AUSTRALIAN SOCIETY FOR BIOPHYSICS

Abstract: 186P

A hybrid approach to membrane disruption based intracellular delivery

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Membrane disruption based intracellular delivery enables the delivery of cargo to cells prior to complete membrane recovery. These approaches are used for delivery of various antibodies and diagnostic molecules, and enable the transfection of cells for various therapeutic, bioindustrial and research applications. Yet current approaches such as electroporation or mechanoporation suffer from heterogeneous cell damage, often having to choose between high cargo delivery with poor cell recovery or visa-versa [1, 2].

Using a hybrid system consisting of sequential mechanoporation and electroporation, we aim to develop a device that exposes cells to synergistic approaches to membrane stress. Here we show how mechano-electroporation (MEP) augments the delivery outcomes compared with using each technique individually, as seen in Figure 1. FITC dextran (4, 50, 150 and 2000kDa) was delivered into three leukocyte lines. Mechanoporation was achieved via mechanic shock by extrusion through micropores <5µm. Then, cells were electroporated using a Neon™ Transfection System. Final delivery and viability were measured using flow cytometry, and cytotoxicity was measured via LDH assay.

The MEP system saw >50% successful delivery of all sizes of cargo into the immune cell representative lines, with peak delivery ~90% when introducing 70kDa FITC dextran to MOLM-13 cells. This delivery was achieved with cells retaining >85% viability. The delivery of different sized dextrans varied, however, all cargoes were successfully introduced. Additionally, variation between replicates was smaller compared to mechanoporation or electroporation alone. Increased cytotoxicity was not observed 48 hrs after MEP, suggesting minimal long-term membrane damage.

MEPs uniform treatment across replicates suggests the permeabilisation is more homogenous compared to the individual techniques separately. Mechanoporation squeezes the cell, applying critical areal strain to the membrane and damaging the actin cytoskeleton causing pores to form [3].

It is hypothesised that when using electroporation after mechanoporation, the former has its cargo delivery amplified because of the compromised cytoskeletal structure [4]. Additionally, charged cargo delivery is hypothesised to be facilitated by the electrophoretic effect created by the pulse, leading to more homogenised delivery between cells than mechanoporation alone [5]. Considering its non-specific and more homogenised delivery, MEP provides an affordable alternative to other methods such as liposomal and chemical-carrier delivery options.

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