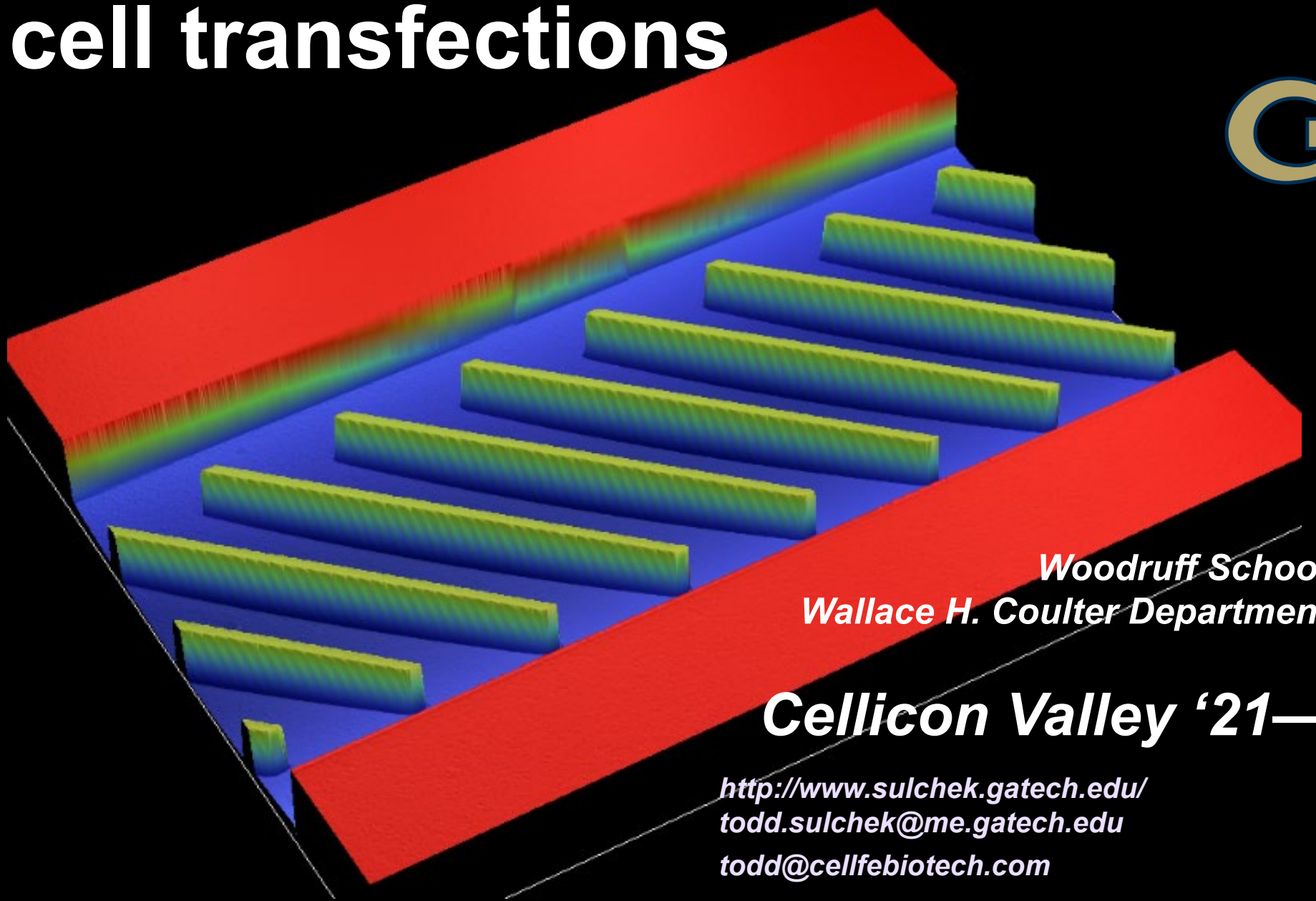
New mechanical approaches to perform cell transfections



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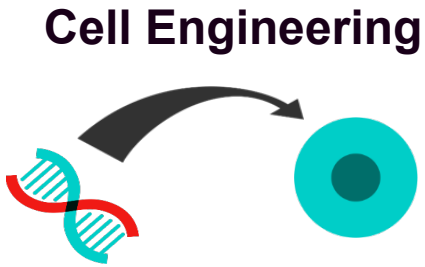
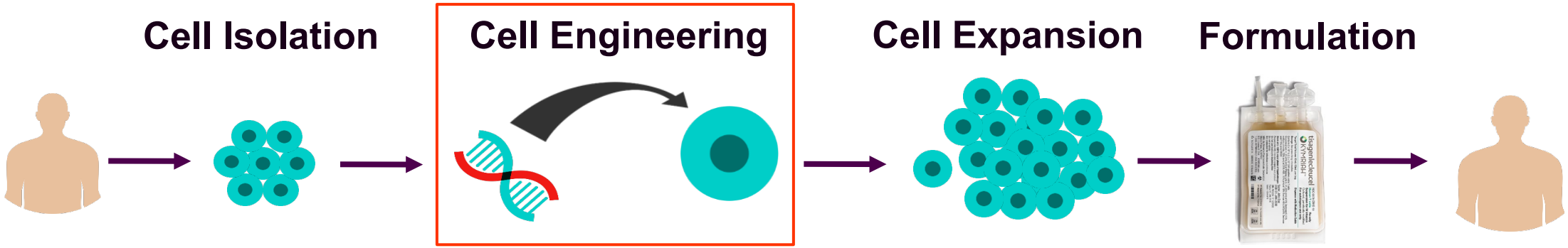
May 6, 2021

Overview of talk



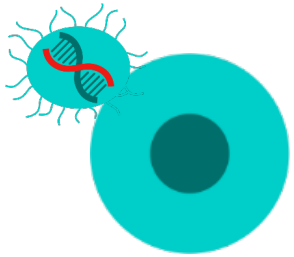
- Review of challenges associated with cell engineering
- History of using mechanical forces for transfections
- Phenomena of using rapid deformations to compress cells and generate active delivery of cargo
- Applications of the technology for cell and gene engineering

The cell therapy roadblocks—payload delivery



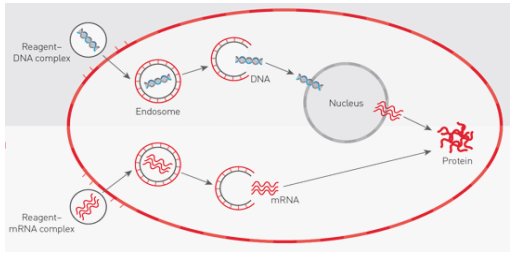
Currently used approaches

Viral Delivery



- Expensive ($50K- $150K / patient)
- Limited access (~1 year wait time)
- Limits to vector size (~ 8 kb)
- Safety concerns

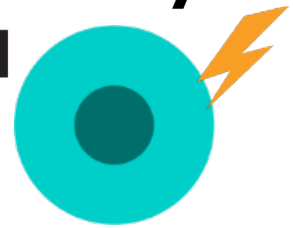
Chemical Membrane Permeabilization



- Relatively cheap
- Not effective for all cell types
- Need for GMP approved reagents

Flow-through electroporation

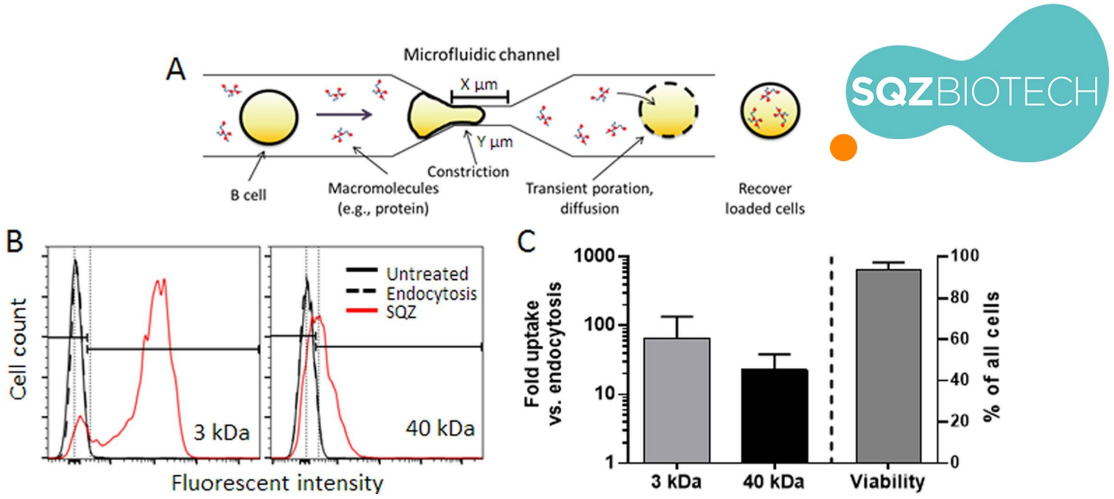
MaxCyte®
Lonza



- Cell loss of >50% of cells common
- Cell phenotype and functional changes
- Challenges to scale from benchtop to clinical scale

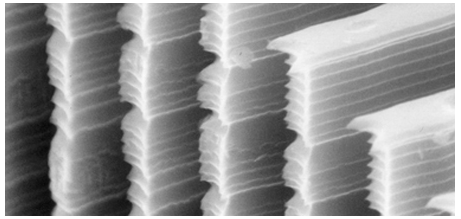
The use of mechanical forces to deliver payloads

Cell Squeezing: PNAS *2012, Sci Rep 2015*

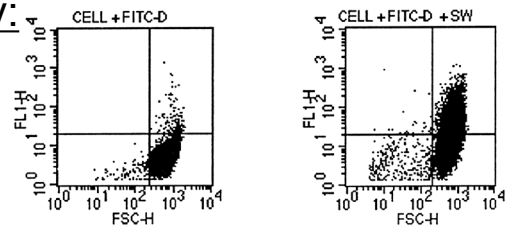


First mechanical approach: "A Technic for the Inoculation of Bacteria and Other Substances Into Living Cells," J. Infectious Diseases (1911).

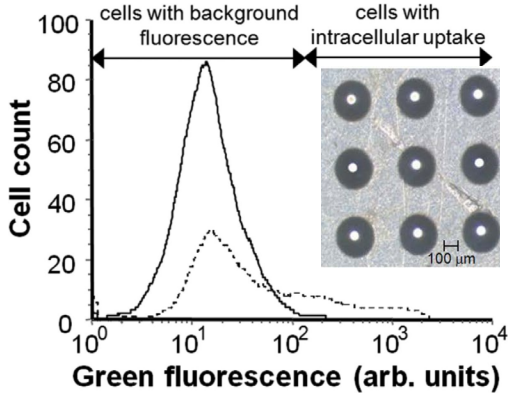
Squeezing: Reagentless Mechanical Cell Lysis. *Lab on a Chip* 2003, **Di Carlo**



Shock wave / acoustic molecular delivery: *Biochimica et Biophysica Acta*, 2002, **Hamblin**
Sci. Reports 2016 **Fedorov**



Shear force molecular delivery: *Biochimica et Biotechnol Bioeng.* 2008, **M. Prausnitz**



A number of other promising techniques…

CRISPR-Cas9 delivery via membrane deformation, *Science Advances 2015,* ***L. Qin***

Intracellular Delivery of Nanomaterials via an Inertial Microfluidic Cell Hydroporator, *Nano Letters 2018,* ***A. Chung***

Intracellular delivery of mRNA to human primary T cells with microfluidic vortex shedding, *Sci Reports 2019,* ***R. Pawell***

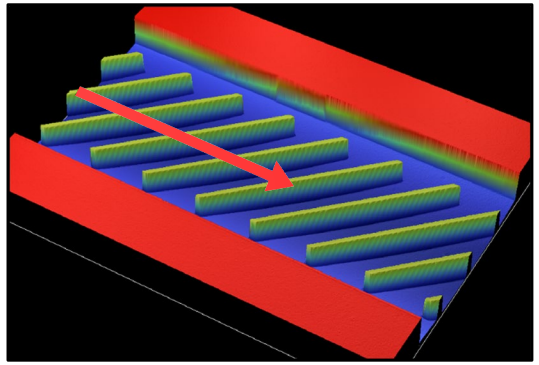
With a few exceptions, mechanical approaches have had limited success with delivery of large payloads

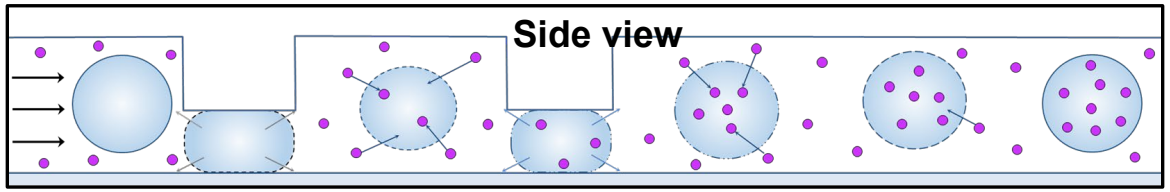
Delivery hypothesis for mechanical forces

For delivery of genetic cargo, we need:

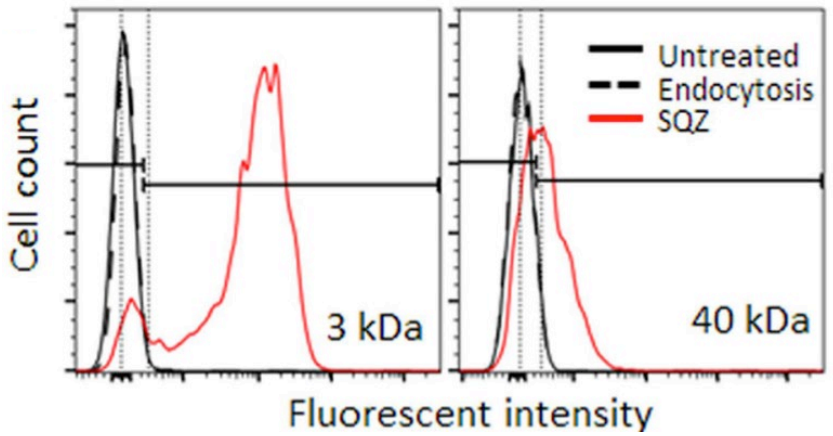
- **A hole in the cell**
- **A transport mechanism**

Diffusion provides limited capacity for transport of large cargo:
$D=T/6\pi\eta a$



