Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing

Jacob A. VanderBurgh, Grant T. Corso, Thomas N. Corso, Stephen L. Levy, Harold G. Craighead
CyteQuest, Inc, Ithaca, NY

https://cytequest.com/
hcraighead@cytequest.com

CyteQuest®

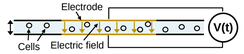
Objective: Scalable transfection of T cells for cell therapy manufacturing

- Viral vectors have enabled CAR-T cell therapies, but drawbacks include:
  - High cost and complex manufacturing
  - Immunogenicity and potential for insertional mutagenesis
  - Incompatibility with CRISPR/Cas9 mediated gene editing
- Electroporation enables non-viral transfection of primary cells, but:
  - Electroporation typically requires difficult empirical optimization
  - Standard electroporation methods are incompatible with automation and large-scale cell manufacturing methods required for cell therapies
- We developed a microfluidic electroporation platform capable of rapid and reproducible electroporation that can seamlessly scale delivery from the research to clinical scale

Overview of electroporation flow chip

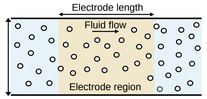
Schematic side view

Channel height and electrode gap: 80 µm

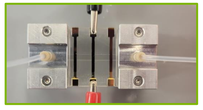


Schematic top view

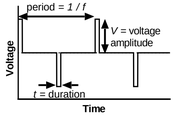
Channel width and electrode width: 2, 10, or 20 mm



- Our platform incorporates a single-use, continuous-flow, microfluidic channel
- The thin channel height (80 µm)
  - Ensures each cell is subjected the same electric field and chemical environment to enable reproducible electroporation
  - Requires relatively low voltage amplitude to achieve electric field strength required to transiently open plasma membrane pores
- The channel width is chosen to achieve the desired experimental throughput
  - Increasing the width (ie. from 2 to 10 mm) increases experimental throughput without changing electric field experienced by the cells
- Flexible electronics permit delivery of any arbitrary electrical waveform
  - Due to low-voltage operation, we are not limited to simple square or exponential pulses
  - Waveforms can be tailored to a particular cell and cargo type to enhance transfection performance



Photograph of a flow cell with three sets of independently addressable electrodes



Example waveform

Plot depicting a bipolar rectangular waveform with frequency *f*, duration *t*, and voltage amplitude *V*

VanderBurgh JA, Corso TN, Levy SL, Craighead HG. Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing. *Sci Rep.* 2023 Apr 26;13(1):6857

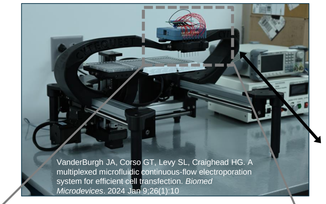
Concept: Two adaptable systems with identical electroporation performance

Optimization Instrument
Research-use-only, flexible, multiplexed low- and mid-volume applications

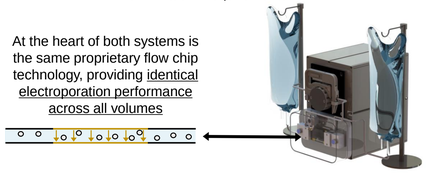
- Multiwell plate processing (96, 48, 24, 6 well plates)
- Multiplexing: 8 independent cell/cargo mixtures
- Flexible choice of processing volume: 20 µL to 20 mL
- Flexible choice of arbitrary waveform
- Rapid optimization: 96 waveforms in minutes
- Robot-controlled liquid handling for automated experimentation

Manufacturing Instrument
Closed system, high volume and/or clinical applications, GMP-compliant

- Single waveform for high volumes (20 mL to >1 L) and high processing throughputs (> 500 million cells/min)
- Rapid processing speed: 1 L in 20 minutes
- Flexible choice of arbitrary waveform
- Bag to bag aseptic processing
- Compliant with good manufacturing practices (GMPs)
- Integrated software records parameters compliant with 21 CFR Part 11 requirements

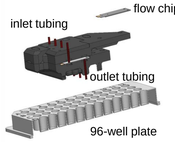


At the heart of both systems is the same proprietary flow chip technology, providing identical electroporation performance across all volumes



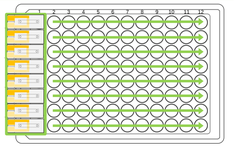
Chip holder with 8 parallel flow chips

Cross-sectional view of chip holder

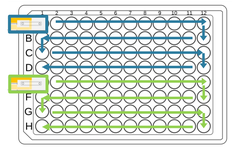


Example configurations with Optimization Instrument

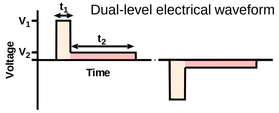
8 chips, 8 liquid chemistries, up to 12 waveforms

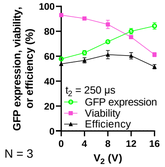


2 chips, 2 liquid chemistries, up to 48 waveforms



Example of flexible waveform design to increase transfection efficiency





Jurkat transfection data from delivery of plasmid DNA-GFP using dual-level waveform

