FRESENIUS KABI

PORTAL

Scaling Multiplexed Cell Engineering

with Portal's MilliBooster and Fresenius Kabi's Lovo® Cell Processing System[1]

Overview

The successful development of cell therapies demands precise cell engineering and scalable, efficient processes. To address these needs, Portal and Fresenius Kabi have partnered to reduce costs and accelerate timelines, enabling seamless translation from small-scale to large-scale manufacturing of next-generation cell therapies. This application note highlights the advanced cell engineering enabled by Portal's mechanoporation technology, which integrates effortlessly with Fresenius Kabi's robust suite of cell therapy instruments—supporting consistent, high-performance results across scales.

Cell Engineering with Portal

Portal's proprietary mechanoporation technology uses mechanical force to deform the cell membrane and allow for delivery of cell engineering cargo into the cytoplasm of the cell (Fig 1). Cargo such as CRISPR RNPs, mRNA, and circular RNA are all efficiently delivered using Portal's technology. One particular advantage of Portal's delivery method is enabling the delivery of multiple mixed cargos simultaneously. For example, in unstimulated human T cells, CRISPR RNPs and mRNA delivered with a dextran tracer yield a combined efficiency of nearly 86% triple positive cells (Fig 2 A&B). Portal's technology is also compatible with newer generation materials, such as circular RNA. In one proof of concept experiment, circular RNA for a CD19 CAR and a membrane-bound IL-12 enhancer (Fig 2 C&D) were delivered simultaneously to create enhanced CAR-T cells. We observe good correlation of CD19 CAR and mbIL-12 expression, and efficient killing >90% at an E:T ratio of 3:1.

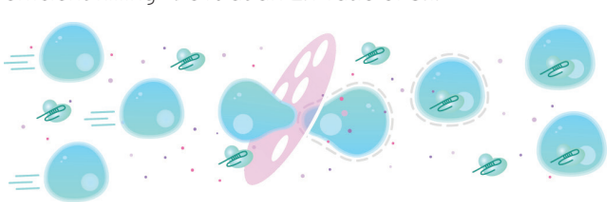

Fig 1. Portal's technology enables rapid mechanical deformation of cells to induce temporary membrane disruption and enable delivery of diverse cargo.

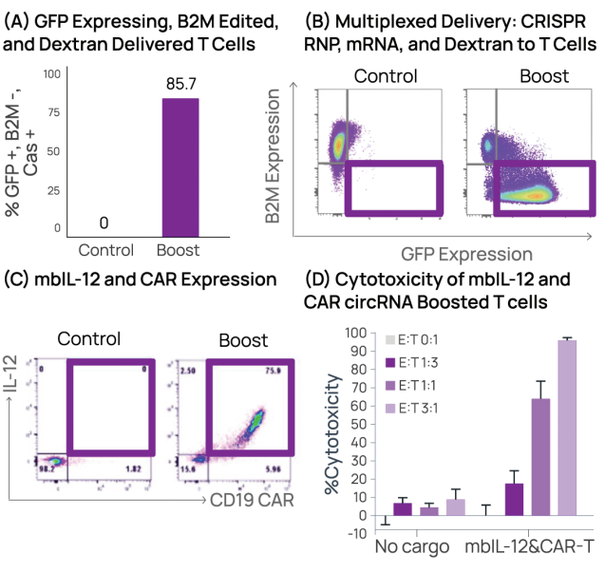

Fig 2. Unstimulated T cells mechanoporated with a mixture of CRISPR RNPs targeting B2M, GFP mRNA, and fluorescent dextran tracer yield a combined delivery efficiency of 86% **(A)**, demonstrating high correlative delivery by flow cytometry **(B)** for all three cargoes. Further, activated T cells mechanoporated with with CD19 CAR circular RNA and membrane bound IL-12 circular RNA yield 76% double positive cells for expression of both transcripts **(C)**. A cytotoxicity assay revealed killing efficiencies >90% at an effector:tumor ratio of 3:1 in cells expressing CD19 CAR and mbIL-12 **(D)**.

Translating to Clinical Scale with Fresenius Kabi

The simple delivery mechanism and controllable parameter space (pore size and cell speed) of mechanoporation enables seamless transition across scales, thus enabling a faster, more de-risked path to the clinic. In our collaborative studies to illustrate scalability, PMBCs were mechanoporated with dextran at both research scale (5M cells) on Portal's Gateway instrument and scaled up to Portal's clinical scale MilliBooster integrated in-line with Fresenius Kabi's Lovo Cell Processing System (400M cells) (Fig 3). Results were consistent across scales: there was good maintenance of viability (> 75%) and high dextran delivery (> 65%) for both Gateway and Lovo runs (Fig 3 A&B).

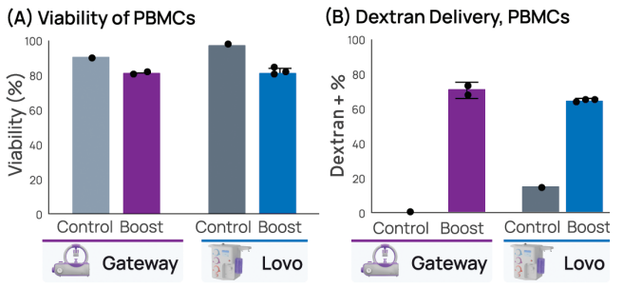

Fig 3. Human PBMCs were mechanoporated to deliver fluorescently tagged dextran tracer at research scale on Portal's Gateway instrument (5M cells) or at clinical scale using Portal's MilliBooster in-line with Fresenius Kabi's Lovo Cell Processing System (400M cells). PBMC viability was comparable at both scales at >75% **(A)**, and dextran delivery was also very similar at both scales >65% **(B)**.

