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A new benchtop system for simple and versatile introduction of macromolecules into human lymphocytes by microfluidic squeezing

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Intracellular delivery of molecules is a key step in biological research that enables the development of emerging generations of cell and gene-based therapies. However, several limitations in existing delivery approaches dramatically restrict the range of deliverable cargos and cell types used to study and treat disease. Furthermore, dysregulated gene expression caused by traditional intracellular delivery methods that can lead to altered cell biology are often overlooked. To address these challenges, we have developed a benchtop research-use-only (RUO) device for gentle, yet efficient delivery of cargos to a range of primary cell types at research scales.

The RUO system is a vector-free, microfluidic platform that relies on mechanical deformation of the cell membrane (also known as mechanoporation) to facilitate intracellular delivery of target materials, including small molecules, nucleic acids, and proteins. This Cell Squeeze® process creates transient disruptions in the plasma membrane that readily enable cargo diffusion directly into the cytoplasm.

We have developed a detailed workflow for use of the system, including cell isolation and resting, cargo preparation, and delivery optimization parameters. These optimized parameters were subsequently applied to successfully deliver eGFP and mCherry mRNA to unactivated T and NK cells, as well as Cas9 gene editing complexes (ribonucleoproteins) targeting *B2M* and *TRAC* genes to unactivated T cells. Functional studies of edited T cells were also performed in order to assess the impact of the RUO system on overall cell quality. Notably, we demonstrated the ability of unactivated T cells manipulated by the system to undergo similar activation and expansion when compared to unmanipulated samples. Furthermore, gene expression analysis revealed that cells manipulated using the RUO system had minimal transcriptional perturbations compared to a high degree of dysregulation measured in cells that had undergone traditional electroporation.

These results underscore the important considerations that need to be made when choosing a suitable delivery method in order to retain appropriate cellular response and function. Efficient yet gentle delivery of cargos to primary cell types by the RUO system offers significant advantages over traditional delivery methods. Particularly, direct cytosolic delivery of macromolecules enabled by the system provides an avenue for multiplexed delivery of heterogeneous cargos. The RUO system provides researchers with a simple and familiar workflow that can be easily integrated as part of their cell therapy research.