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

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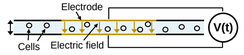
Objective: Scalable transfection of T cells for cell therapy manufacturing

- 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

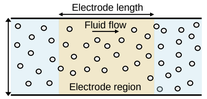
Schematic side view

Channel height and electrode gap: 80 µm

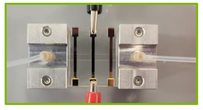


Schematic Top view

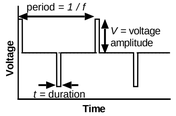
Channel width and electrode width: 2, 10, or 20 mm



- 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



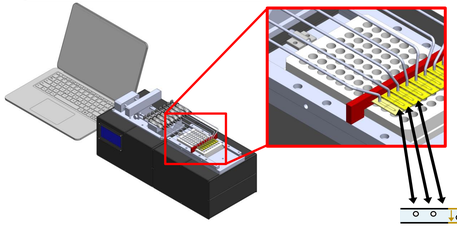
Example waveform

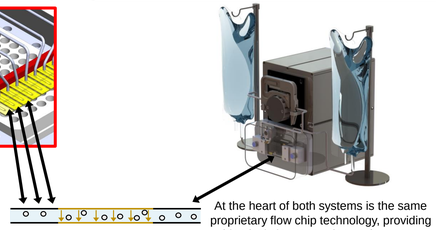
Plot depicting a bipolar rectangular waveform with frequency *f*, duration *t*, and voltage amplitude *V*

Concept: Two adaptable systems with identical electroporation performance

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

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





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

Designed for rapid optimization: can screen 96 unique parameters in less than a minute with less than 1 mL of cell solution per cell/cargo mixture

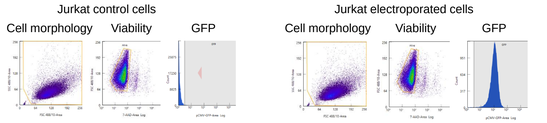
- Multiwell plate processing (96, 48, 24 well plates)
- 8 independent cell/cargo mixtures
- 12 different electrical waveform settings
- Robot-controlled liquid handling for automated experimentation

Designed for cell therapy manufacturing: can process 1 L of cell solution within 20 minutes with a processing throughput of 500 million to 1 billion cells/minute

- Compliant with good manufacturing practices (GMPs)
- Integrated software records parameters compliant with 21 CFR Part 11 requirements
- Bag to bag aseptic processing
- Single waveform

Automated liquid handling allows rapid optimization: 56 waveforms in 2 minutes
Flexible waveform design allows tailoring waveform for particular cell/cargo types

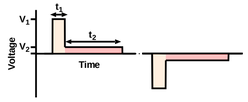
Representative flow cytometry plots from control and electroporated cells



$$Viability = \frac{N_{viable}}{N_{total}} \qquad GFP\ expression = \frac{N_{expressing}}{N_{viable}} \qquad Viability\ ratio = \frac{Viability}{Viability_{zero-voltage\ control}}$$

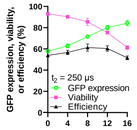
Flexible waveform design for adapting electroporation for particular cell/cargo(s), used to deliver plasmid DNA GFP to Jurkat cells

Plot depicting a dual-level electrical waveform with:
1) A high amplitude, short duration for opening pores
2) A low amplitude, long duration for electrophoresis



$$Efficiency = GFP\ expression * viability$$

By tuning voltage of long duration segment (V_2), we can favorably balance GFP expression and viability



N = 3

Automated liquid handling used to rapidly optimize delivery of nanoplasmid DNA GFP to Jurkat cells

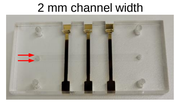
- 56 unique electrical waveforms
- 2 minute experiment time
- 2 mL of Jurkat cell solution used for experiment
- Waveform: Bipolar rectangular wave with 33 Hz frequency and varying duration and voltage amplitude

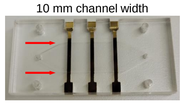
GFP expression	25 µs	50 µs	75 µs	100 µs	125 µs	150 µs	175 µs
10V	1%	4%	5%	9%	9%	14%	12%
15V	4%	12%	16%	25%	26%	34%	33%
20V	8%	23%	31%	41%	45%	52%	52%
25V	14%	34%	45%	56%	58%	65%	64%
30V	22%	40%	57%	65%	67%	75%	72%
35V	28%	49%	63%	70%	72%	79%	78%
40V	35%	52%	68%	74%	77%	81%	80%

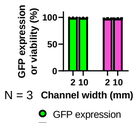
Viability	25 µs	50 µs	75 µs	100 µs	125 µs	150 µs	175 µs
10V	97%	95%	95%	94%	94%	91%	91%
15V	96%	95%	95%	94%	94%	90%	91%
20V	95%	94%	95%	93%	93%	91%	91%
25V	96%	92%	95%	93%	94%	91%	92%
30V	95%	94%	94%	92%	92%	90%	85%
35V	96%	94%	94%	93%	92%	89%	84%
40V	95%	93%	94%	93%	89%	87%	79%

Seamless scalability through scaling channel width and other parameters

Scaling delivery of mRNA GFP to Jurkat cells

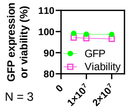






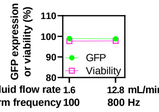
N = 3

Scaling the channel width by factor of five increases throughput without impacting transfection performance



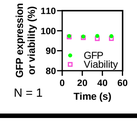
N = 3

Increasing the cell concentration up to 20 million cells / mL in electroporation buffer increases throughput without impacting transfection performance



N = 3

Proportionally increasing the fluid flow rate and waveform frequency increases throughput without impacting transfection performance



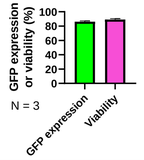
N = 1

Demonstration of mRNA delivery at 256 million cells per minute.
Transfected 240 million cells in 56 s

Clinical scale delivery: 500 million cells/minute and volumes up to 1 L

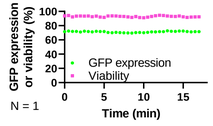
Clinical scale delivery of nanoplasmid GFP to Jurkat cells

Processing speed 500 million cells/minute



N = 3

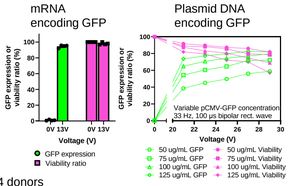
Processing volume 1 L of cell suspension in 18 minutes



N = 1

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

Delivery of mRNA or plasmid DNA encoding GFP to primary human T cells



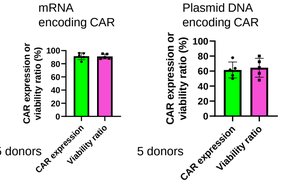
4 donors

1 representative donor

mRNA GFP
>95% GFP expression
98% viability ratio

Plasmid DNA GFP
~80% GFP expression
~70% viability ratio

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



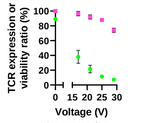
5 donors

5 donors

mRNA CAR
92% CAR expression
91% viability ratio

Plasmid DNA CAR
62% CAR expression
64% viability ratio

Delivery of CRISPR ribonucleoproteins to primary human T cells



3 donors

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

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
  - Automated experimentation for efficient and easy experimentation
  - Seamless scaling from research to clinical scales by scaling the channel width, cell concentration, and fluid flow rate

Acknowledgements

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