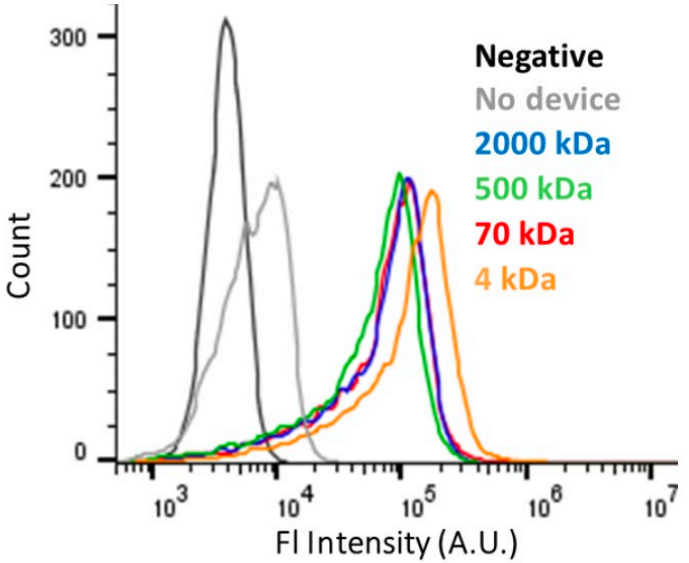


Diffusive-driven dextran delivery



Szeto, Sci Reports *2015*

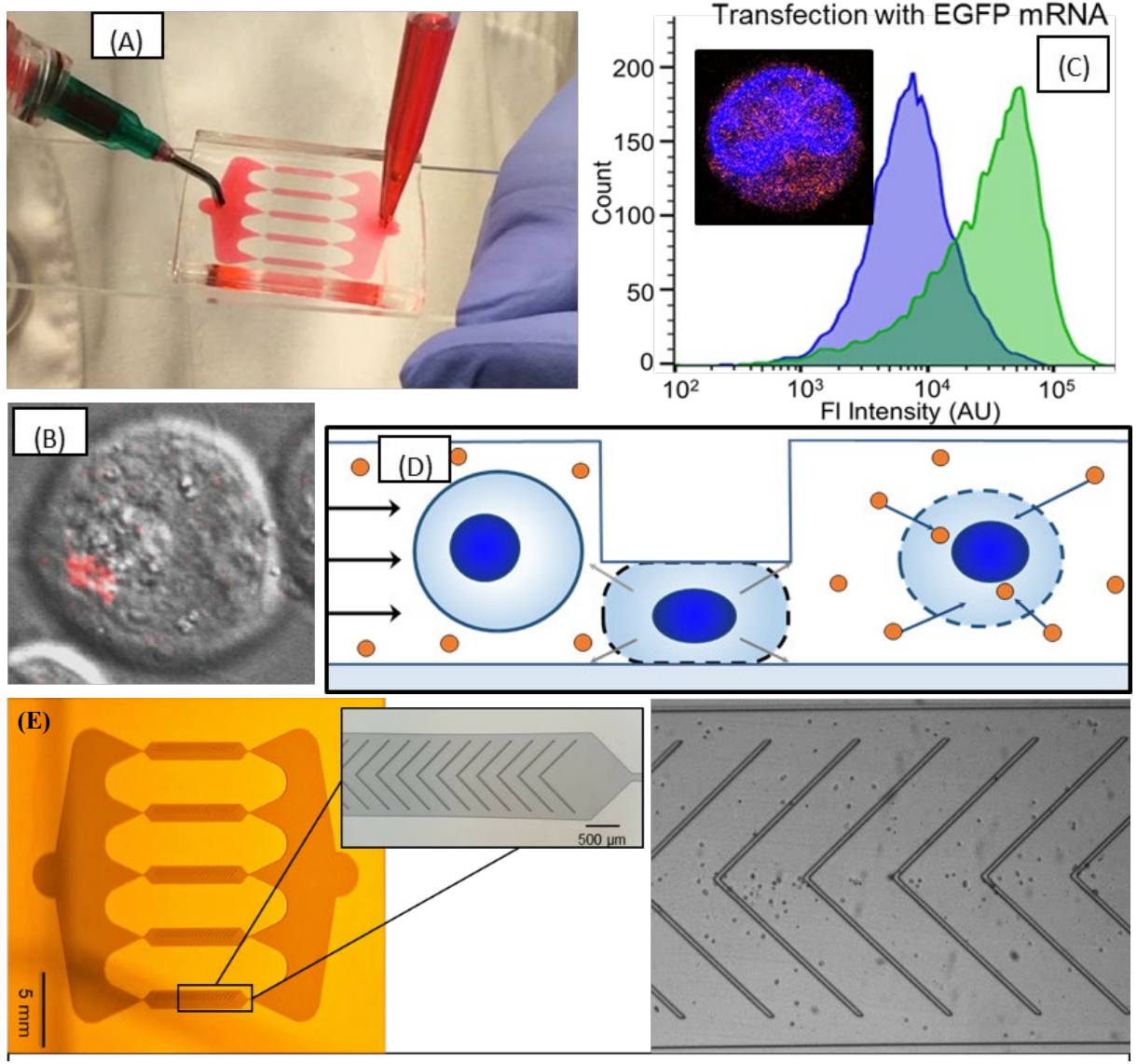
Active transport dextran delivery



- **Delivery via ridged microfluidics did not show decrease with size expected from diffusive transport**
- **Mechanical mode is more of an impact and deformation**
- **Opportunity to leverage new transport mechanisms**

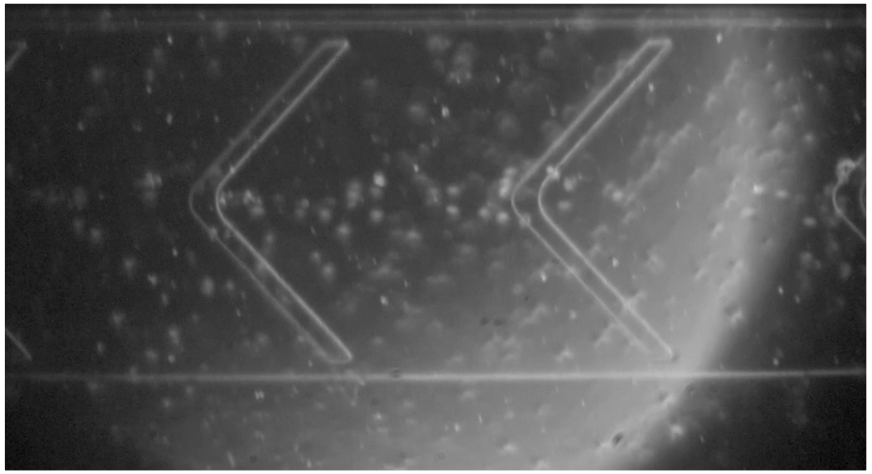
Liu, Materials Today *2018*

Active **molecular delivery** without electric fields—volume exchange for convective transfections (VECT)

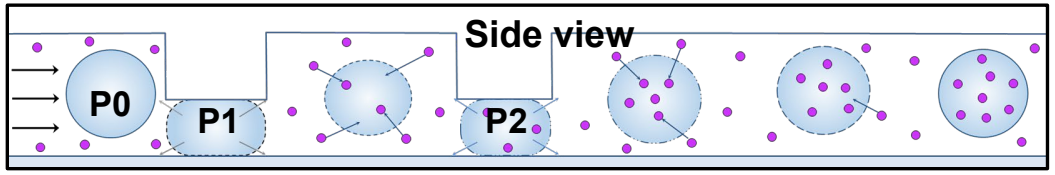


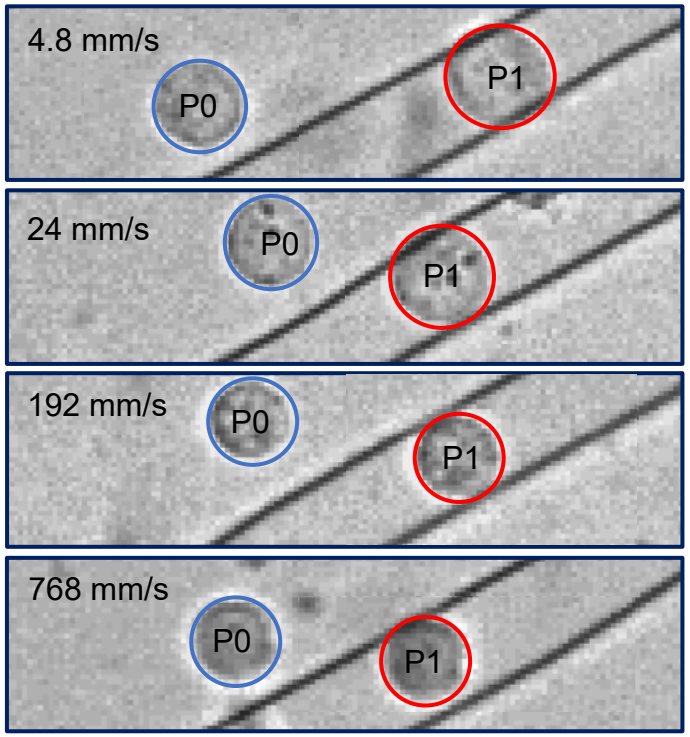
Liu, **Materials Today** 2018

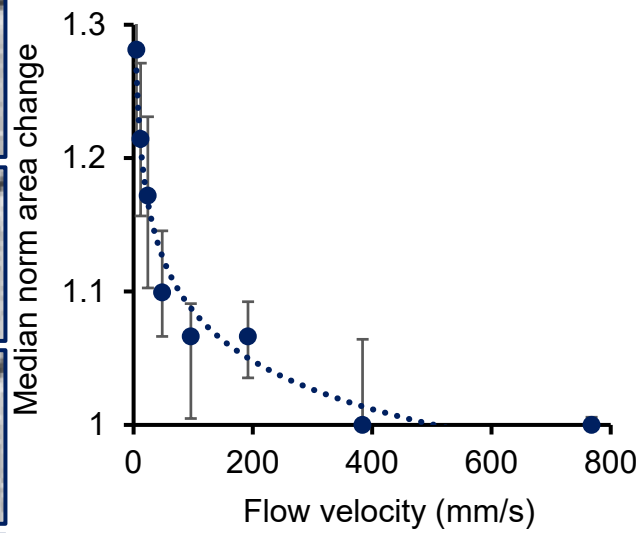
- Ultrafast, microfluidic cell compressions cause cell volume exchange for convective transfer (VECT) of molecules.
- Convective delivery is (relatively) independent of molecule size.
- a wide array of macromolecules and particles can be delivered.
- Rapid (10^7 cells/min/channel),
- Simple to scale from bench to clinical throughputs



Delivery mechanism hypothesis: compressed state of cell due to rapid deformation





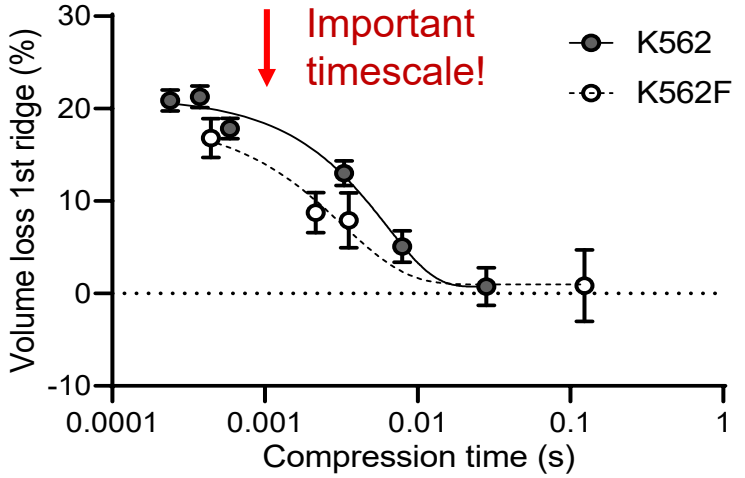


Liu, **Small** 2019

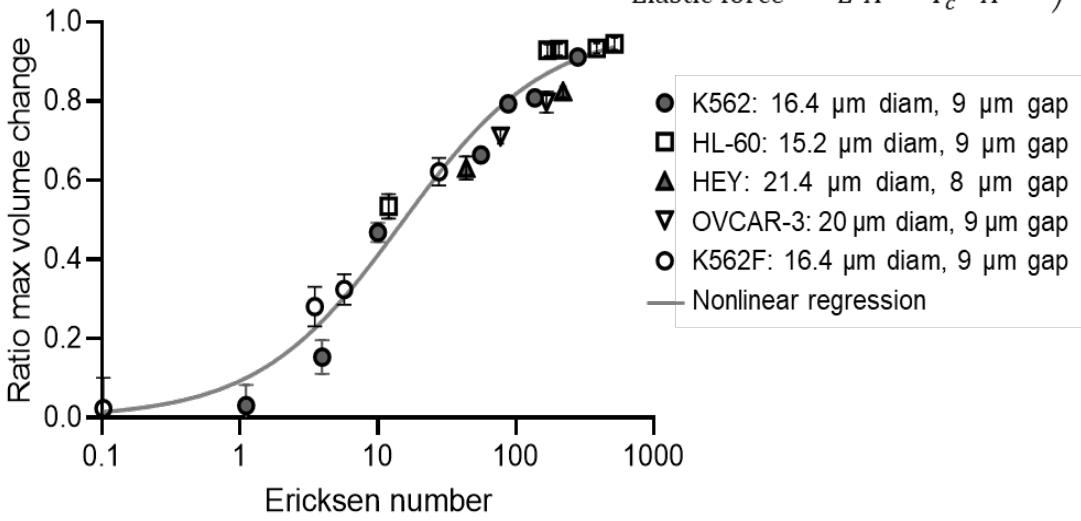
Delivery Step 1

- Cells expand under the ridge at low velocity, but not at fast velocity
- Volume loss increases with faster flow

