New mechanical approaches to perform cell transfections

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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

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

Currently used approaches

Viral Delivery

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

Chemical Membrane Permeabilization

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

Flow-through electroporation
The use of mechanical forces to deliver payloads


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

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

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

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:
\[ D = \frac{T}{6\pi\eta} \]

Diffusive-driven dextran delivery

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

Szeto, Sci Reports 2015
Active **molecular delivery** without electric fields—volume exchange for convective transfections (VECT)

- 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

Liu, *Materials Today* 2018
Delivery mechanism hypothesis: compressed state of cell due to rapid deformation

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

Liu, Small 2019
Design of “mechanical programs” to hone mechanical and hydrodynamic forces to elicits desired cell responses

1) Ridge gap height is key parameter

Computational modeling of solid and fluid responses in channel

2) Yet, ridge spacing can be tuned to cell relaxation response
   - Step 1: volume loss
   - Step 2: convective delivery via cell relaxation

Channel length $l$, Channel width, $w$, Channel height $H$, Gap height, $h$
Ridge angle, $\alpha$, Ridge spacing, $L$, Ridge width, $b$, Number ridges, $N$

Cell viscous responses

courtesy Prof. Allen Liu, U Michigan

Mean Fl Intensity vs. Ridge Gap ($\mu$m)

Mean Fl Intensity (A.U.) vs. Time between ridges (ms)
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
- IncRNA

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

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
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.
Productization and Operational Capacity
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
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.

**Data set courtesy: Dr. Sewoon Han (CellFE)**

**Total cell number: 100M cells**

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

![Graph showing viability and transfection percentages](image)

- Viability: 90%, 81.7%, 80.6%
- CD4⁺ Cells Transfection: 100%, 81.7%, 80.6%
- CD8⁺ Cells Transfection: 90%, 81.7%, 80.6%
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**

- **FUNCTIONAL, HEALTHY CELLS**
- **HIGHLY SCALABLE**
- **PAYLOAD AGNOSTIC**

**CHALLENGES ADDRESSED**

**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

**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

- 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?

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

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

- **Sulchek BioMEMS Group:** Anna Liu, Dr. Muhynim 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

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- 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.

**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 hematopoietic stem cells using ultra-fast physical deformations”, Loo et al., in review.

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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)
**Immunophenotype**

**Immunophenotype CD4+**

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**Immunophenotype CD8+**

Data set courtesy: Dr. Miguel Calero-Garcia (CellFE)
Flexible in a Wide Range of Cell Densities and Payload Concentrations

**mRNA 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.

**CRISPR/Cas9 RNP Transfection in T Cells**

- Data set courtesy: Dr. Sewoon Han (CellFE)
Delivery and Co-Delivery of mRNA into Primary Human CD34+ HSPCs

- 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)
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

Add Cells to CRISPR/Cas9 RNP Complex

VECT Cells through CellFE Device

Add cells directly to well plate

Simplified Workflow Enables Rapid Manufacturing

VECT with CellFE Device
The cell therapy roadblocks

Cell Isolation  →  Cell Engineering  →  Cell Expansion  →  Formulation

Label-free Separations

- Stiff cells
- Soft cells

Label-free Sensing

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

Media Exchange

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

A unique opportunity to apply microfluidics technologies to integrate workflows