
4) Transduction efficiency is assessed by determining GFP expression on a Nucleocounter® NC-3000® Image Cytometer and compared to conventional transduction performed in bulk.

Transduction efficiency was assessed at decreasing virus concentration (MOI) while keeping picodroplet size constant. Transduction efficiency in pico droplets was higher than controls at all MOIs tested. Longer incubation in pico droplets resulted in increased transduction efficiencies. At low MOIs the beneficial effect of pico droplets was more pronounced.

The effect of pico droplet size was investigated at a constant MOI of 0.1. At the lowest pico droplet volume tested (300 pL), stimulation of transduction was strongest (>2-fold), probably due to a closer confinement of cells and viral particles inside the pico droplet.

Results 2: Human T-cells require small cell populations for efficient transduction in pico droplets

Transduction efficiency in human T-cells was assessed under conditions optimised for Jurkat cells. Two different T-cell activation reagents (ImmunoCult™ and TransAct™) were tested and co-encapsulated with cells and virus.

Unlike with Jurkat cells, we observed considerably lower transduction efficiencies in pico droplets, possibly caused by inefficient T-cell activation in the single cell scenario.

In larger pico droplets, at higher cell occupancy, transduction efficiencies in pico droplets are comparable to levels obtained with transduction in bulk, indicating efficient activation of T-cells (TransAct™ used for activation in all cases).

At lower MOI, transduction in pico droplets appears to perform better than bulk transduction again, suggesting a plateau effect at MOI=1.

Conclusion:
Two pico droplet microfluidic-based approaches were tested for viral transduction of Jurkat T-lymphoma and primary human T-cells - at the single cell level in pL volumes of fluid, or with small population of cells using larger volumes (up to mL size). While the model Jurkat cell line was efficiently transduced at the single cell level, primary human T-cells required small cell populations in pico droplets to achieve levels of transduction similar to or better than conventional methods. These new technologies will provide users and manufacturers with useful information and precision needed to develop better cell therapy products.