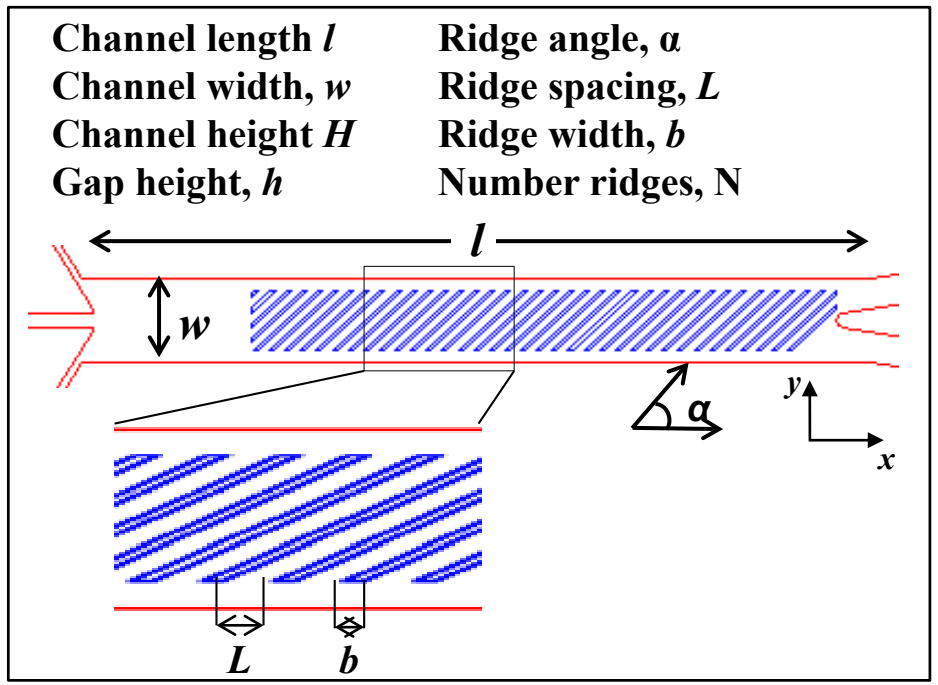
Cell biophysical responses



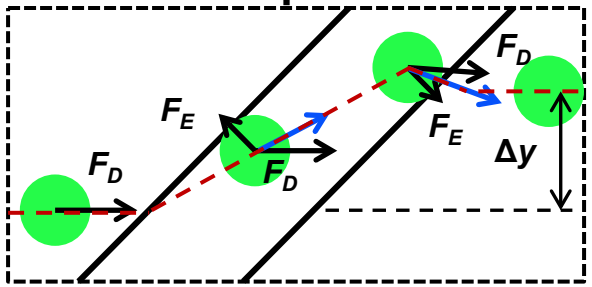
$$\text{Ericksen number} = \frac{\text{Viscous force}}{\text{Elastic force}} = \frac{\mu V L}{E\,A} = \frac{T_v}{T_c}\frac{\Delta L\, L}{A}$$



Design of “mechanical programs” to hone mechanical and hydrodynamic forces to elicits desired cell responses



Computational modeling of solid and fluid responses in channel

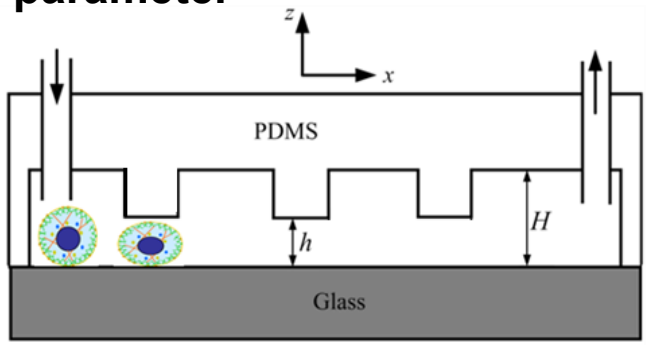


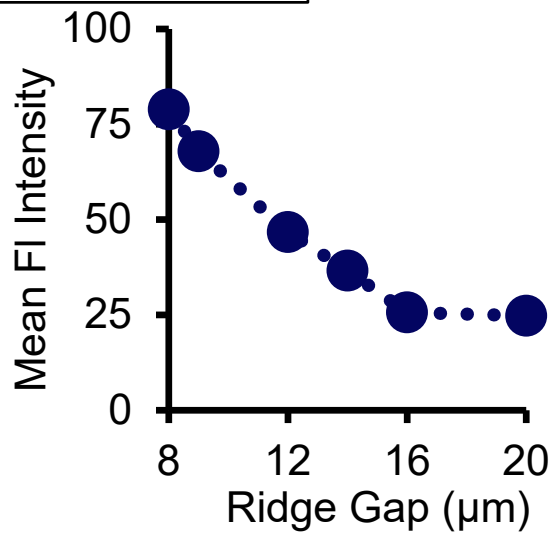
Cell viscous responses

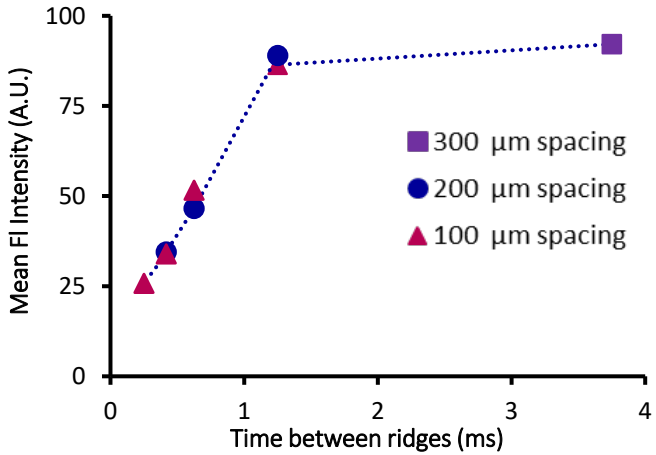
2) Yet, ridge spacing can be tuned to cell relaxation response

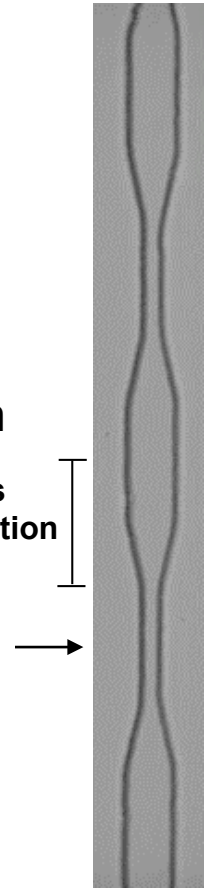
- Step 1: volume loss
- Step 2: convective delivery via cell relaxation

1) Ridge gap height is key parameter





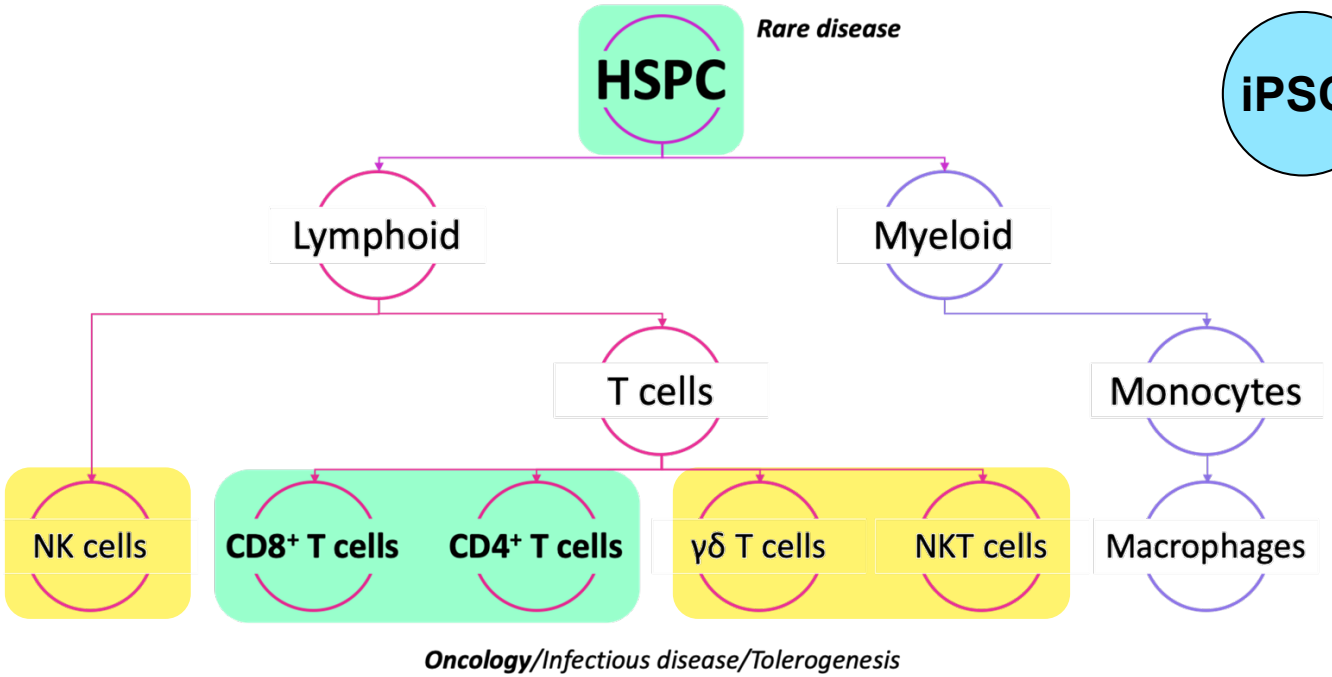




courtesy Prof. Allen Liu, U Michigan

Transfection Results in Primary Cell Carriers

Cell types and payloads tested by CellFE and academic collaborations



We have focused on HSPC and T cell genetic engineering with preliminary data in up-and-coming cell carriers

DNA

Plasmid
- Delivery of large size plasmids
- Delivery of gene editing DNA templates

Minicircles and dbDNA

RNA

mRNA
- Therapeutic genes
- Reprogramming factors
- CRISPR/Cas9

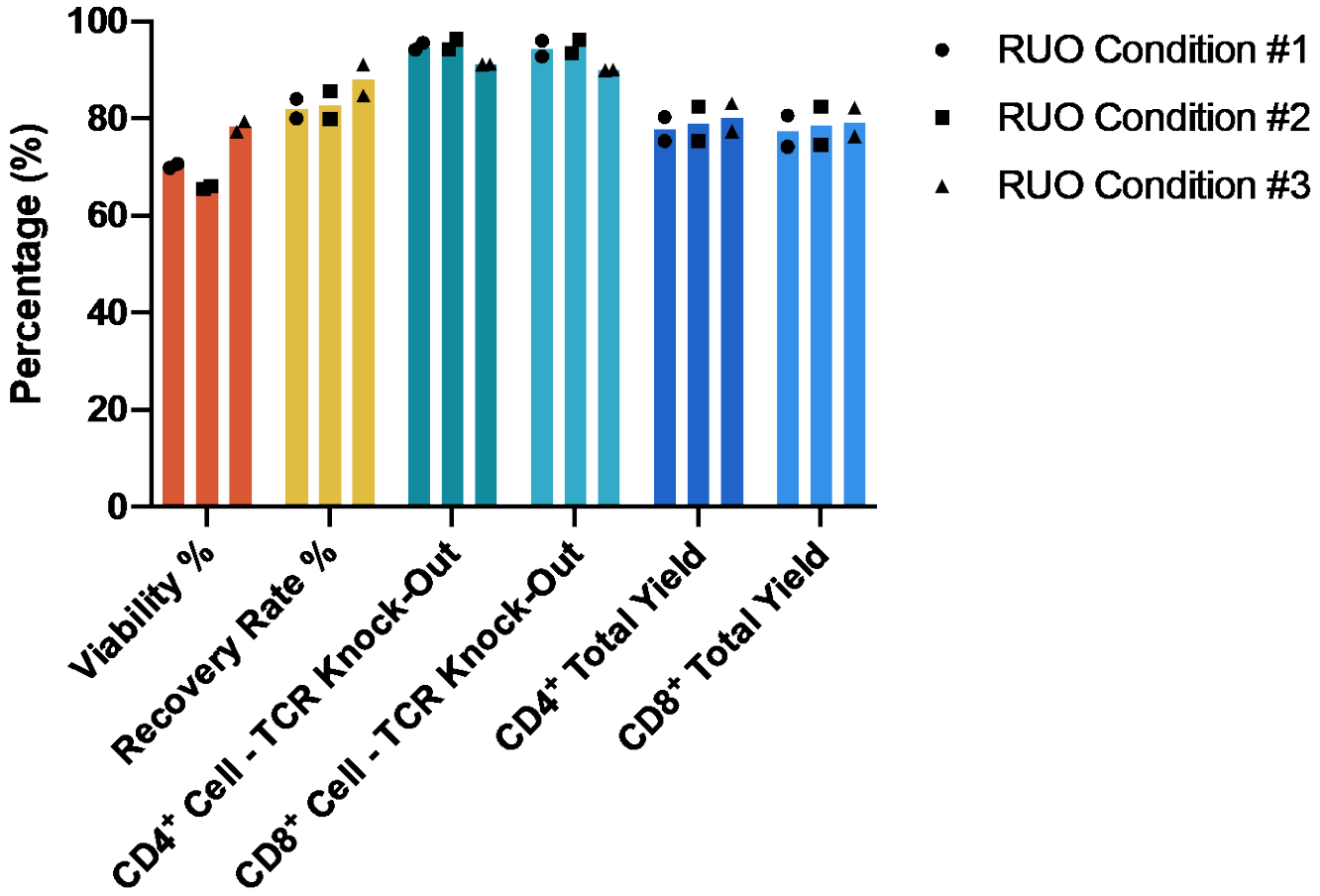
lncRNA

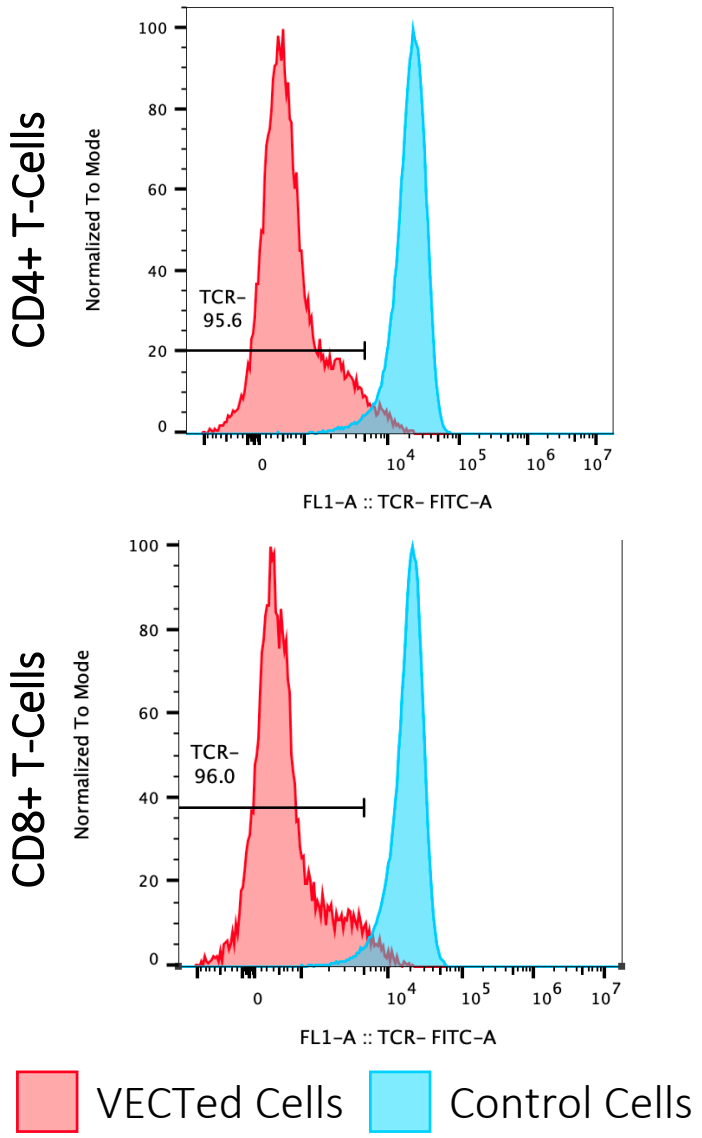
Protein

CRISPR/Cas9 RNP
- Single, tandem or multiplexed CRISPR RNPs

Labelling antibodies

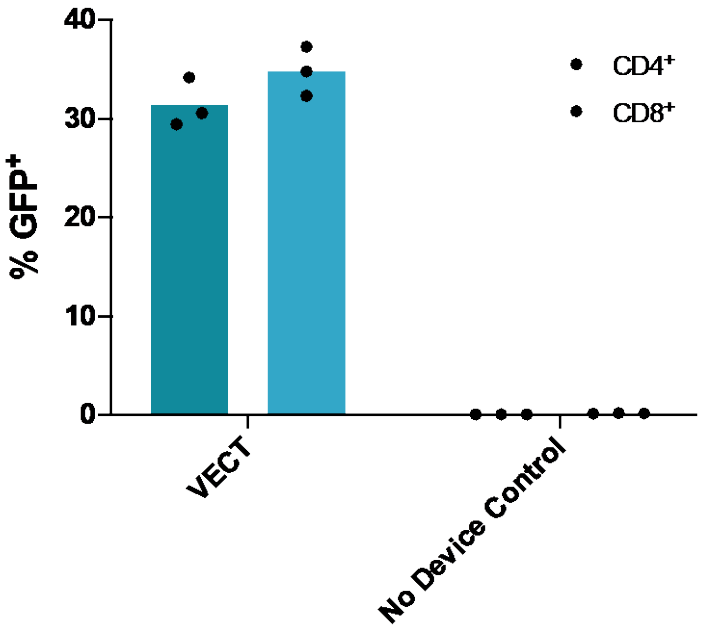
Instrument and Consumable Leads to High(er) Efficiency RNP Mediated Knock Out in T Cells

