Enabling Novel Assays with Intracellular Delivery on the HighRes PDII - Powered by Portal

Introduction to Portal Bio

Portal's technology uses mechanical deformation to deliver a variety of cargo types into diverse cell types while maintaining cell health. Cells are mechanoporated (or "boosted") through pores in a silicon membrane, causing temporary disruption of the cell membrane and allowing the target cargo to enter the cytosol before the cell membrane reseals. Portal has developed instruments for mechanical delivery across scales including the bench top research-scale R&D system (Gateway) and a high-throughput instrument (Galaxy) using simple cell type-specific consumables with a defined pore size for optimal intracellular delivery. The cell type-specific consumables also enable seamless integration into existing instrumentation at scale. This simple process enables high-throughput and scalable mechanoporation that has the potential to enable new work flows in both drug discovery and cell therapy.

Applications for High-Throughput Drug Discovery

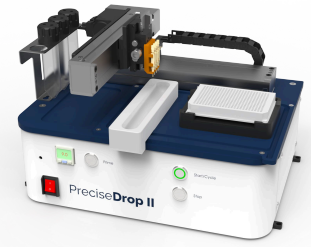
Many early discovery workflows are conducted in biochemical and lysate-based systems where the environment is unrepresentative of a live cell. Portal's approach to intracellular delivery enables direct-to-biology live cell assays, and the simple mechanical delivery strategy facilitates implementation at scale for automated screening. Using a single cartridge containing cell type-specific pores, Portal's mechanoporation technology can deliver multiple cargos simultaneously into cells across 96- and 384-well plates. This enables cytosolic delivery of otherwise impermeable cargo including small molecules, peptides, PROTACs, DELs, macrocycles, probes, nucleic acids, and CRISPR RNPs to both primary cells and cell lines. Portal's delivery technology supports early pharmacological validation of impermeable compounds in primary cells and cell lines while maintaining cell function.

Purpose of Experiments

The low operating pressures required for mechanoporation enable seamless integration of Portal's technology into existing fluidics. To address the need for delivery of impermeable cargo into cells for high-throughput screening, Portal and HighRes have partnered to integrate Portal's mechanoporation cartridges with the HighRes Biosolutions Precise Drop II dispenser. These experiments demonstrate the ability to consistently deliver diverse cargo classes into cell lines and primary cells at scale into multi-well plates using this integrated technology.

HighRes PDII with Portal Tech Integration

The integration with the HighRes Biosolutions Precise Drop II (PDII) Dispenser enables high-throughput screening by automated dispensing into plates pre-loaded with cargo. The Precise Drop II is a non-contact dispenser that accurately and precisely dispenses cells and other reagents from 50 nl to hundreds of milliliters with a simple interface. Integration of Portal's cartridges into common dispensers including the Precise Drop II enables dispensing into multi-well plates with increased throughput while minimizing errors. The existing fluidics in the Precise Drop II are used to apply pressure to the cells as they transit through the membrane in the Portal cartridges. Cargos are pre-plated and cells are mechanoporated ("boosted") through the in-line Portal cartridge immediately prior to dispensing into wells (Figure 1). Cell membranes remain open during the dispense to facilitate efficient material delivery upon entering the well. The simplicity of the mechanical delivery strategy facilitates implementation at scale and a simple integration with the HighRes Biosolutions Precise Drop II, requiring only the addition of the cartridge. The high degree of access to the dispensing manifold allows users to configure the device for consistent dispensing at a range of pressures, enabling consistent delivery across diverse cell types. This integration can deliver cargo into cells dispensed across multi-well plates for screening applications.



The Precise Drop II (PDII) is a low-volume, non-contact dispenser designed for efficient and reproducible cell and reagent dispensing as a part of genomics, drug discovery, and synthetic biology workflows.

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Figure 1:Enabling High-Throughput Screening by Automated Dispensing into Pre-loaded Cargo Plates

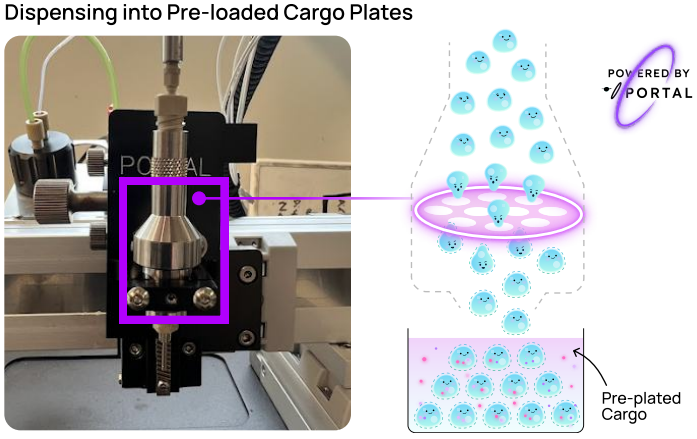

Figure 1: Enabling High-Throughput Screening by Automated Dispensing into Pre-Loaded Cargo Plates. The Portal cartridge (purple box) is integrated in-line with the existing HighRes Biosolutions Precise Drop II (PDII) (Left). Cells are porated as they transit through the silicon membrane into multi-well plates pre-plated with cargo (Right). The porated cells take up material upon dispensing into the well before membrane recovery.

