Flowfect™ Transfection of iPSCs Maintains Cell Pluripotency

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OBJECTIVE

Both allogeneic and autologous iPSC-based therapies are becoming a reality. The ability of iPSCs to differentiate into numerous cell types and to proliferate indefinitely make these cells ideal candidates for gene therapy. However, hurdles in transiting iPSC-derived therapies to the clinic can include high toxicity and loss of pluripotency after editing. We developed *Flowfect*™, a non-viral process that combines continuous fluid flow with electric fields, to transfect iPSCs. *Flowfect*™ transfection of eGFP-mRNA in adherent iPSCs resulted in highly functional cells which maintained stem cell lineage markers and differentiative properties.

Why *Flowfect*™ – Kytopen's non-viral *Flowfect*™ technology is identical at the research and manufacturing scales

Click to zoom in

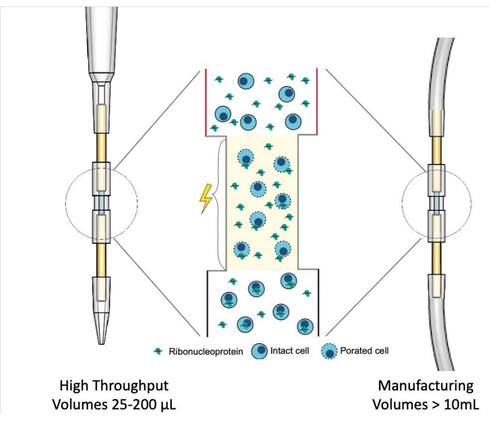

Figure 1: Schematic of our continuous flow non-viral *Flowfect*™ platforms. Briefly, cells & payload are suspended in our proprietary buffer. As the cells & payload flow through a channel they are exposed to low energy electrical pulses to induce transient cell membrane disruption and simultaneous delivery of mRNA or RNPs into the cells. Transfected cells are then dispensed directly into growth media for cell recovery. The same technology is used in our high throughput research platform (Array) and our manufacturing platform (Tx).

Rapid optimization – T cell editing represents a high value cell type vital to the advancement of cell therapy

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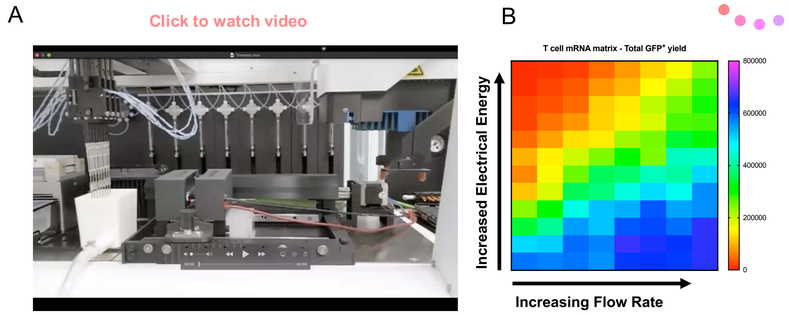

Figure 2: *Flowfect*™ high throughput Array platform can be used to optimize primary T cells. Primary T cells were transfected with mRNA encoding GFP using the *Flowfect*™ Array platform (A). After 24 hours, the cells were analyzed for GFP positive yield (GFP⁺ live cell counts) to determine optimal transfection conditions (B).

Optimization can utilize multiple parameters – *Flowfect*™ transfection of iPSC works at multiple cell densities

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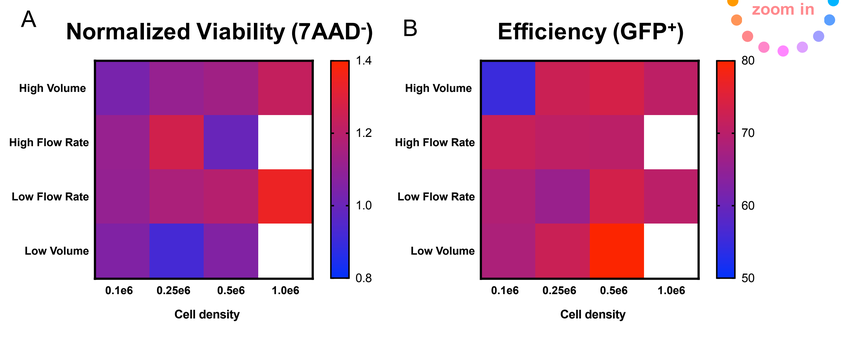