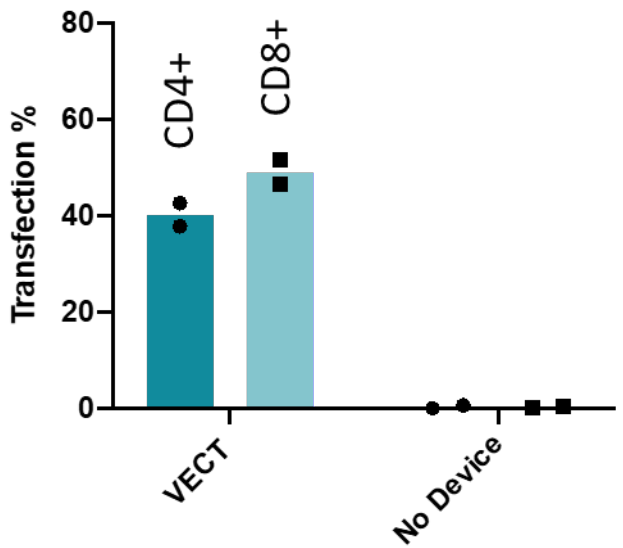


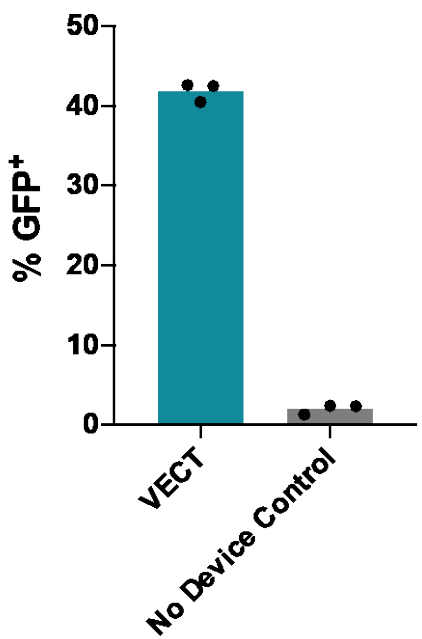


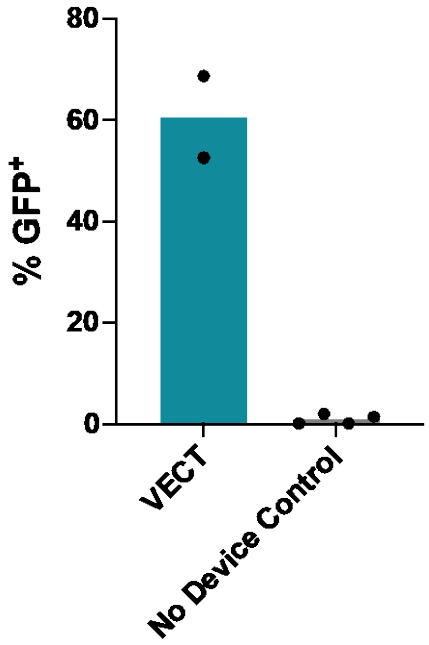
Data set courtesy: Dr. Sewoon Han (CellFE)

Delivery of pDNA into Primary Human Cells and Cell Lines









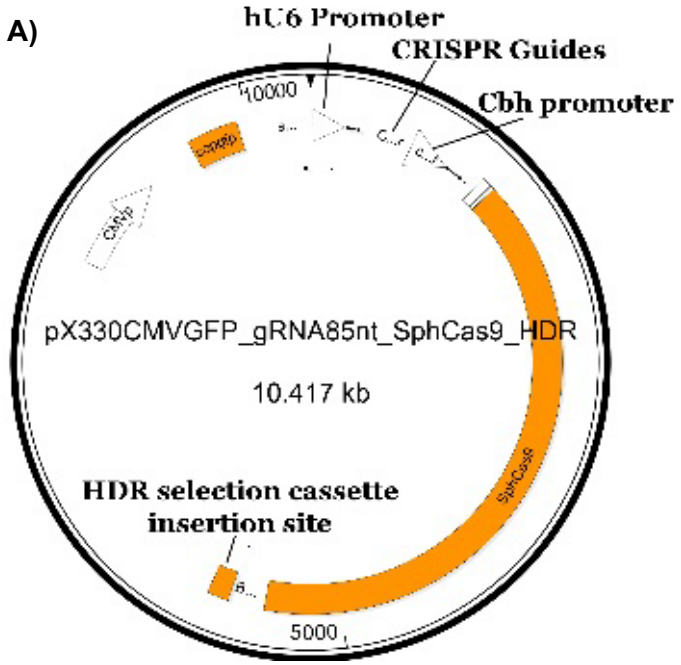
Preliminary data shows successful pDNA transfection across a variety of cell types, both primary cells (T cells and HSPCs cells) and cell lines (Jurkat).

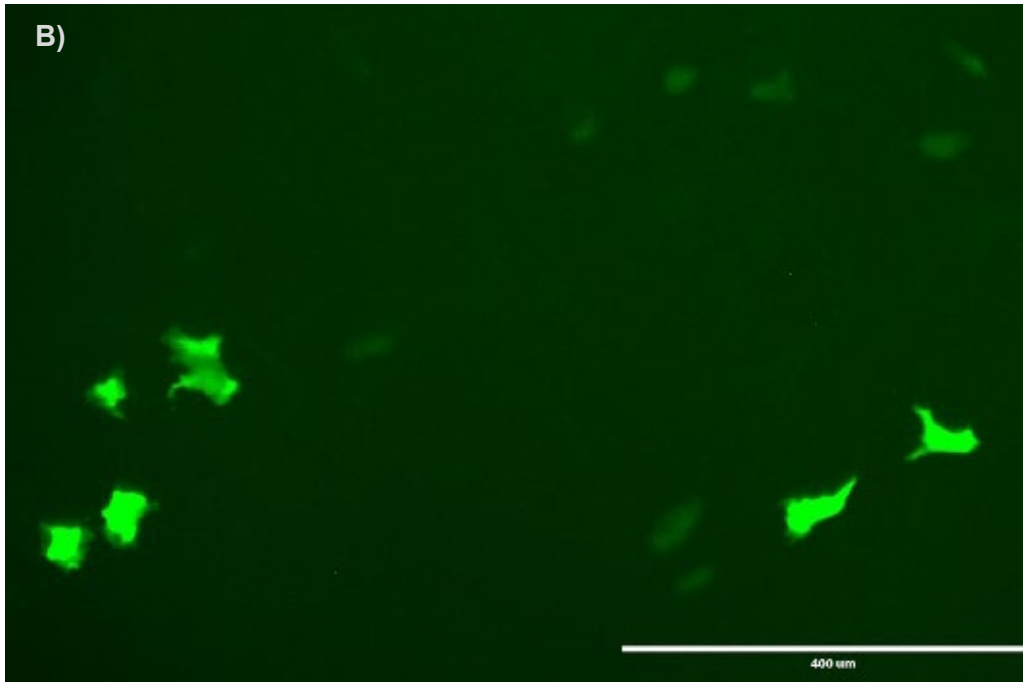
n = 2-5
24hrs post-transfection

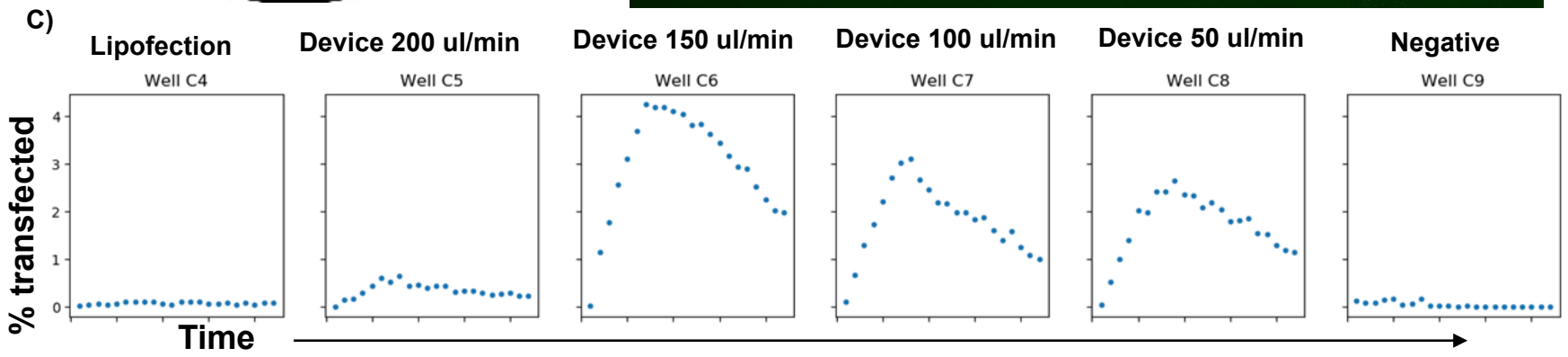
Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

Delivering large vectors for gene correction of large transgenes in induced pluripotent stem cells

- Large transgenes and vectors (i.e. >8 kilobases) express poorly using viral or lipofection approaches.
- An **emerging need exists for large vector delivery** (e.g. large therapeutic transgene, plasmids with multi-functional cassettes).
  - Several genetic diseases (e.g. dystrophin: 11 kb; Factor VIII: 7 kb, Ush2A: 15 kb)





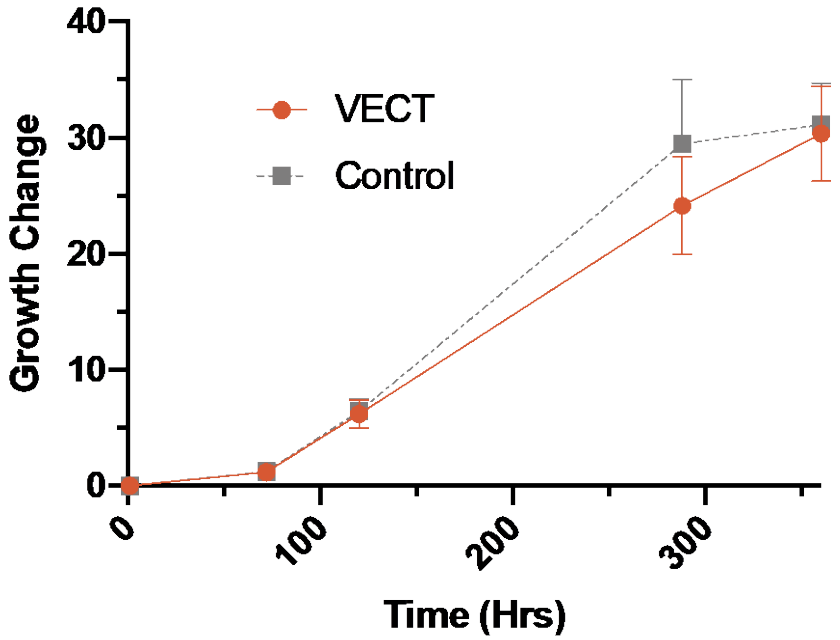


Data set courtesy: Dr. Budd Tucker (U Iowa)

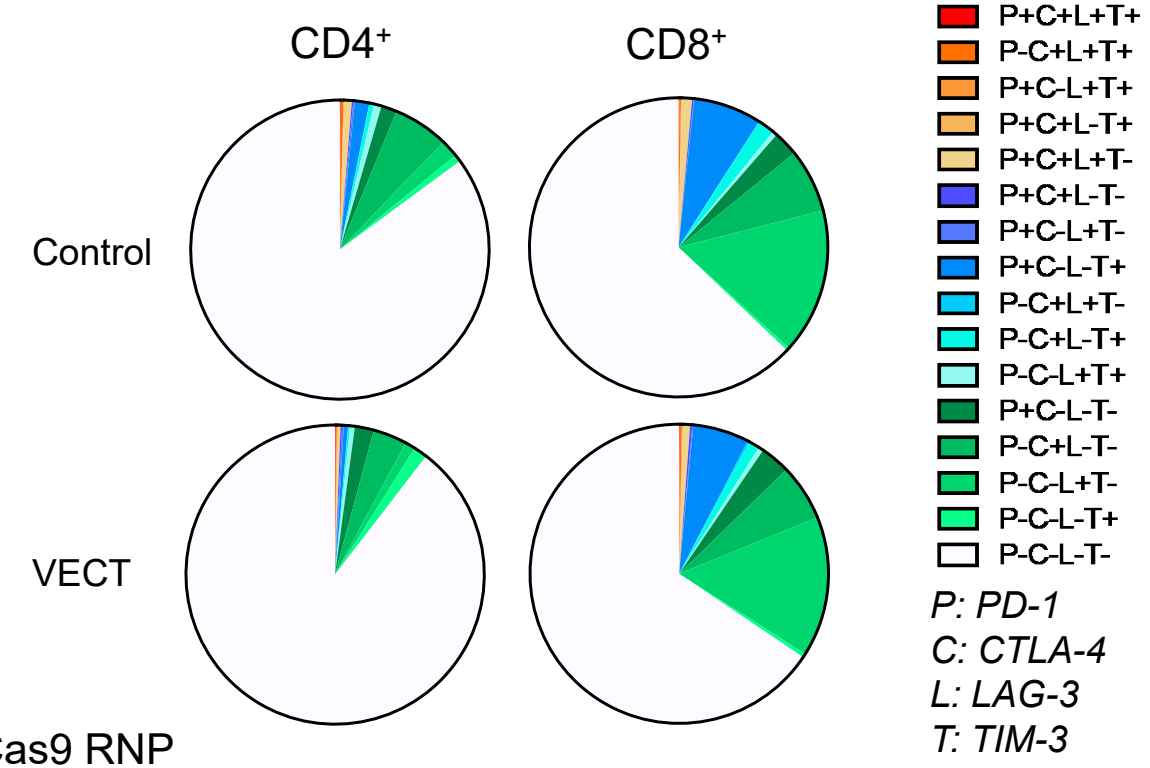
Preserved Cell Functionality

T Cell Functionality is Preserved when Transfecting RNP (permanent modification)