Minimal optimization was required for scaleup; the pore size driving the mechanoporation process remained consistent across scales, and only minor optimization was needed to determine 40 mL/min as the preferred Lovo flow rate.

Scalable mRNA and RNP Delivery in T Cells

To showcase compatibility with a more complex workflow, we established a T cell scale-up study. We first optimized mechanoporation parameters for unstimulated T cells at research scale on Portal's Gateway instrument. T cells displayed maintenance of viability (>75%) and high (93%) mRNA expression (Fig 4 A&B). We then designed a more complex workflow in which we delivered a CRISPR RNP targeting B2M to unstimulated T cells at research scale using Portal's Gateway instrument followed by an activation and expansion step to obtain >10^9 cells (Fig 4D). Editing efficiency for the research scale edit was high (>60%) as measured at endpoint (Fig 4D). Cell expansion was robust following activation, and viability was well maintained (Fig 4 E&F). We then delivered GFP mRNA at scale using Portal's clinical scale MilliBooster integrated into the Lovo cell processing system (Fig 4D). Expression of mRNA was consistent with the high efficiency observed at small scale, with 94% expression in the activated T cells following Lovo mediated delivery (Fig 4G). This workflow demonstrates a multi-cargo and multi-scale delivery approach for potential use in cell therapy that is easily optimized and scaled for high performance.

From Research to Clinical Workflows

The breadth of compatible cell types and cargoes, coupled with the simplicity of Portal's scaling and integration with Fresenius Kabi's Lovo Cell Processing System illustrate how one can design complex cell therapies at research scale and reliably transition to clinical workflows. A simple sterile weld is sufficient to connect the Portal MilliBooster to the Lovo kit and enable this process. Through our ongoing collaboration, Portal and Fresenius Kabi intend to further simplify cell therapy production across scales while unlocking novel capabilities in cellular engineering to enable next generation therapies.

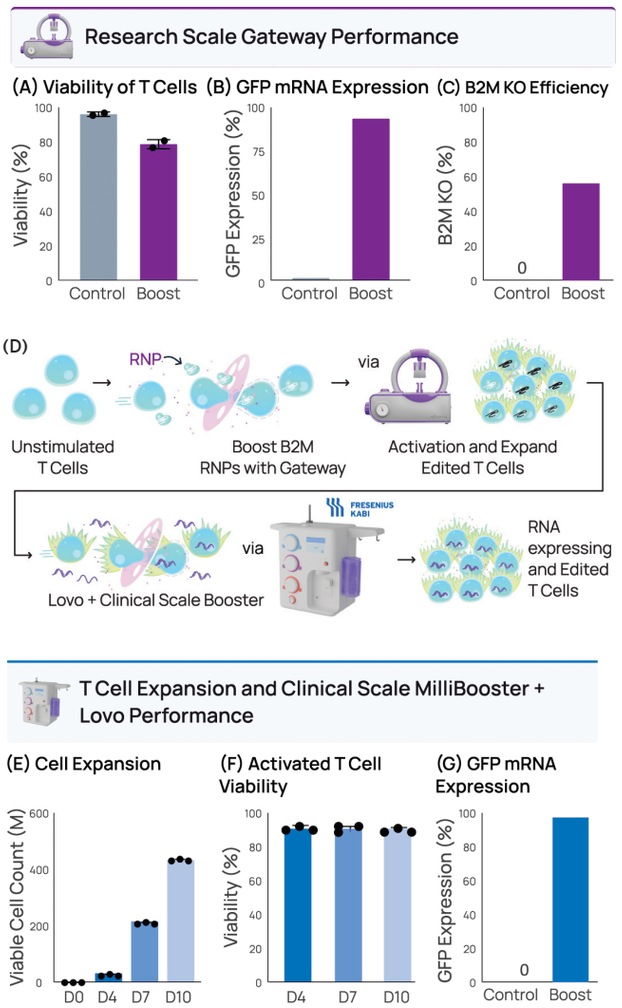

Fig 4. In a functional workflow, we first optimized mechanoporation parameters for unstimulated human T cells at research scale using Portal's Gateway instrument with GFP mRNA. Viability was >75% **(A)** and mRNA expression was 93% **(B)**. We then performed a scaled experiment in which we mechanoporated unstimulated human T cells with CRISPR RNPs targeting B2M using Portal's Gateway instrument at research scale, then activated and expanded before mechanoporating with GFP mRNA at clinical scale using Portal's MilliBooster integrated with Fresenius Kabi's Lovo Cell Processing System instrument **(D)**. Editing efficiency measured at the study endpoint was high (nearly 60%) **(C)**. Following the initial mechanoporation, cells exhibited robust expansion **(E)**, and maintained high viability throughout the study **(F)**. At endpoint, mRNA expression at clinical scale was 94% **(G)**, which was consistent with optimization at research scale.

The Lovo Cell Processing System is for non-clinical laboratory and research use only. User must obtain appropriate regulatory clearance for clinical use. Refer to the Lovo Cell Processing System Operator's Manual for a complete list of warnings and precautions associated with the use of these products.

Note: The term 'Boost', as shown in Figures 2-4 refers to cells that have undergone mechanoporation.

[1]Data on file Fresenius Kabi USA

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