

Scalable Continuous Flow Electroporation Platform

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Primary CNF Tools Used: Odd-Hour Evaporator

Abstract:

Viral vectors are a bottleneck in the manufacturing of cell therapies. To bypass viral vectors, electroporation has emerged as a non-viral transfection method for primary cells. However, standard cuvette-style approaches are limited by difficult optimization and incompatibility with large-scale cell manufacturing. Here, we present and fabricate a novel electroporation platform that can efficiently transfect small volumes of cells for research and process optimization and scale to volumes required for applications in cellular therapy. We demonstrate of messenger ribonucleic acid (mRNA) to primary human T cells with high efficiency and viability at research scale and we demonstrate seamless scaling of delivery by increasing experimental throughput by a factor of five.

Report:

To address limitations associated with cuvette-style electroporation, CyteQuest has developed a scalable electroporation platform to optimize transfection parameters and deliver cargo efficiently and reproducibly at high throughput. Our approach incorporates a single use, continuous-flow fluidic system designed to integrate with automated cell processing approaches.

The prototype electroporation flow cell consists of a planar flow chip with a thin slab geometry. It contains a single fluid inlet/outlet that receives cells suspended in electroporation buffer containing the cargo to be delivered. Electrodes are patterned on the top and bottom flow cell surfaces in order to apply a uniform electric field perpendicular to the flow direction (Figure 1A). The thin slab geometry of the device ensures that each cell is subject to the same electric field and the same chemical environment enabling reproducible electroporation. The shallow height (50 -100 μm) also means that we can achieve the necessary electric field strength to open pores in the cellular and nuclear membranes by applying relatively low voltages (~ 15 V) compared to the high voltage of traditional commercial systems. The width (1-100 mm) of the device is much larger than its depth to allow for

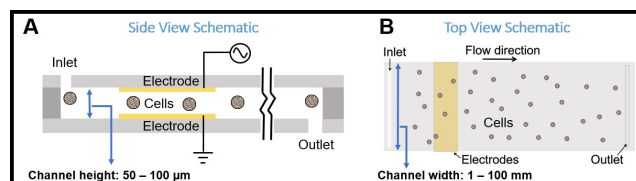


Figure 1: (A) Side view schematic of the flow cell (not to scale). (B) Top view schematic of the flow cell (not to scale).

rapid and continuous flow of the cells through the chip (Figure 1B). Importantly, the width of the device can be varied to match the desired experimental throughput without changing the electric field experienced by the cells. As such, our planar geometry enables us to seamlessly scale from small volumes of precious sample to determine optimal transfection parameters to large volumes for delivery at clinical scales.

Electroporation flow chips are constructed from a three-layer stack of polymer substrates. All three layers are laser cut with a small beam spot, high resolution carbon dioxide laser. The top and bottom layers, cut from 1 mm thick acrylic slabs, create the floor and sealing channel

surfaces. The middle layer is a spacer that defines the channel depth and width and is composed of a thin pressure sensitive adhesive tape. To fabricate the chip, the bottom and top acrylic layers are laser-cut into 1" x 2" pieces. The pieces are then laser-cut to add fluid inlet/outlet ports and alignment holes for use during the assembly process. Afterwards, a thin film electrode of gold is deposited by physical vapor deposition on the inside surface of each acrylic piece at the CNF using the odd-hour evaporator. The middle layer is cut to shape and also receives the corresponding alignment holes via the laser cutting process. The three-piece (acrylic, spacer, acrylic) sandwich assembly is then compression bonded in a press.

Cells with the cargo to be transfected are loaded into a syringe and injected through tubing into the flow cell by a syringe pump. As cells flow through the electrode pair, they experience a spatially uniform electrical field produced by a computer-controlled function generator and amplifier. The applied voltage waveform and resultant current are monitored via an oscilloscope. The electroporated cells then exit the flow cell and are dispensed into wells of a well-plate by a robotic fraction collector. Computer-controlled waveform selection and robotic sampling enable rapid sweeping of waveform parameters such as the voltage amplitude or waveform shape.

Using this system, CyteQuest has delivered mRNA encoding green fluorescent protein (GFP) to primary human T cells with high efficiency and high viability, observing > 95% transfection efficiency with < 2% loss of cell viability compared to control cells (Figure 2). To demonstrate the ability of our device to scale experimental throughput, we increased the width of the device from 2 to 10 mm and the volumetric flow rate from 320 $\mu\text{L}/\text{min}$ to 1.6 mL/min (Figure 3A-B). Scaling both the channel width and flow rate by a factor of five produced identical GFP expression and viability values in both channel widths for Jurkat cells transfected with mRNA encoding GFP (Figure 3B). Overall, these data demonstrate the ability of our platform to efficiently deliver mRNA to cells and seamlessly scale-up delivery without changing delivery performance.

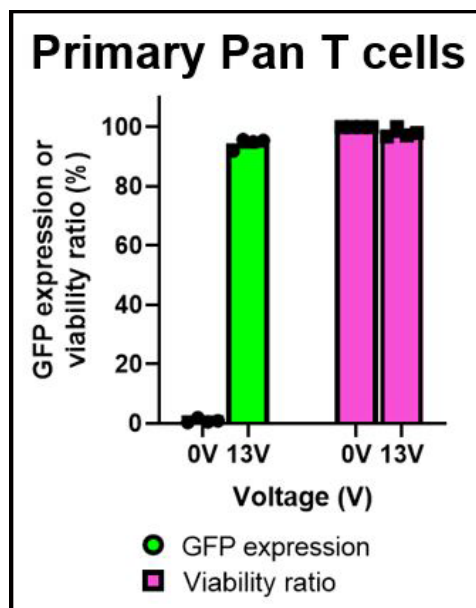


Figure 2: Delivery of mRNA to primary human T cells. Data from primary T cells from four healthy donors ($n = 4$). Data shown as mean \pm standard deviation.

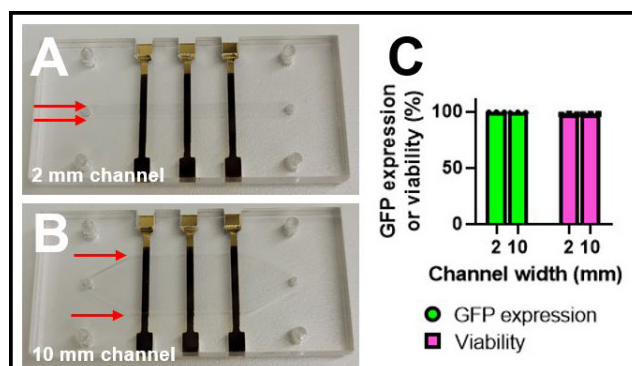


Figure 3: Scaled-up delivery of mRNA to Jurkat cells. (A) Photograph of a 2 mm and (B) 10 mm electroporation flow cell. Red arrows highlight the channel width. (C) Plot of GFP expression and viability values from Jurkat cells transfected with mRNA encoding GFP in either the 2- or 10-mm channels ($n = 3$). Data shown as mean \pm standard deviation.