Proliferation



Exhaustion



Donor **B**
Day 9 Post-Activation

- Cell proliferation is not affected by transfecting CRISPR/Cas9 RNP using CellFE's device.
- RNP delivery using VECT has no impact on the exhaustion profile of the final T cell product.

n = 3
Automatic cell counting in NC-3000

Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

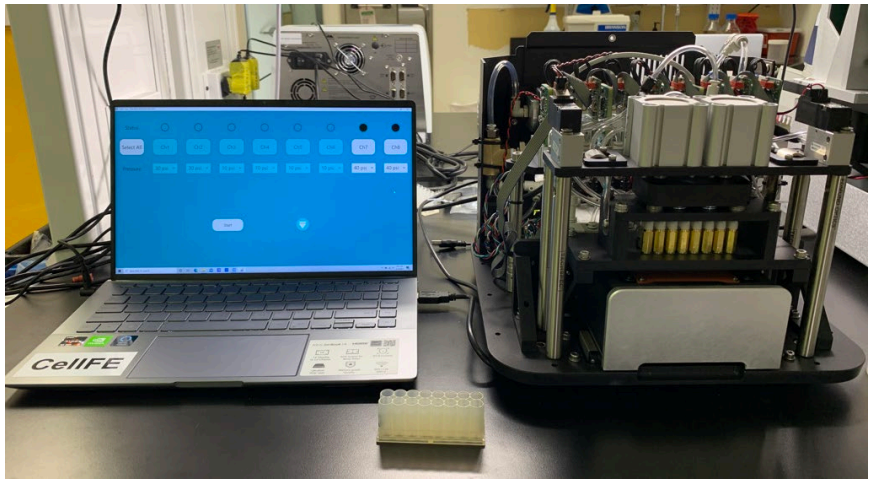
Productization and Operational Capacity

RUO Instrument & Consumable Development

RUO Consumable

- 8 channels
- Each channel processes 0.1–1 ml at a cell density of 1 – 8M cells/mL (processing time: 10 sec - 1 min)
- Automatic processing of 8 samples

RUO Alpha

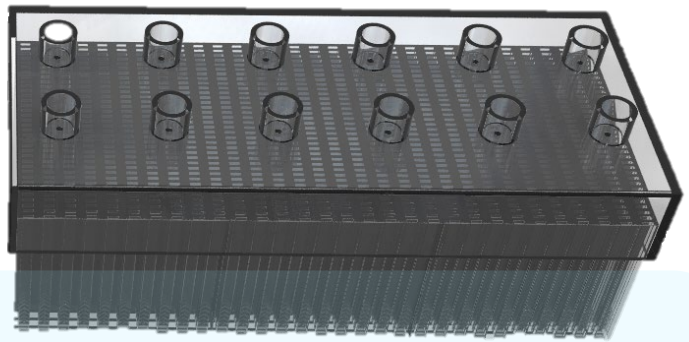


Elegant device design can be adapted to any workflow to enable seamless transition from discovery to clinical manufacturing

SINGLE CHANNEL
10M cells

TENS OF CHANNELS
100M+ cells

THOUSANDS OF CHANNELS
10B+ cells



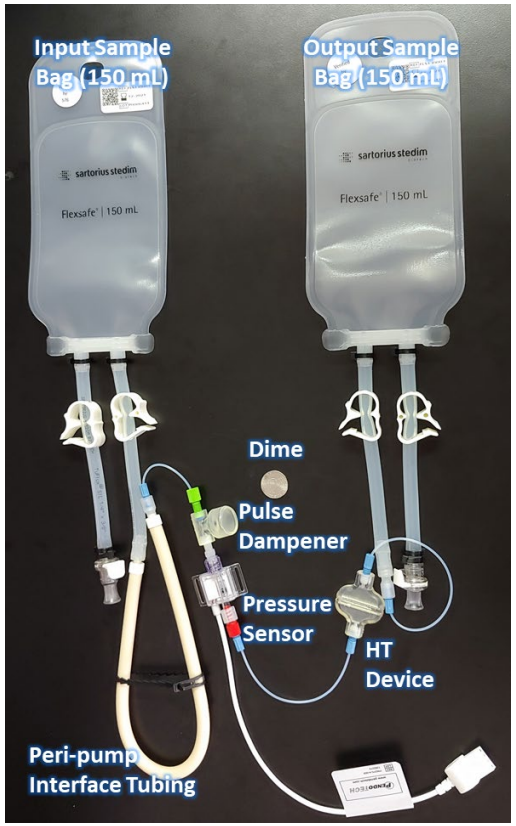
Discovery Research

Autologous Therapies

Allogeneic Therapies

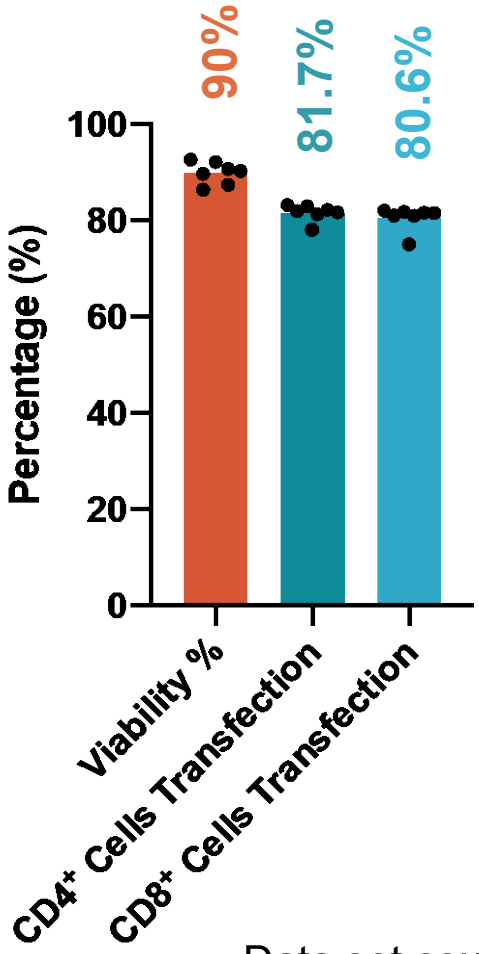
High Throughput Consumable Development

- **High Throughput consumable**
  - Processing tolerates broad range of cell densities
  - Multichannel-processing results in equivalent product yield
  - 330 channels demonstrated.



<u>Total cell number: 100M cells</u>

Cell density: 4 M cells/mL
mRNA concentration: 50 µg/mL
Applied pressure: 5 bar
Gap size: 5 µm

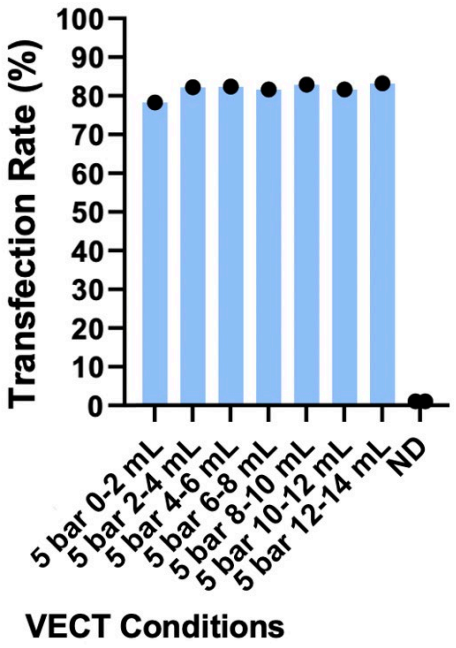


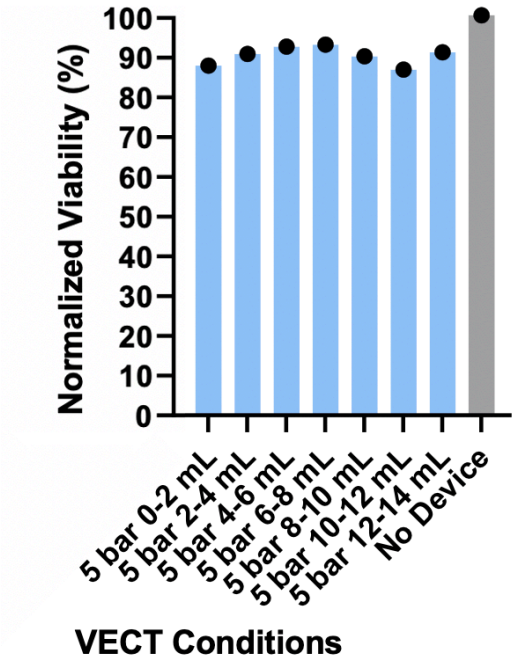
Data set courtesy: Dr. Sewoon Han (CellFE)

1B cells, 1 step, 1 minute

Scaled to clinically relevant throughput for cell therapy

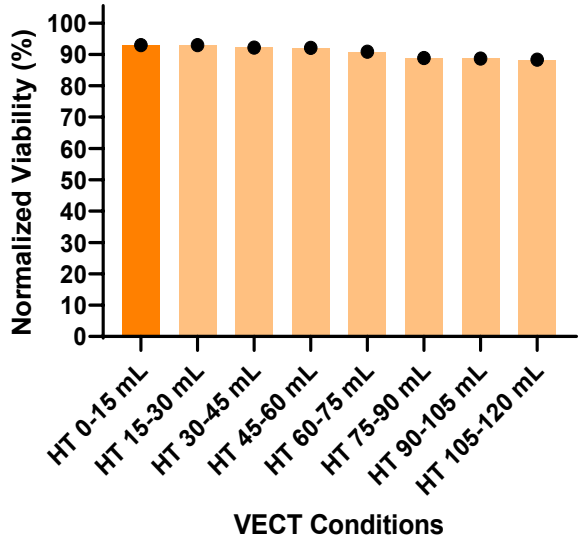
Naïve PBMC, 100M cells

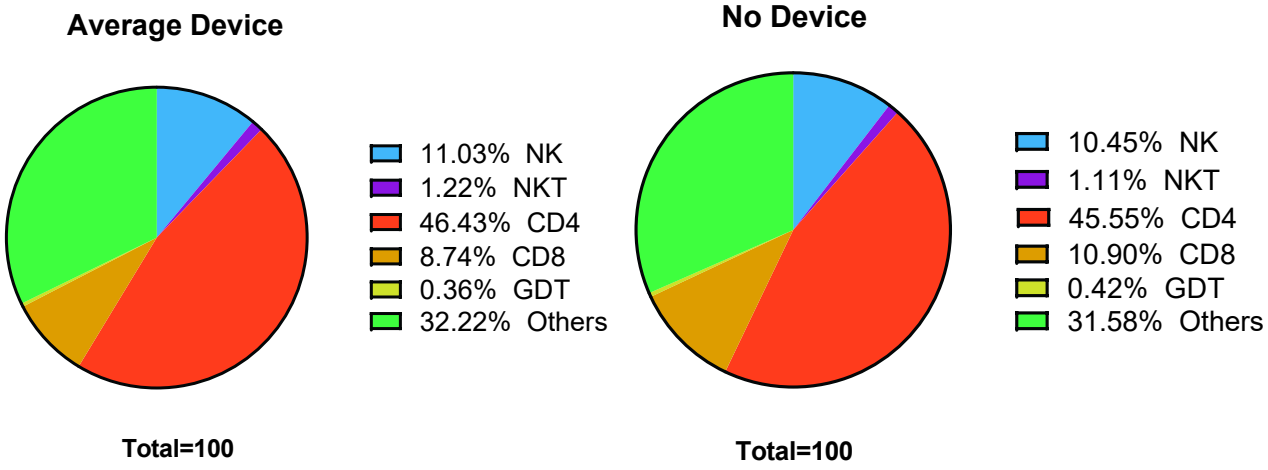




Consumable is scaled through multiplexing channels
Viability and transfection rate are maintained

Naïve PBMC, 1B cells



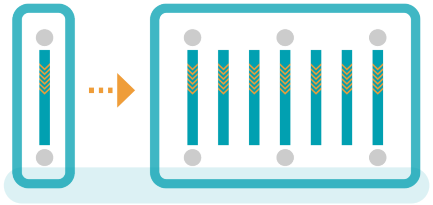
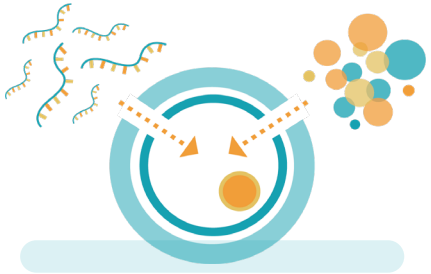


The average cell type distribution consistent with the No Device, the un-VECTed sample.

