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Intracellular delivery of mRNA to human primary T cells with microfluidic vortex shedding

Ryan Pawell
Indee Labs, USA

Intracellular delivery is a critical process in biology and medicine. During intracellular delivery, different constructs (e.g., functional macromolecules such as DNA, RNA, and protein, and various complexes) are delivered across the cell membrane and into the cytosol. Herein, we use a microfluidic post array to induce hydro-dynamic conditions for cell membrane poration with microfluidic vortex shedding (μVS). μVS is used for the intracellular delivery of mRNA to primary human pan T cells. The specific microfluidic device used in this study contains a 960 μm by 40 μm deep flow cell capable of processing more than 2x106 cells per second at volumes ranging from 100 μL to 1.5 mL. We demonstrate efficient mRNA expression of enhanced green fluorescent protein (EGFP) (e.g., 57.4±6.8% of viable, recovered cells, mean ± stdev) after mRNA delivery to pan T cells derived from human PBMCs. High cell viability (e.g., 83.7±0.7% of recovered cells), high cell recovery (e.g., 96.3±1.1% of processed cells), and minimal alteration to T cell activation profile were characteristics of these EGFP expressing pan T cells. The optimal yield were observed at mRNA concentrations of 80 μg ml-1. These results demonstrate that μVS is a rapid intracellular delivery platform with promising potential for cytosolic delivery of mRNA to human primary T cells for clinical applications, where larger volumes of cells are required and demonstrated value and for research applications, where rapid screening and minimal reagent consumption is preferred.

taylor@indeelabs.com