Data and Conclusions

To showcase Portal's integration with the HighRes PDII dispenser, we simultaneously delivered several impermeable cargos to HeLa cells across five 96 well plates. 3 kDa fluorescent dextran polymer, fluorescently-labeled 21 base oligonucleotide, and fluorophore-conjugated IgG antibody were pre-plated in wells of 5, 96 well plates. HeLa cells were lifted into single cell suspension and boosted into the cargo-containing 96 well plates using the PDII dispenser integrated with the Portal mechanoporation cartridge. HeLa cells showed high intracellular co-delivery of all three cargos (>70% dextran and oligo delivery; >50% antibody delivery to live cells) that was consistent across 5 plates (Figure 2). Intracellular delivery using the PDII in-line integration was comparable to delivery using the Portal R&D system and significantly higher than the "no boost" control (<5%) in which cells were dispensed directly into the cargo without the in-line Portal cartridge. This shows that the in-line integration of Portal's mechanoporation technology enables consistent and high delivery of impermeable cargos of different sizes across several plates.

Figure 2: PDII In-line integration enables scalable and Consistent High-Throughput Delivery

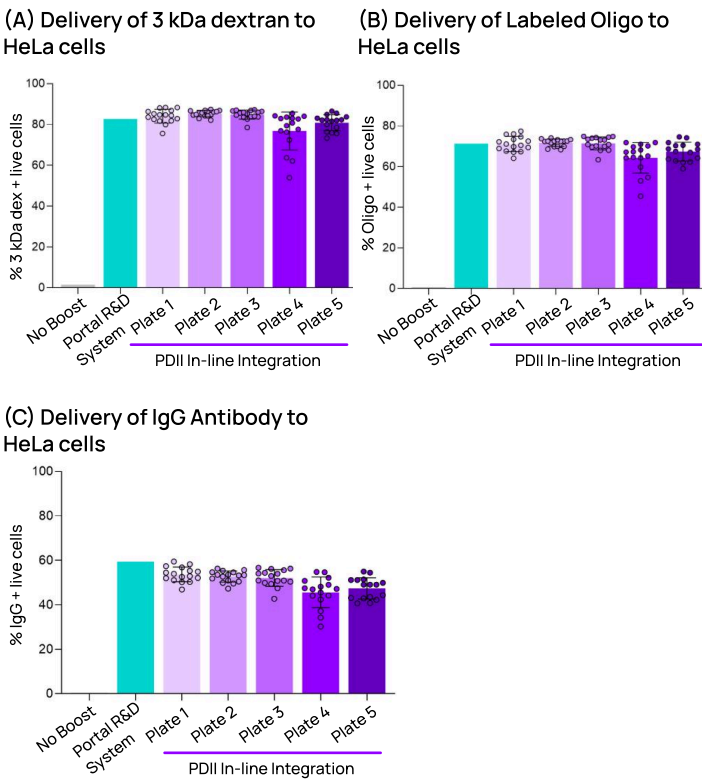

Figure 2: PDII In-line Integration Enables Scalable and Consistent High-Throughput delivery. HeLa cells were mechanoporated ("boosted") using either the Portal R&D system (blue) or the Portal cartridge integrated into the PDII dispenser into wells of 5, 96-well plates containing (A) 3 kDa fluorescently labeled dextran, (B) fluorescently labeled oligonucleotide, and (C) IgG antibody, each at 0.1 mg/ml. Intracellular delivery to live cells was measured by flow cytometry.

This performance is transferable to a wide variety of cell types. We boosted HEK293 cells into plates containing fluorescent oligo and IgG antibody through the Portal cartridge integrated with the PDII dispenser. The HEK293 cells demonstrated consistently high intracellular delivery across five plates (>60% oligo delivery, >50% antibody delivery), comparable to mechanoporation using the Portal R&D system (Figure 3A, B). We boosted human Peripheral Blood Mononucleated Cells (PBMCs) through the Portal cartridge integrated with the PDII dispenser into a 96-well plate containing fluorescently labeled dextran. Dextran was delivered to >70% of human PBMCs in both the PDII integration and Portal R&D systems, as compared to <5% in the no boost control (Figure 3C). Thus, the Portal cartridge integration into the PDII can be used for intracellular delivery of impermeable cargos into both cell lines and primary cells.