Data set courtesy: Dr. Sewoon Han (CellFE)

Future Directions and Specific Challenges

VECT platform is uniquely positioned to address transfection needs in cell therapy

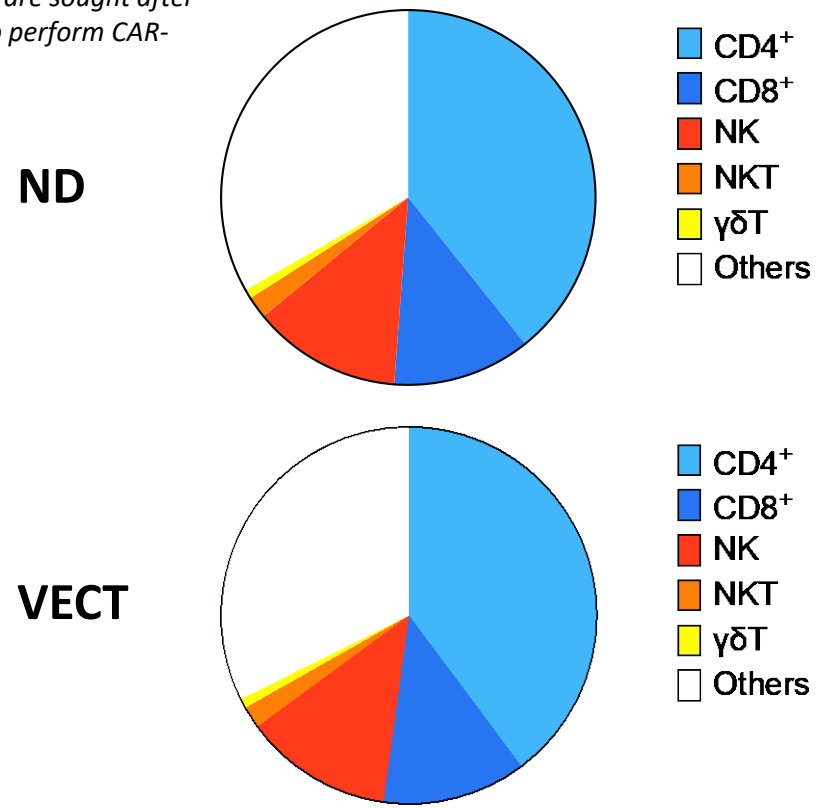
CELLFE CAPABILITIES	CHALLENGES ADDRESSED
 FUNCTIONAL, HEALTHY CELLS	AUTOLOGOUS CELL THERAPY  • Enables complicated genetic engineering • Reduces risk of manufacturing failures • Reduces manufacturing time and cost • Enables point of care systems • 1B cells in 1 minute
 HIGHLY SCALABLE	ALLOGENEIC CELL THERAPY  • Reduces manufacturing time and cost • Enables efficient scale up • Can perform multiplexed knock-outs more safely
 PAYLOAD AGNOSTIC	

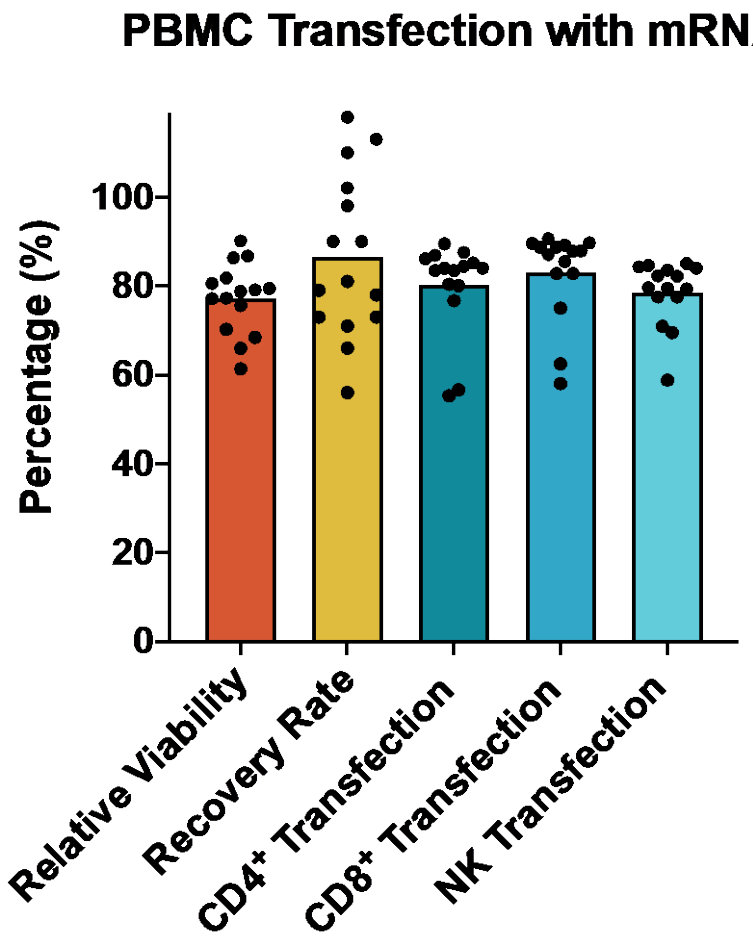
Future Challenges

- Rapid manufacturing workflows with minimal unit operations or need for cell expansion steps;
- Safer multiplexed editing;
- Solutions to visualize therapeutic cell trafficking *in vivo*.

Rapid Manufacturing workflows: T Cells and NK Cells Can Be Transfected As PBMCs With mRNA at High Efficiency

In this donor, NK compartment is as large as CD8⁺ compartment. Both populations are sought after for their ability to perform CAR-mediated killing.





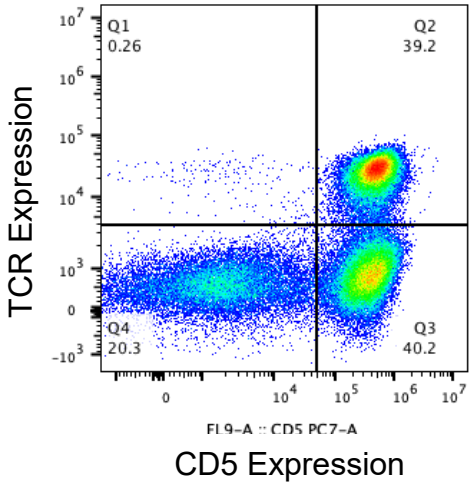
PBMC populations are maintained through the transfection process.

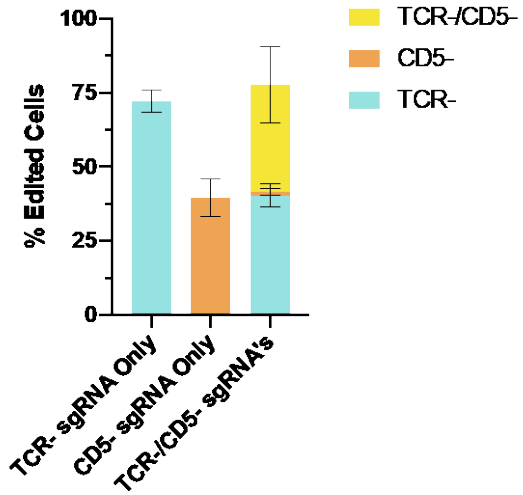
T cells and NK cells can be transfected at high efficiency when processed as raw PBMCs

VECT Efficiently Co-Transfects Multiple CRISPR/Cas9 RNPs in T Cells

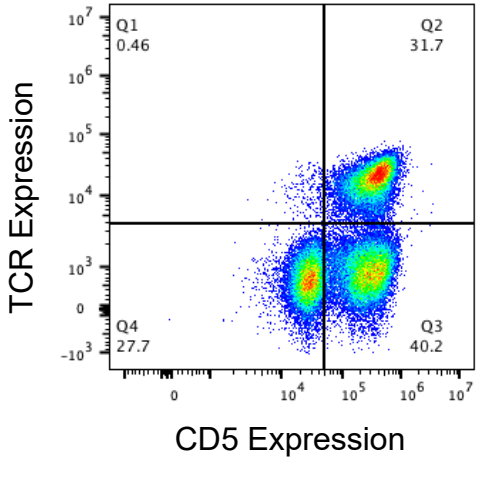
RNP Co-Transfection in T Cells

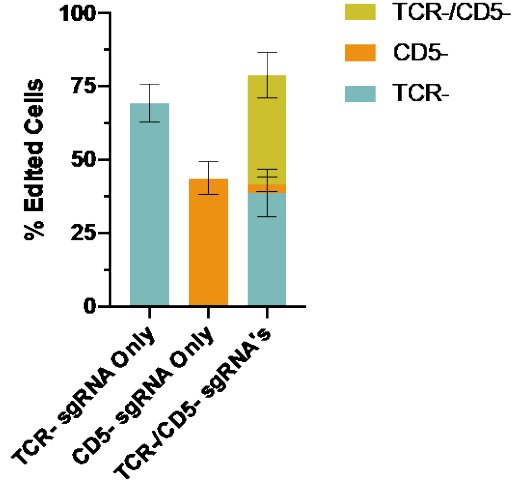
CD4⁺





CD8⁺

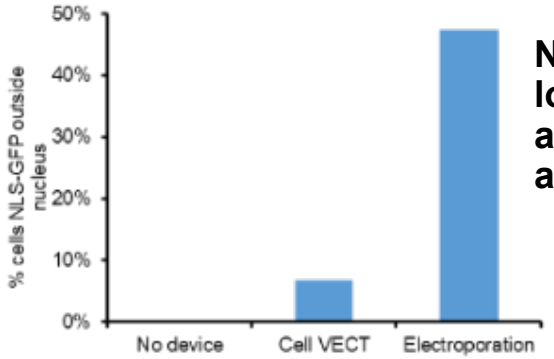




n = 4-6 (2 donors)
5+ days post-transfection

- Efficient co-delivery of CRISPR/Cas9 RNPs, as exemplified by the generation of TCR/CD5 double KO cells.
- Can new transfection tools alter the modality of multiplexed edits to avoid risks of genomic instability and translocations?

Liu and Sulchek, **Small** 2019



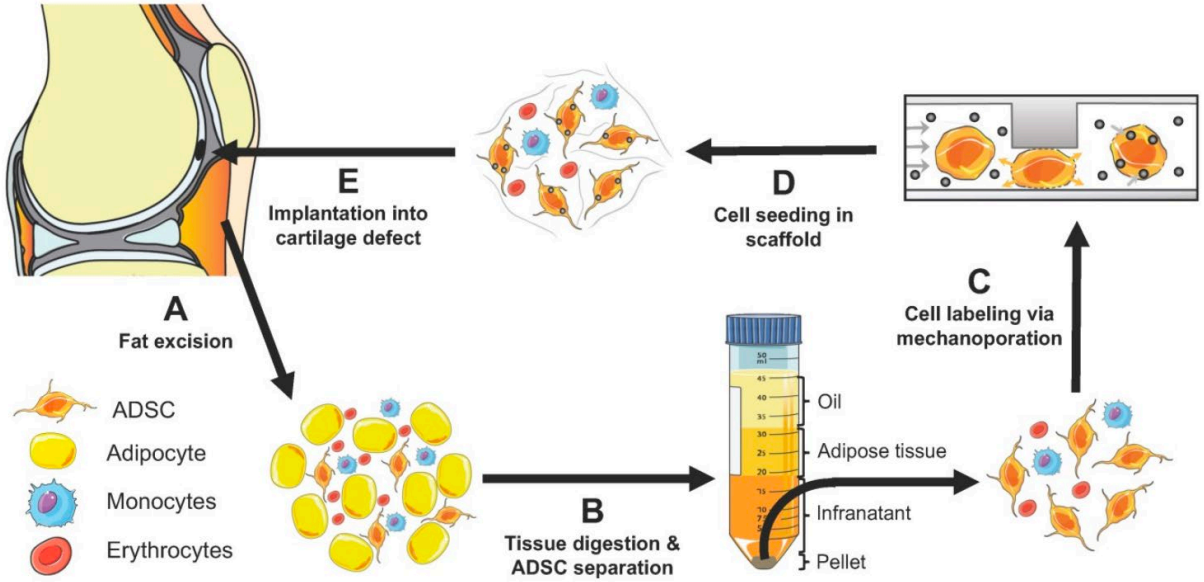
NLS: nuclear localization signal after electroporation and VECT

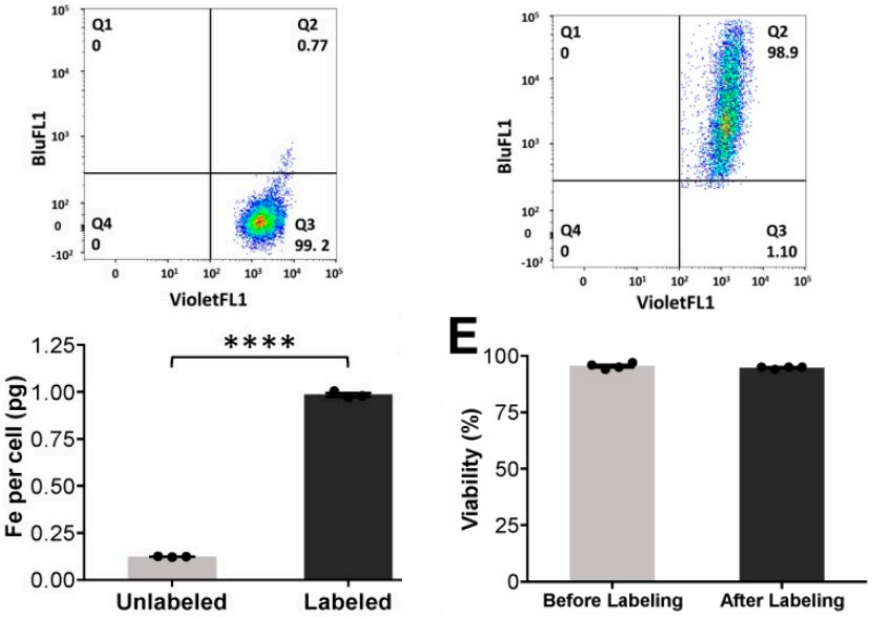
Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

Project in collaboration with S. Raikar, J. Fraietta

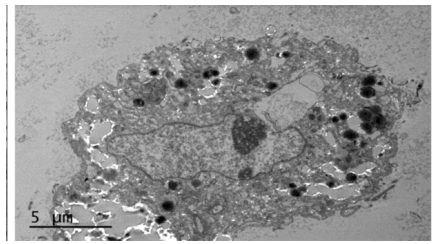
“One-step” labeling of cell therapies for in vivo tracking of homing

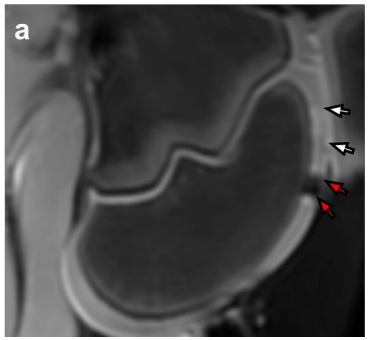
VECT can be used to deliver 1 pg/cell ferumoxytol magnetic nanoparticles to 30M cells in 15 mins to enable *in vivo* imaging



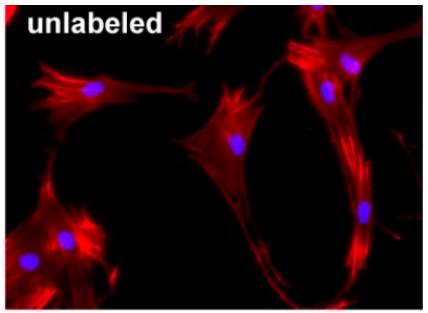


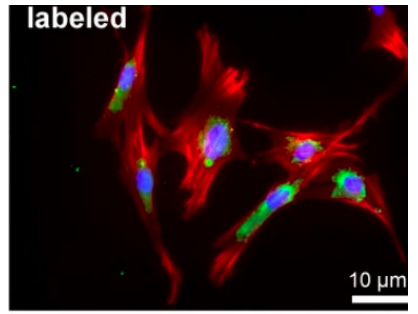
100 nm silica PET payloads





Negative contrast MRI Imaging





MRI data set courtesy: Dr. Heike Daldrup-link (Stanford)
PET data set courtesy: Dr. Guillem Pratx (Stanford)

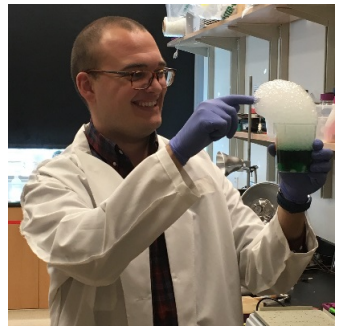
- Nejadnik et al., *Theranostics* 2020, Vol. 10, Issue 13
- “Tracking of stem cell using instant mechanoporation with radiolabeled MSNs in PET” Jung et al., in review
- “In vivo imaging of nanoparticle labeled CAR T-cells”, Kiru et al. in review

Summary and Thanks!

