

Enhancing Non-viral Engineering of Cells for Cell Therapy Manufacturing – Optimizing Yields for Peak Cell Quality with the Flowfect Tx™ Platform

A Comparative Study Conducted by the Cell Therapy CDMO Services Group at Charles River International, Inc

OBJECTIVES

Here we present a head-to-head study conducted by Kytopen and an independent, world-class CDMO, comparing Kytopen’s Flowfect Tx™ platform with two leading, commercially available electroporation platforms (denoted here as Competitor A and B). Specifically, a 10-day gene knockout study (Cas9 RNP targeted to TRAC locus) was performed with primary T cells using manufacturer recommended settings but without further optimization. Knockout efficiency, cell viability, and cell expansion were measured in this study.

RESULTS

Upon completion of side-by-side testing of the platforms, the following results were observed.

Cell Viability: Measured on days 2 (pre-transfection), 4, 7, and 10.

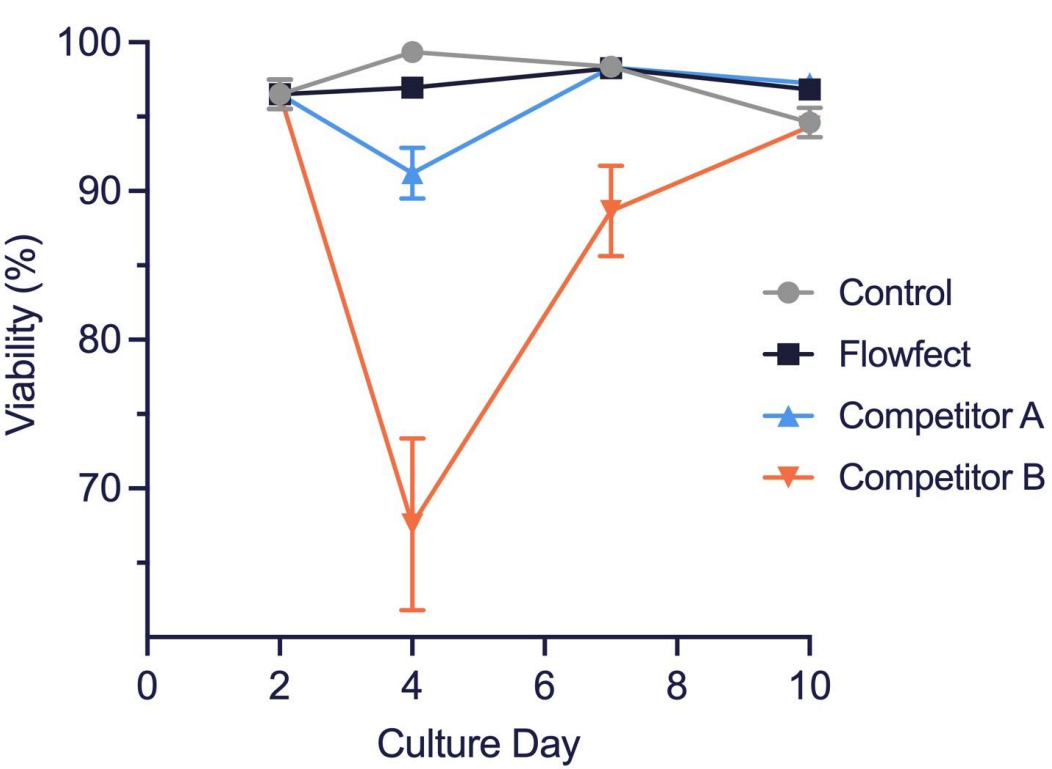


Figure 1: % Viability. The cell viability for each condition was sampled on culture days 2 (prior to EP), 4, 7, and 10 (at harvest). Data shown = mean+/-SD for n = 2 donors.