Figure 3: Adherent iPSCs transfection using *Flowfect*™ technology. Human iPSC were transfected with mRNA encoding GFP using the *Flowfect*™ Array platform. After 96 hours, the cells were lifted and analyzed for A) 7AAD⁻ viability (normalized to no transfection controls) and B) GFP⁺ efficiency. Data points on heatmap are shown as Averages (n=6).

Transfection with optimized conditions – *Flowfect*™ transfected iPSC can be lifted as early as 48 hours

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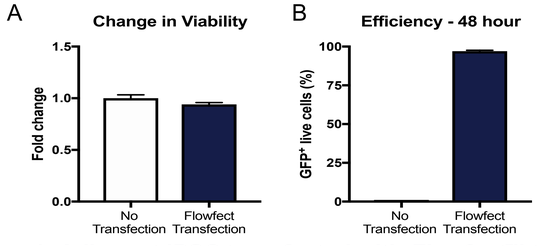

Figure 4: Adherent iPSC transfected with *Flowfect*™ technology show no loss of viability at 48 hours. iPSC were transfected with mRNA encoding GFP using the *Flowfect*™ platform. After 48 hours, the cells were lifted and analyzed for A) 7AAD⁻ viability and B) GFP⁺ efficiency. Graphs are shows as Mean±SEM (n=5).

Pluripotency preserved after *Flowfect*™ transfection – iPSC retain markers and morphology after transfection

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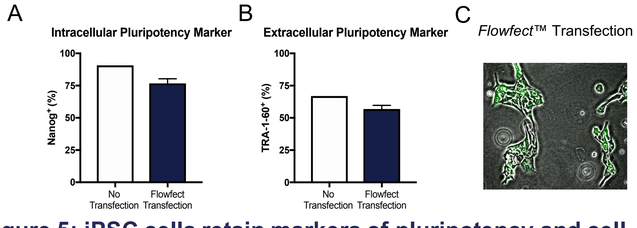

Figure 5: iPSC cells retain markers of pluripotency and cell morphology after *Flowfect*™ transfection. iPSC were transfected with mRNA encoding GFP using the *Flowfect*™ platform. After 48 hours, the cells were lifted and analyzed for A)internal (Nanog) and B) extracellular (Tra-1-60) lineage marker expression. Graphs are shown as Mean±SEM (n=5). C) Microscope images (10x) are shown as GFP/bright field overlay.

iPSC retain their differentiative capability after *Flowfect*™ transfection – *Flowfect*™ iPSC exhibit preserved function

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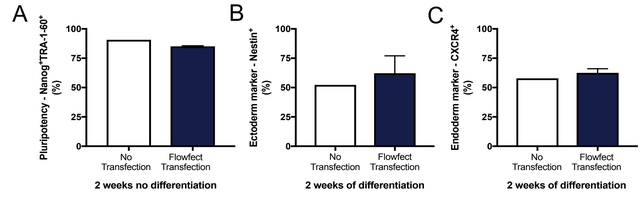

Figure 6: *Flowfect*™ transfected iPSC retain the ability to differentiate into ectoderm and endoderm. Adherent iPSC transfected with mRNA encoding GFP using the *Flowfect*™ platform. After 24 hours, the cells were cultured for 2 weeks in A) standard media (MeTeSR) or STEMdiff media for B) ectoderm and C) endoderm differentiation. Graphs are shown as Mean±SEM (n=3).

CONCLUSION

Compelling transfection data confirms that the Flowfect™ technology will enable rapid optimization of iPSC-derived therapeutic innovations without loss of function.