- **Sulchek BioMEMS Group:** Anna Liu, Dr. Muhymin Islam, Nick Stone, **Academic Collaborators:** GT: Alex Alexeev, Peng Qiu, Emory: Wilbur Lam, Edmund Waller, Sunil Raikar, Trent Spencer, Stanford: Heike Daldrup-Link, Guillem Pratx, U Iowa: Budd Tucker, CUNY Albany: Alexander Shekhtman, UPenn: Bruce Levine, Joseph Fraietta
- **Funding (GT & CellFE):**
  - **NSF CBET (2010-2013); NSF CMMI/BMMB (2015-2018); NIIMBL (2020-2021); NSF/CMAT (2020) NIH/SBIR NCI; NIH/SBIR NHLBI; NIH/SBIR NCI**

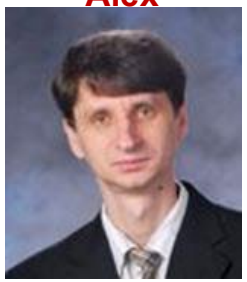
- Mechanical forces can be used to deliver large payloads provided the deformation forces act at fast timescales
- Large deformation forces acting on short time scale can avoid altering cell phenotype.
- Productization of precise microfluidic components into silicon and plastic are possible
  - Improves the reliability of results
- Scaleup of processing rates to 1B PBMCs/min is possible
- Addressing manufacturing challenges of therapies can unlock access.

Nick



Anna

Alex



Edmund

Budd

Publications:

- A Liu, “Microfluidic generation of transient cell volume exchange for convectively driven intracellular delivery of large macromolecules” Materials Today 2018
- A. Liu “Cell mechanical and physiological behavior in the regime of rapid mechanical compressions that lead to cell volume change” Small 2019
- H. Nejadnik “Instant labeling of therapeutic cells for multimodality imaging” Theranostics 2020
- N. Stone “Microfluidic processing of stem cells for autologous photoreceptor cell replacement” under review
- “Tracking of stem cells using instant mechanoporation with radiolabeled MSNs in PET” Jung et al., in review
- “In vivo imaging of Nanoparticle labeled CAR T-cells”, Kiru et al., in review
- “Microfluidic transfection of mRNA into human primary lymphocytes and hematopietic stem cells using ultra-fast physical deformations”, Loo et al., in review.

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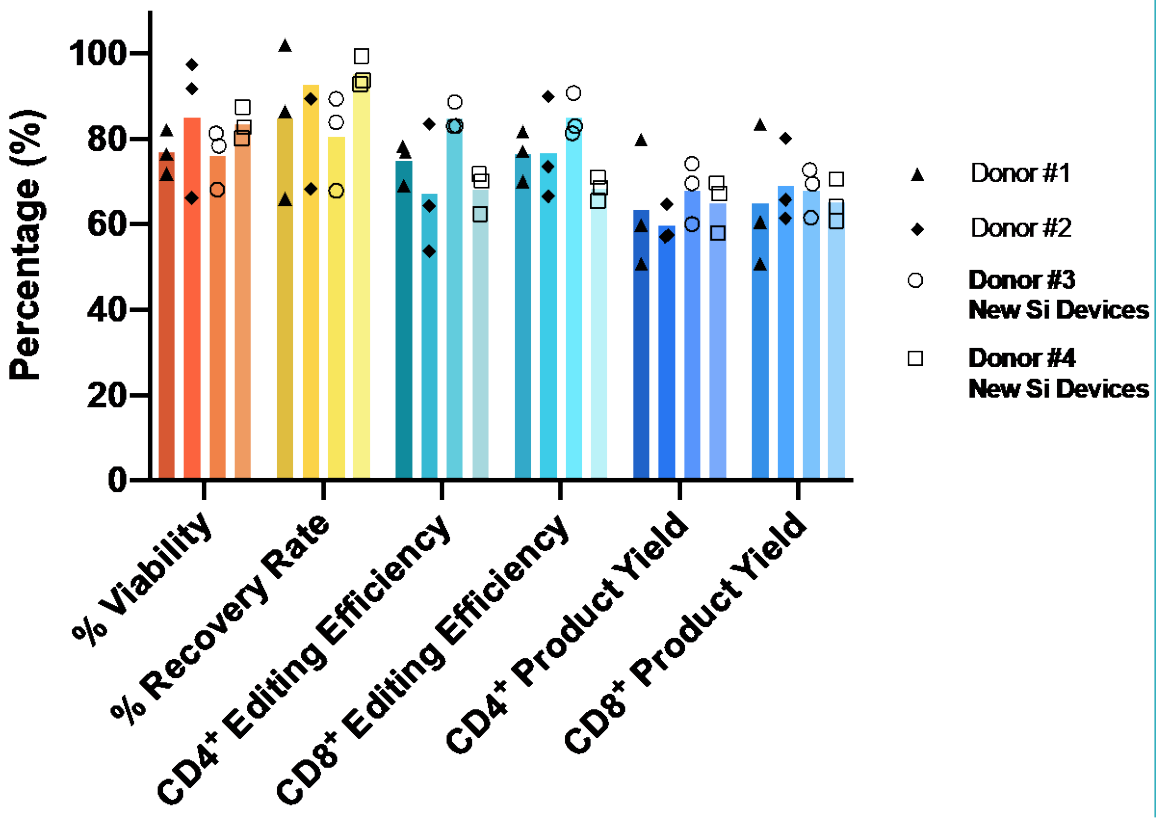
Sulchek BioMEMS & Biomechanics Lab

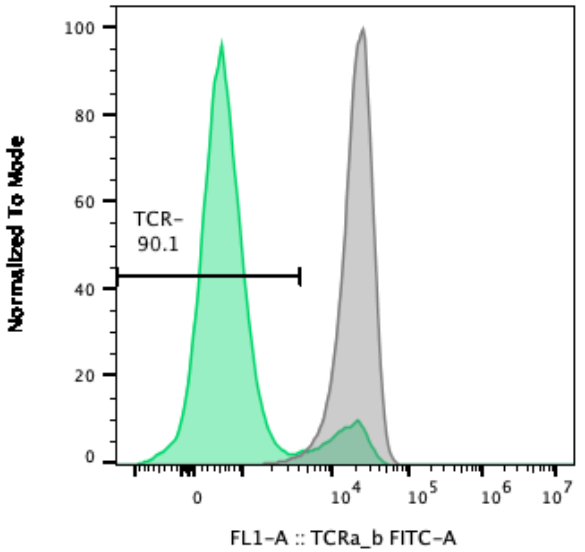
CellFE

High Efficiency RNP Mediated Knock Out in T Cells and Consistent Across Donors

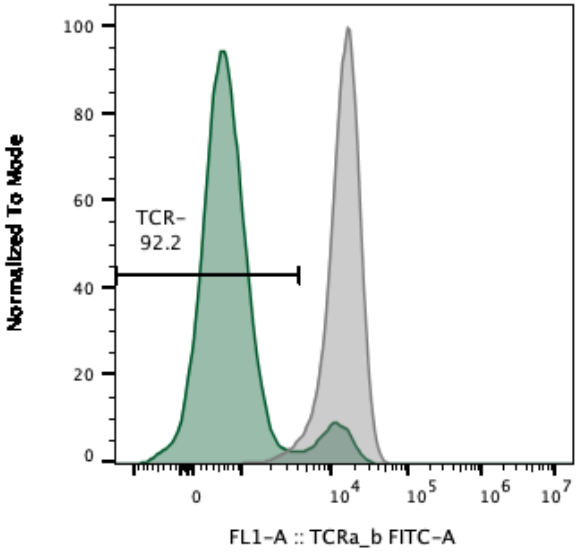
RNP Transfection in T Cells



CD4+ Cells



CD8+ Cells



CD4+ Population CD8+ Population Control Population

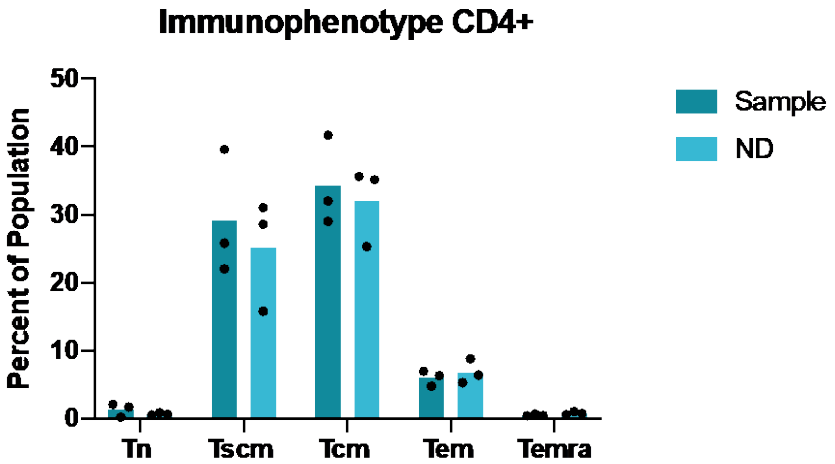
n = 3
5+ days post-transfection

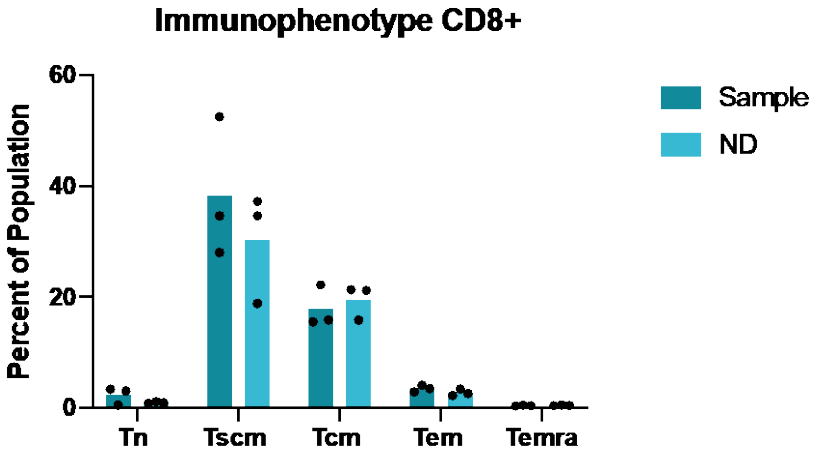
Highly efficient delivery of CRISPR/Cas9 RNPs, as measured by the generation of TCR KO T cells.

Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

T Cell Immunophenotype after Transfecting RNP for TCR KO (permanent modification)

Immunophenotype



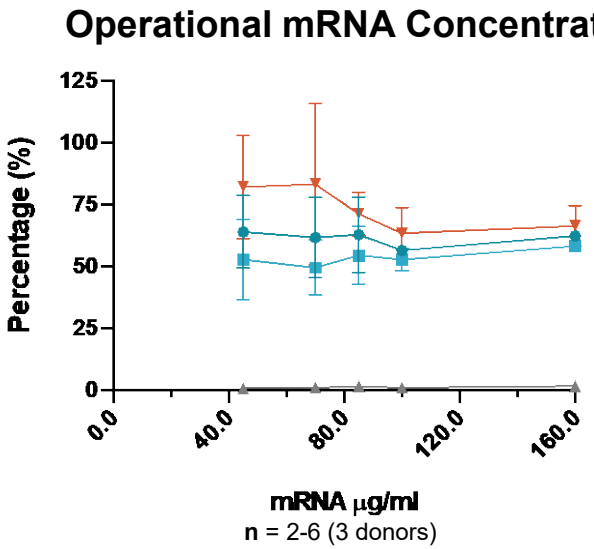


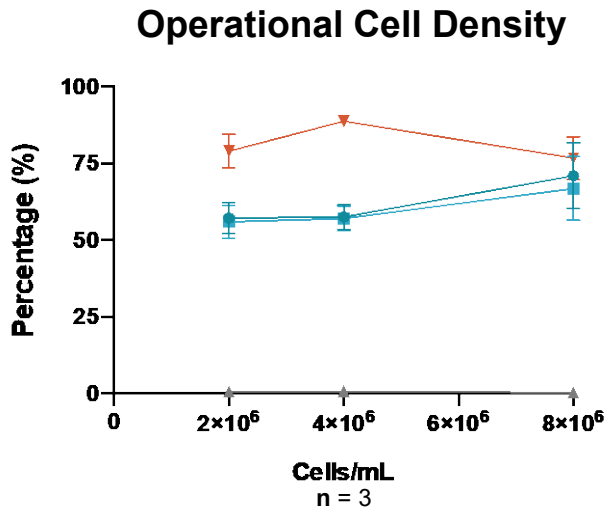
	Tn	Tscm	Tcm	Tem	Temra
CD45RA	+	+	-	-	+
CD45RO	-	-	+	+	-
CD62L	+	+	+	-	-
CD95	-	+	+	+	+

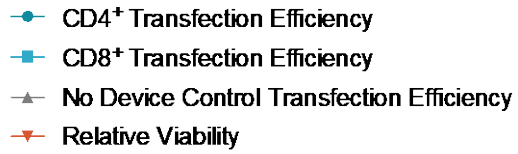
Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