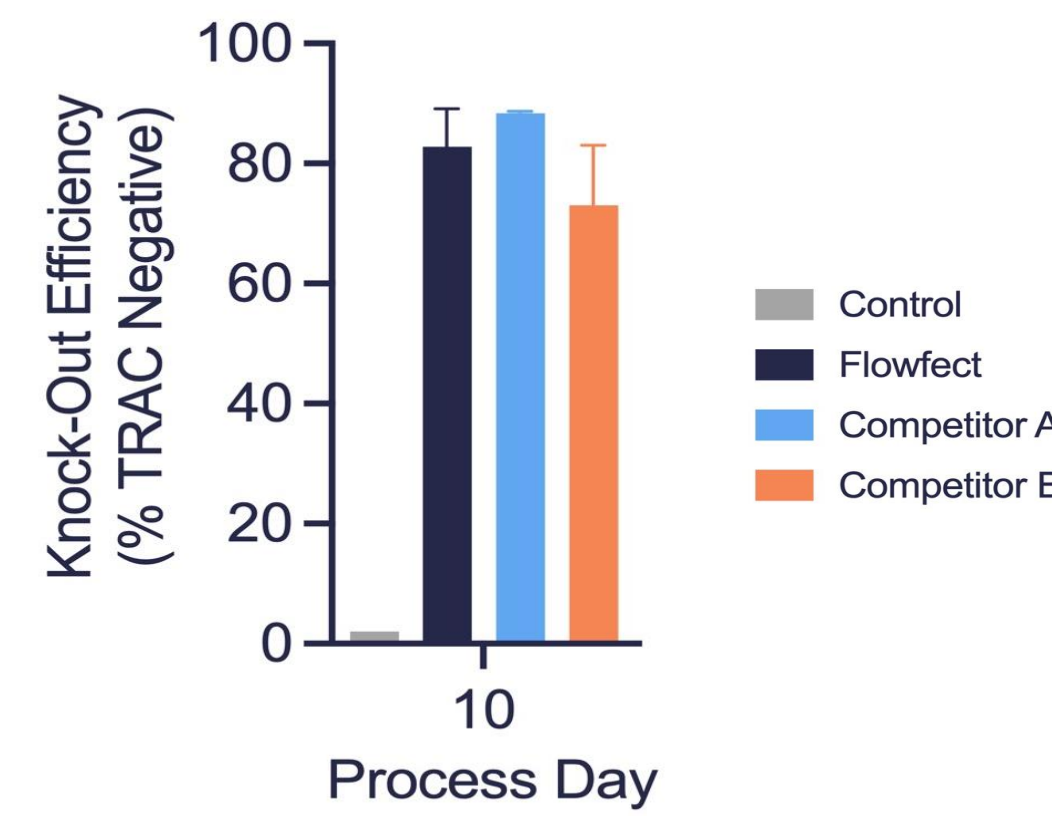


Figure 2: Knock-Out Efficiency. % TRAC Negative cells for each EP device was assessed using flow cytometry on day10. Data shown = mean+/-SD for n = 2 donors.