N = 3

$$Efficiency = GFP\ expression * viability$$

Automated liquid handling used to rapidly optimize waveform parameters (1 chip, 56 waveforms)

GFP expression

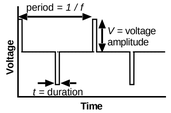
	25 µs	50 µs	75 µs	100 µs	125 µs	150 µs	175 µs
10V	1%	4%	5%	9%	9%	14%	12%
15V	4%	12%	16%	25%	26%	34%	33%
20V	8%	23%	31%	41%	45%	52%	52%
25V	14%	34%	45%	56%	58%	65%	64%
30V	22%	40%	57%	65%	67%	75%	72%
35V	28%	49%	63%	70%	72%	79%	78%
40V	35%	52%	68%	74%	77%	81%	80%

Viability

	25 µs	50 µs	75 µs	100 µs	125 µs	150 µs	175 µs
10V	97%	95%	95%	94%	94%	91%	91%
15V	96%	95%	95%	94%	94%	90%	91%
20V	95%	94%	95%	93%	93%	91%	91%
25V	96%	92%	95%	93%	94%	91%	92%
30V	95%	94%	94%	92%	92%	90%	85%
35V	96%	94%	94%	93%	92%	89%	84%
40V	95%	93%	94%	93%	89%	87%	79%

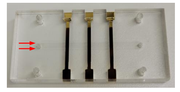
- 1 chip / liquid chemistry
- 56 unique electrical waveforms
- 2-minute experiment time
- 2 mL of Jurkat cell solution
- Plasmid DNA-GFP
- Waveform: Bipolar rectangular wave with 33 Hz frequency and varying duration and voltage amplitude

Bipolar rectangular waveform

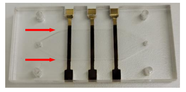


Seamless scalability through scaling channel width

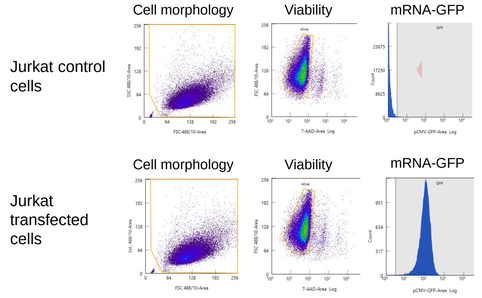
2 mm channel width



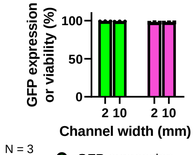
10 mm channel width



Representative flow cytometry plots from control and electroporated cells (mRNA-GFP)



$$GFP\ expression = \frac{N_{expressing}}{N_{viable}} \qquad Viability = \frac{N_{viable}}{N_{total}}$$



N = 3

GFP expression
Viability

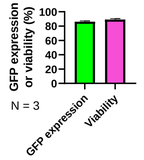
Scaling the channel width by factor of five increases throughput without impacting mRNA-GFP expression or cell viability

VanderBurgh JA, Corso TN, Levy SL, Craighead HG. Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing. *Sci Rep.* 2023 Apr 26;13(1):6857

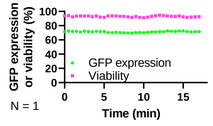
Clinical scale delivery with Manufacturing Instrument

Delivery of plasmid DNA-GFP to Jurkat cells

Processing speed 500 million cells/minute



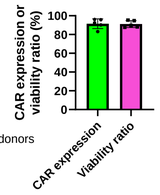
Processing volume 1 L of cell suspension in 18 minutes



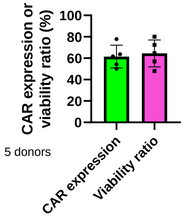
High performance transfection of human primary T cells with CAR and CRISPR RNPs

Delivery of mRNA or plasmid DNA encoding HER2-CAR to primary human T cells

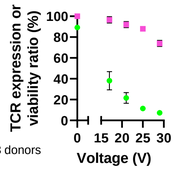
mRNA encoding CAR



Plasmid DNA encoding CAR



Delivery of CRISPR ribonucleoproteins to primary human T cells



Primary T cells with CRISPR RNPs:
88% knockdown
88% viability ratio

VanderBurgh JA, Corso TN, Levy SL, Craighead HG. Scalable continuous-flow electroporation platform enabling T cell transfection for cellular therapy manufacturing. *Sci Rep.* 2023 Apr 26;13(1):6857

Conclusions

- CyteQuest offers two systems, for optimization and manufacturing, that use the same proprietary flow electroporation chip to deliver identical electroporation performance
- Our demonstrated capabilities include:
  - Efficient and flexible transfection of hard-to-transfect primary cells
  - Rapid optimization using small volumes of material at the research scale
  - Up to 8 simultaneous transfection chemistries
  - Flexible choice of electrical waveforms to improve transfection performance
  - Automated, efficient, and easy experimentation
  - Seamless scaling from research to clinical scales by scaling the channel width

Acknowledgements

This work was supported by a Phase I SBIR grant from the NIH. This work was performed in part at the Cornell NanoScale Facility, an NNCI member supported by NSF Grant NNCI-2025233.