Flexible in a Wide Range of Cell Densities and Payload Concentrations

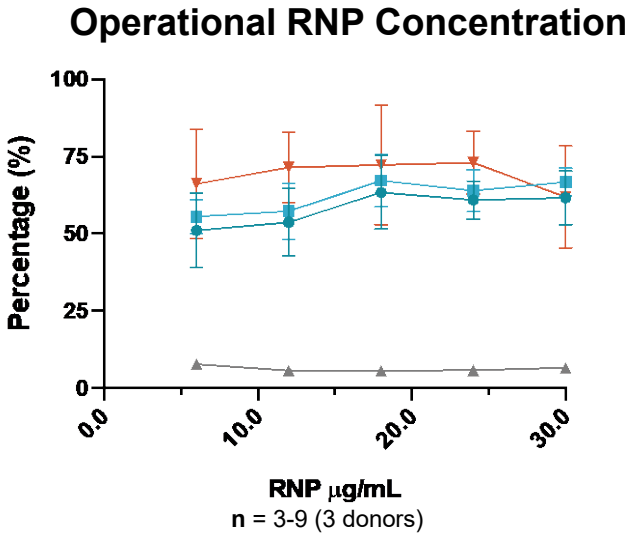
mRNA
Transfection in T Cells

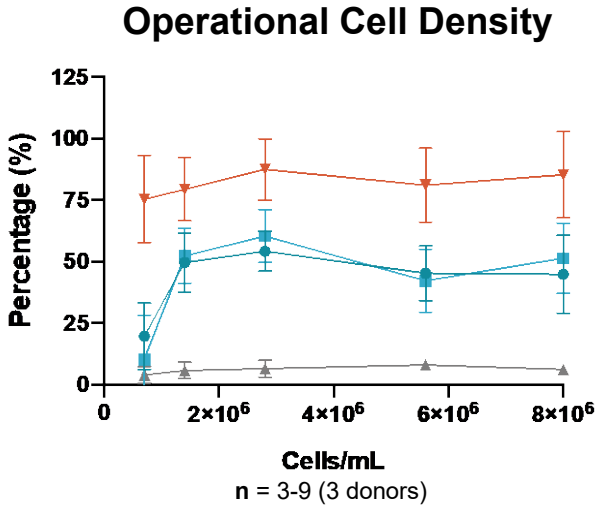


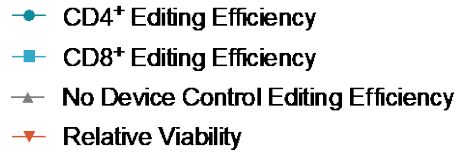




CRISPR/Cas9 RNP
Transfection in T Cells





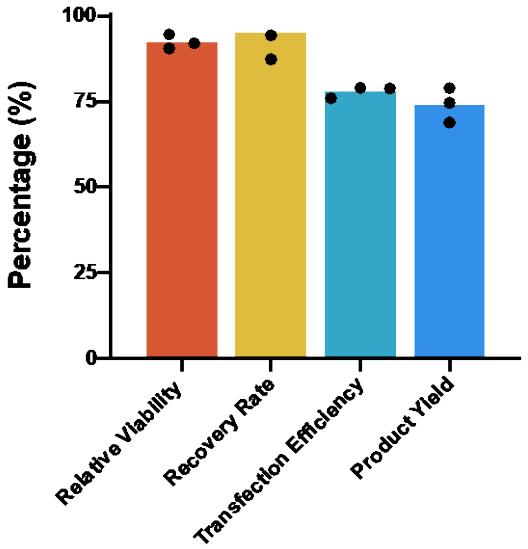


- Transfection is successful with as little as 40µg/mL mRNA, or 6µg/mL RNP.
- Consistent mRNA and RNP transfection with sustained high viability across a wide range of cell concentrations.

Data set courtesy: Dr. Sewoon Han (CellFE)

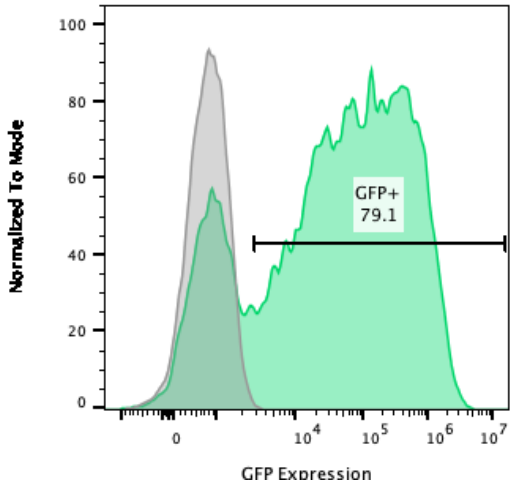
Delivery and Co-Delivery of mRNA into Primary Human CD34+ HSPCs

mRNA Transfection in CD34⁺ HSPCs

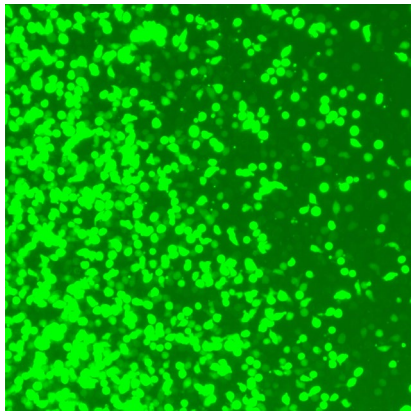


n = 3
24 hrs post-transfection
Recovery Rate = Output Live Cell # / Input Live Cell # x 100%
Product Yield = Recovery Rate x Transfection Efficiency

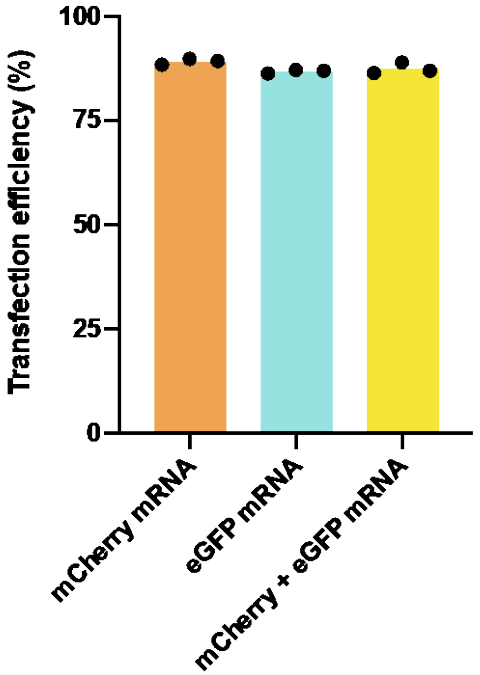
Gray: No Device Control
Green: VECT Transfected Cells

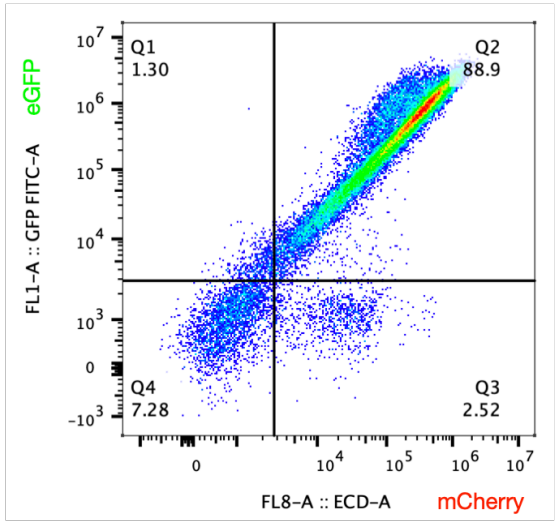


Representative image of VECT transfection in HSPCs



mRNA Co-Transfection in Human CD34⁺ HSPCs



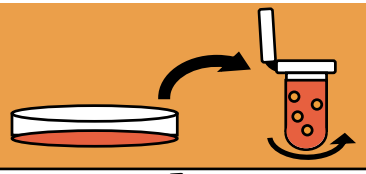
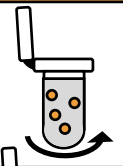
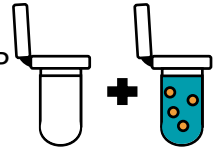
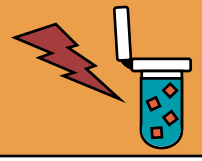
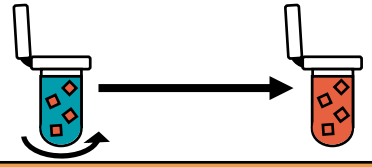
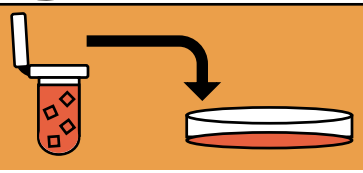


n = 3
24 hrs post-transfection

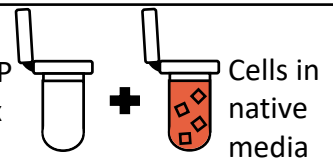
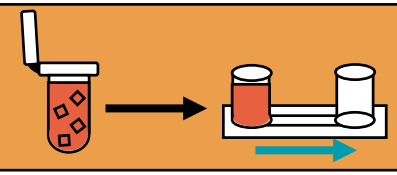
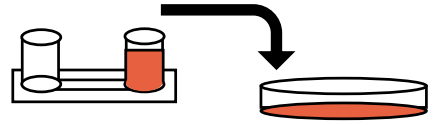
- Highly efficient delivery of mRNA into HSPCs
- High viability and recovery of the cell product
- Co-transfection is equally successful, and results in co-localization of both mRNA molecules

Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)

Simplified Workflow Enables Rapid Manufacturing

Spin down cells	
Wash cells with DPBS	
Aspirate DPBS and resuspend with electroporation reagent	
Add Cells to CRISPR/Cas9 RNP Complex	
Electroporate Cells	
Perform media exchange on cells	
Add cells to pre-incubated media in well plate	

VECT with CellFE Device

Add Cells to CRISPR/Cas9 RNP	
VECT Cells through CellFE Device	
Add cells directly to well plate	

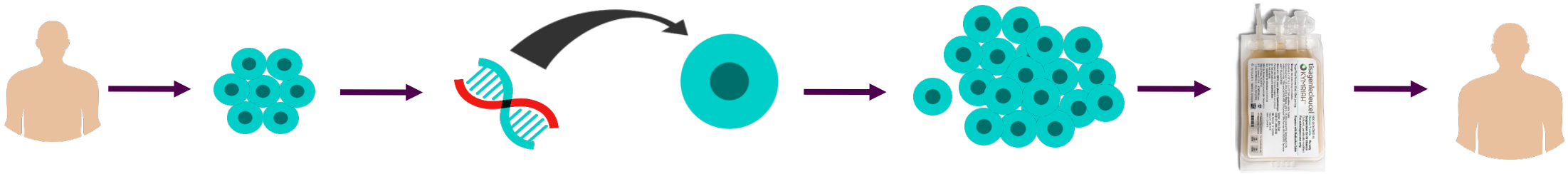
The cell therapy roadblocks

Cell Isolation

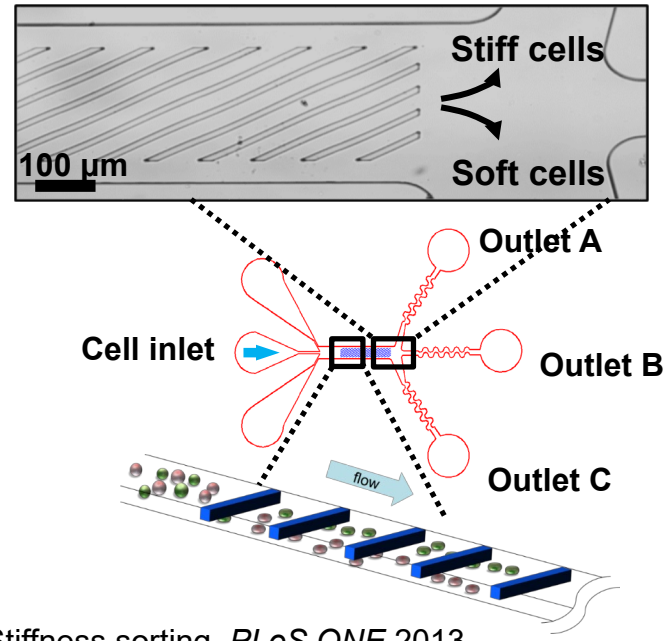
Cell Engineering

Cell Expansion

Formulation

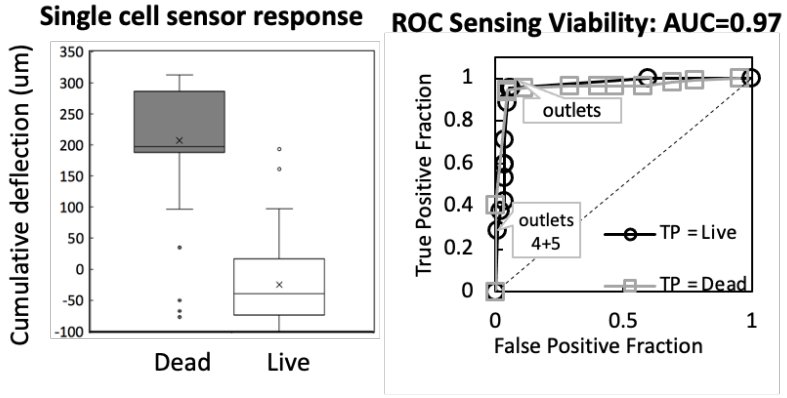


Label-free Separations



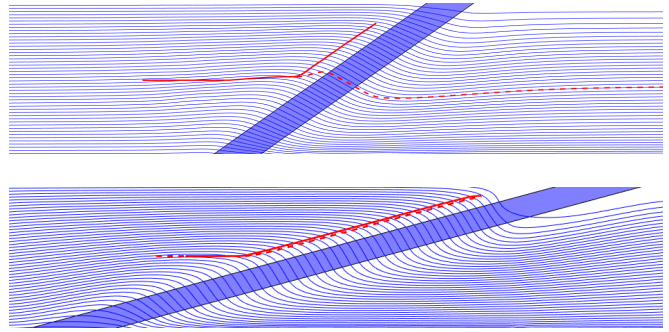
- Wang, Stiffness sorting, *PLoS ONE* 2013.
- Wang, Viscoelastic sorting, *Lab on a Chip* 2014.
- Islam, Viability-based sorting *Scientific Reports*, *Cell Death and Disease* 2017, 2018
- Tasadduq, Size sorting, *Scientific Reports* 2017
- Bongiorno, Stemness-based sorting, *PLoS One* 2018
- Stone, photoreceptor cell isolation, *Experimental Eye Research* 2020

Label-free Sensing



- Chrit *et al*. (in review). Real-time viability sensing
- Tasadduq, Continuous adhesion-based sensing, *Analytical Chemistry* 2017

Media Exchange



- Hanasoge *et al*. (in review). Liquid exchange

A unique opportunity to apply microfluidics technologies to integrate workflows