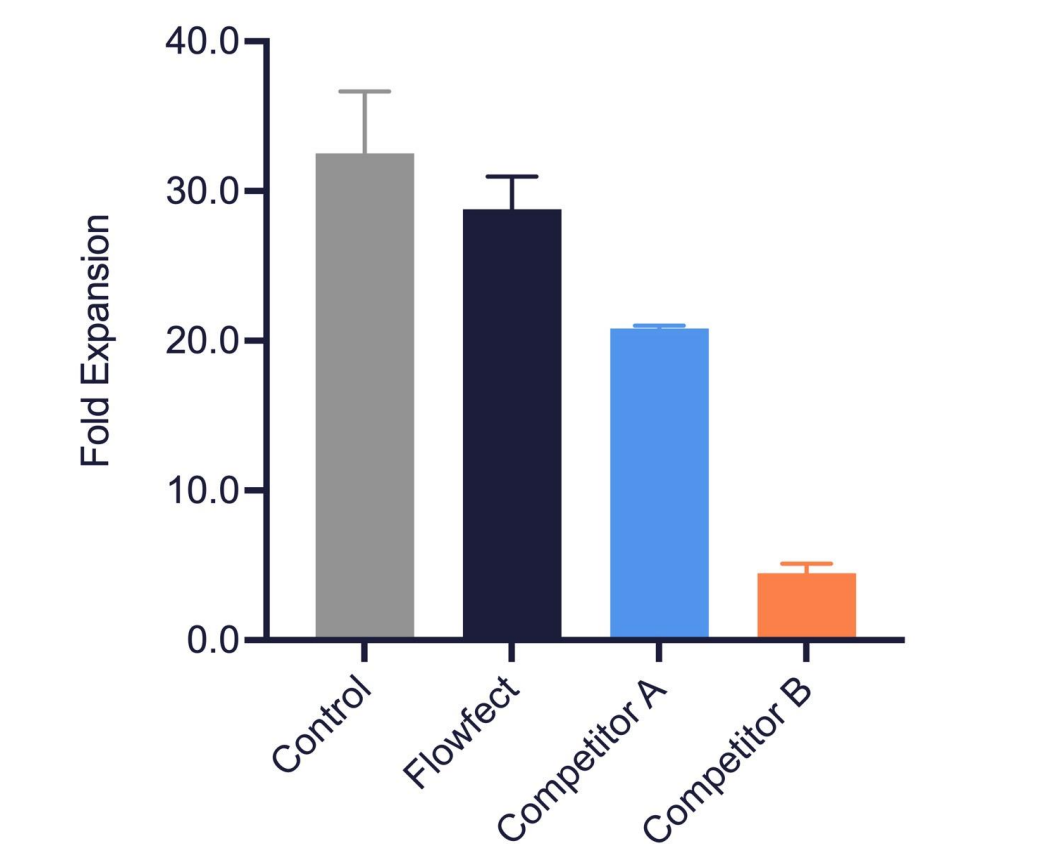


Figure 3: Fold Expansion. The fold expansion of cells for each condition was computed using the cells electroporated on day 2 and the final total viable cell density. Data shown = mean+/-SD for n = 2 donors.

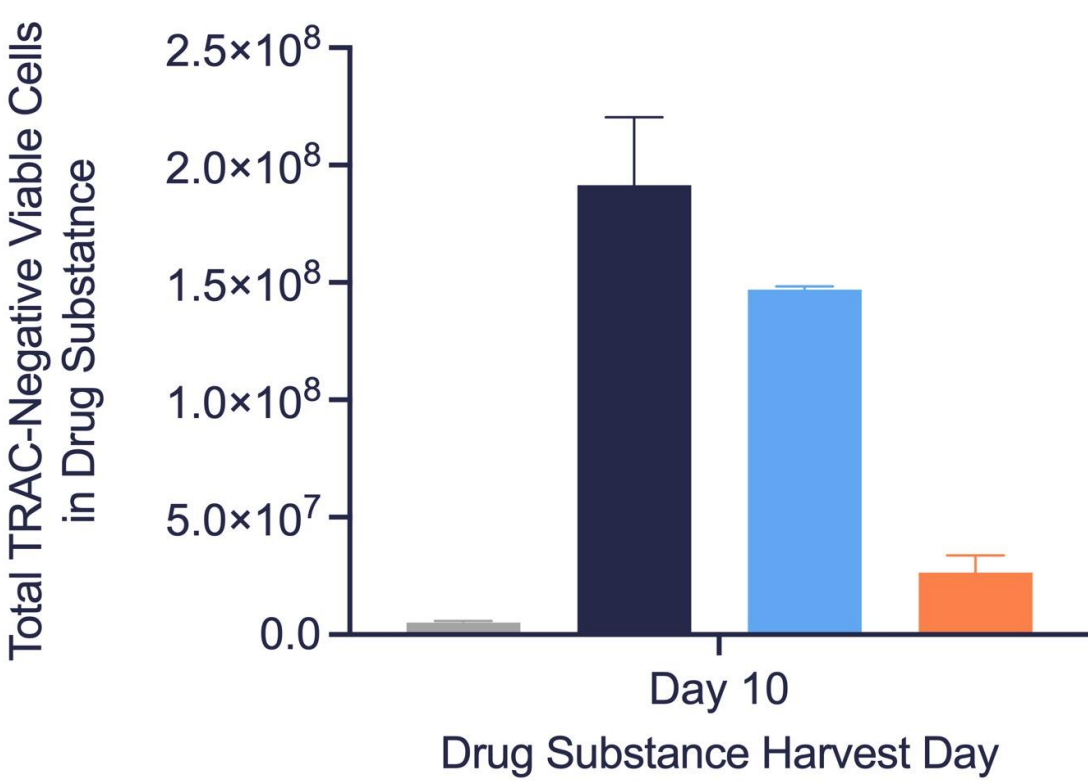
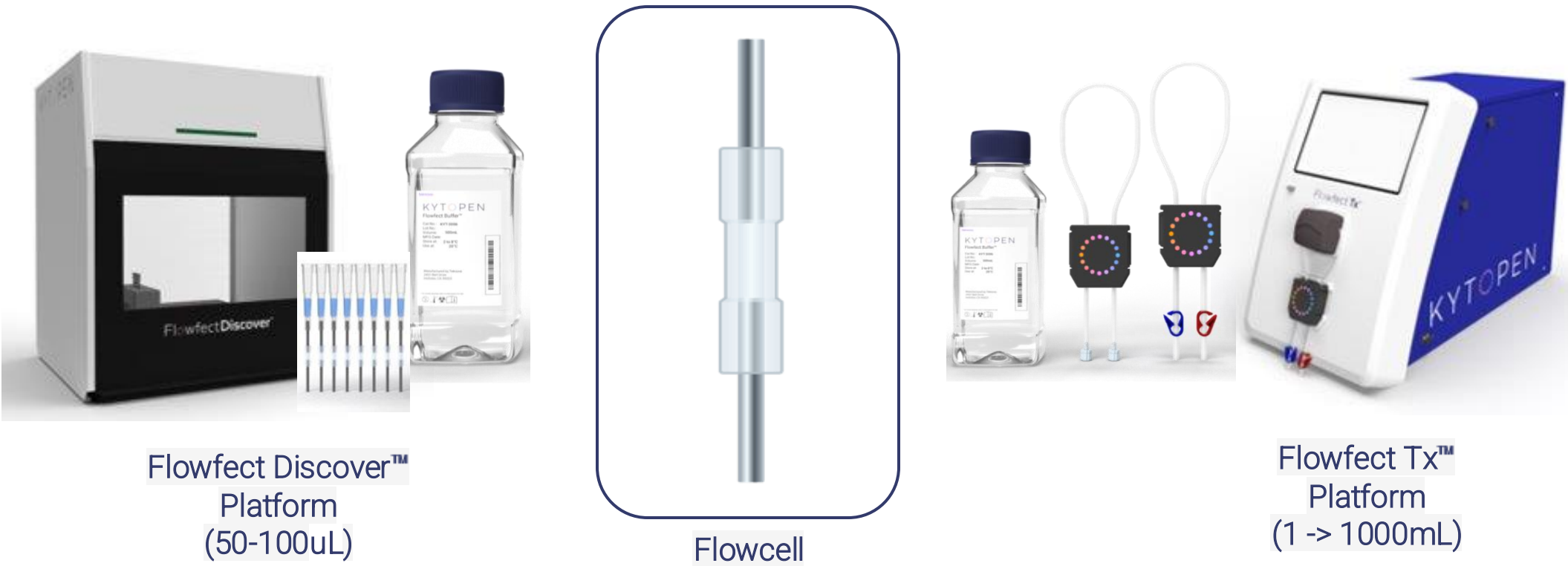


