Introduction

Cell Squeeze® Technology Enables Efficient Primary Immune Cell Editing with Preserved Function

- With our microfluidic approach to intracellular delivery, cell membranes are deformed as the cells pass through constrictions in channels, resulting in payload diffusion from the surrounding buffer directly into the cytosol (1).
- The translational potential and scalability of cell-based therapies are often limited by complications related to effective engineering and manufacturing functional cells, including low efficiency, cytotoxicity, and off-target effects (2).
- Cell Squeeze® technology reduces cell toxicity and off-target effects observed with conventional delivery methods by eliminating the need for electrical fields or exogenous vectors such as viruses, differentiating our approach as potentially transformative for enabling cell therapies that have been historically challenging to engineer.

Complex Cell Engineering Capabilities Enabled by the Cell Squeeze® Technology

- Cell Squeeze® technology can efficiently deliver a variety of cargo types including proteins, peptides, nucleic acids, ribonucleaseprotein (RNP) complexes, and large molecules including whole AAVs.
- Co-delivery of multiple cargos of the same or different molecule type may be delivered in a single mechanical deformation with minimal loss in delivery efficiency of either cargo.
- We developed a sequential squeezing method in which cells may be repeatedly squeezed in order to introduce multiple cargos with increased efficiency, preservation of cell function, and desired phenotype.
- By using mechanical deformation via Cell Squeeze® technology, we can achieve efficient multi-gene editing with minimal perturbation of gene expression or cytokine secretion, and preserved T cell function in vitro.

Cell Squeeze® Technology Preserves Cell Function While Electroporation Non-specifically Activates T Cells

- Human T Cell Proliferation is Unaffected by Cell Squeeze® Technology but Delayed Following Electroporation
- No Disruption in Cytokine Secretion Following Squeeze
- Activated Human T Cells Proliferate Similarly to Untreated

High Efficiency Multiplex Engineering of Cells and Materials Using Cell Squeeze® Technology

- Gene Misregulation Also Observed Following EP in CD34+ Cells
- Broad Applicability of Genome Engineering Beyond T Cells

Summary

- Cell Squeeze® technology enables delivery of genome engineering material to primary T cells with minimal effect on cell phenotype and health.
- Squeezed cells perform more similarly to untreated cells in vitro in terms of gene expression profiles, cytokine production, and expansion rate.
- In contrast, electroporated cells exhibit large changes to gene expression which include cytokine upregulation and delays in expansion.
- Similar Squeeze performance and characteristics in a variety of cell types suggests broad applicability.
- Multiplexed editing is efficiently achieved through sequential squeezes.
- Both multiplexed and sequential applications of Cell Squeeze® technology allow for broad uses in genome engineering.
- Sequential application of genome editing reduces potential for risk of multiple simultaneous cuts with minimal effect on cell health and expansion.