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Figure 3: Performance Transferable to HEK293 Cells and PBMCs

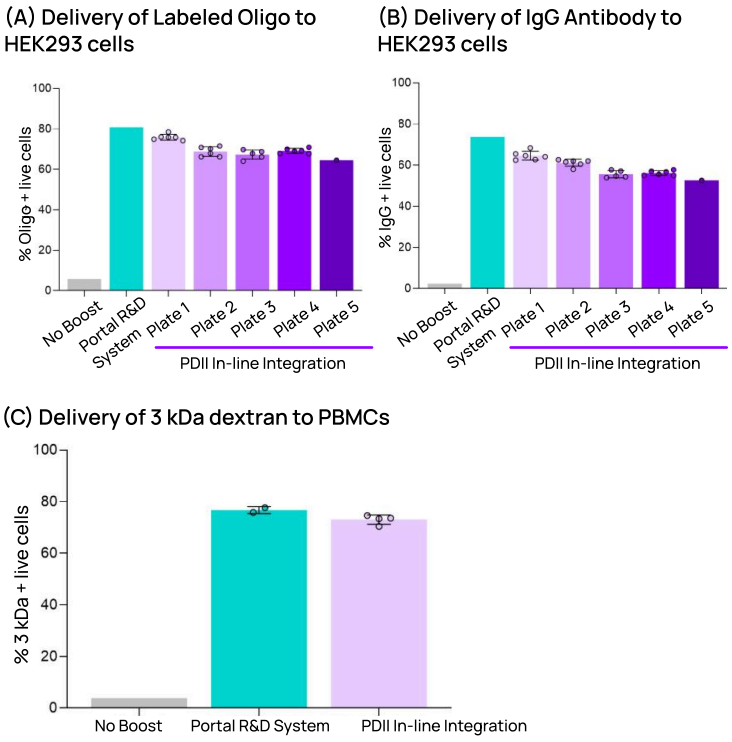

Figure 3: Performance transferable to HEK293 and Human Peripheral Blood Mononucleated Cells (PBMCs). HEK293 cells were mechanoporated ("boosted") using either the Portal R&D system (blue) or the Portal cartridge integrated into the PDII dispenser into wells of 5 96-well plates containing (A) fluorescently labeled oligonucleotide and (B) IgG antibody, each at 0.1 mg/ml. (C) Human PBMCs isolated via leukapheresis were boosted using either the Portal R&D system (blue) or the Portal cartridge integrated into the PDII dispenser into a multi-well plate containing fluorescently labeled 3 kDa dextran (0.1 mg/ml). Intracellular cargo delivery to live cells was measured by flow cytometry.

To demonstrate the delivery of complex cargo types, we delivered GFP mRNA to HeLa cells by boosting them through the Portal cartridge in line with the PDII dispenser into wells of a 96-well plate (Day 0). On Day 1 after mechanoporation, HeLa cell viability was consistently high (>90%) with >65% of cells expressing GFP (Figure 4A, B). In addition to consistent mRNA delivery across wells, mRNA expression was also high (Figure 4C, D). Portal's integration with the HighRes PDII enables consistent delivery of complex cargo for direct to biology screening.

In summary, Portal's mechanoporation cartridge can be seamlessly integrated with the HighRes Biosolutions Precise Drop II Dispenser for consistent intracellular delivery across cargo classes and cell types for high-throughput screening of impermeable cargos. The integration of these technologies enables live cell assays to provide novel insights, de-risk targets, and minimize probability of biological failures after lead optimization.

Figure 4: HeLa GFP mRNA Data

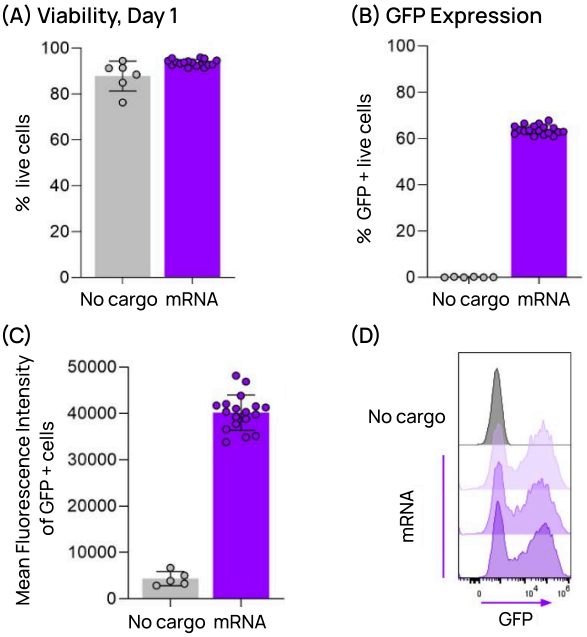

Figure 4: PDII In-line Integration Enables Delivery of Complex Cargo to Cells. HeLa cells were boosted using the Portal cartridge integrated into the PDII dispenser into wells containing either No Cargo or GFP mRNA (0.1 mg/ml). Flow cytometry was used to measure (A) viability and (B) GFP expression 20 hours after delivery (Day 1). C) Mean GFP fluorescence intensity (MFI) and histograms of GFP expression in boosted cells 20h after delivery.