Figure 4: Total TRAC-Negative Viable Cells in Final Drug Substance. The total number of TRAC-Negative Viable Cells at harvest for each EP device were computed using knock-out efficiency (% TRAC-negative cells) and the total number of cells harvested on Day 10. Data shown = mean+/-SD for n = 2 donors.

Automated, HTP Screening
96 conditions in < 15 min

Flowfect® Technology

Closed, Large Scale
Billions of cells in minutes



FLOWFECT® CONTINUOUS FLOW TECHNOLOGY

One Technology - Unlimited Volumes

The Flowfect Discover™ 96-well Optimization platform and the Flowfect Tx™ GMP-Ready system support proof-of-concept testing through process development to clinical and commercial manufacturing scale in just two weeks.

The cell experience is the same from discovery to GMP manufacturing, allowing for quick scale-up with minimal process development.

FLOWFECT® TECHNOLOGY FEATURES:

- **Mechanical, electrical, and chemical forces**, provide a gentler process to deliver high yields of healthy, gene-edited cells.
- Overcomes the **limitations of conventional electroporators**, which can overheat, damage cells, and increase the risk of contamination during batch processing.
- A **highly tunable system**, allows for the adjustment of multiple parameters to maximize transfection efficiency, cell health, and cell yield.
- A **continuous flow platform**, enables the processing of liters of material to produce hundreds of billions of healthy, high-quality engineered cells in just minutes.

CONCLUSIONS

- **Scalability and improved cell health:** Flowfect® technology outperformed against the leading electroporators in terms of cell viability, notably at day 4, and fold expansion at day 10.
- **Increased recovery and yield of Final Drug Substance:** The total number of TRAC-negative viable cells was considerably higher after transfection with the Flowfect Tx™ platform at day 10, showing the ability of the Flowfect® technology to retain cell health post transfection and to deliver larger amounts of gene-edited cells.

