Microfluidic Platform For High Throughput Single-Cell Drug Delivery Using Mechano-Electroporation And Real-Time Flow Control

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1 Abstract

The delivery of therapeutic molecules into individual cells is central to gene therapy, personalized medicine, and advanced diagnostics. Traditional techniques such as electroporation and mechanoporation face limitations regarding cell-type specificity, membrane integrity, and cell viability. In this work, we present a novel microfluidic platform that integrates mechanoporation and nano-localized electroporation with real-time flow control for efficient and scalable single-cell drug delivery. The system features piezoelectric actuation, embedded microvalves, and ITO-based electrode interfaces to provide precise intracellular access. The device maintains high cell viability while achieving controlled transfection with minimal user intervention, making it applicable in both research and clinical environments.

2 Introduction

Efficient intracellular delivery of biomolecules remains a major challenge in biomedical engineering. Electroporation, widely used to permeabilize cell membranes using electric fields, often necessitates high voltages, leading to potential DNA fragmentation or apoptosis. Mechanoporation, based on physically deforming cells through microchannel constriction, presents a safer alternative but has limited applicability for stiffer or less deformable cells. To overcome these limitations, we propose a hybrid microfluidic platform that integrates mechanoporation and localized electroporation in a single chip. The platform utilizes flow-based cell alignment, piezoelectric constriction, and low-voltage electric pulses to induce transient pores in the membrane, facilitating drug or gene delivery. Real-time microvalve control enables automation, making the platform suitable for high-throughput applications.

3 Objectives

The primary objective of this research is to design and fabricate a microfluidic device that enables high-throughput single-cell drug delivery through the sequential application of mechanical and electrical stimuli. Specifically, the system is intended to align and deform cells using constriction zones, followed by nanolocalized electroporation

via integrated electrodes. The goal is to improve cargo delivery efficiency while ensuring cell viability, cross-cell-type applicability, and reusability. A user-friendly and automated control scheme is also envisioned to minimize technical barriers and maximize operational consistency.

4 Device Model and Working Mechanism

The microfluidic system consists of an inlet for sample introduction, a constriction zone for mechanoporation, and an electroporation region with patterned ITO electrodes. Cells suspended in buffer are introduced into the device and guided toward the constriction region using pressure differentials controlled by pneumatic microvalves. The constriction causes mechanical deformation of the cell, weakening the membrane. A piezoelectric actuator (PZT) integrated into the channel walls is triggered at this point, enhancing membrane strain. Once the cell reaches the center of the constriction, upstream and downstream microvalves close to isolate the cell. A square-wave pulse is then applied through ITO electrodes (Figure 1), creating a localized electric field that transiently permeabilizes the membrane. This facilitates the diffusion of drug molecules into the cell.

Finally, the valves reopen and the treated cell is flushed toward the outlet. The full sequence, from loading to release, is shown in the flow process diagram (Figure 2).

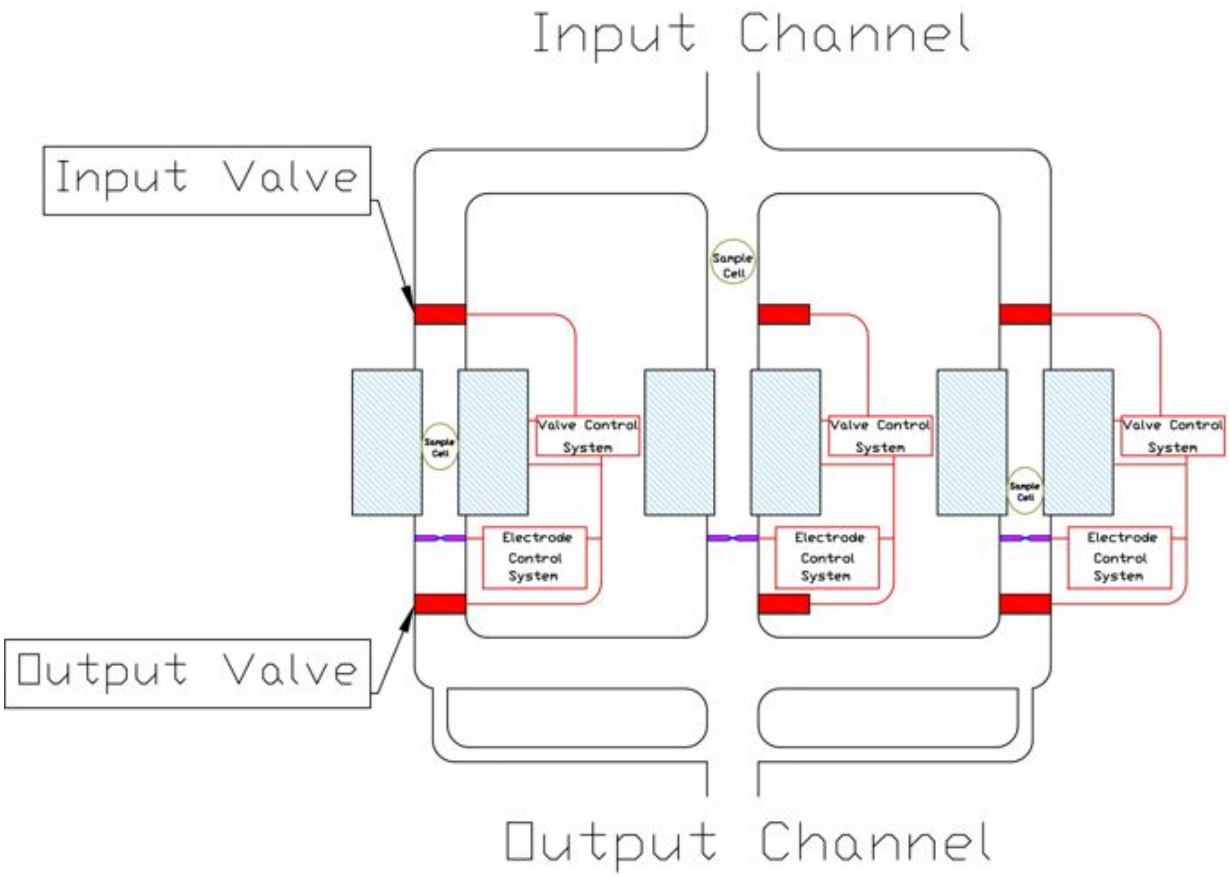

Figure 1: Schematic of the microfluidic device showing constriction zones, microvalves, PZT actuator, and ITO electrodes.

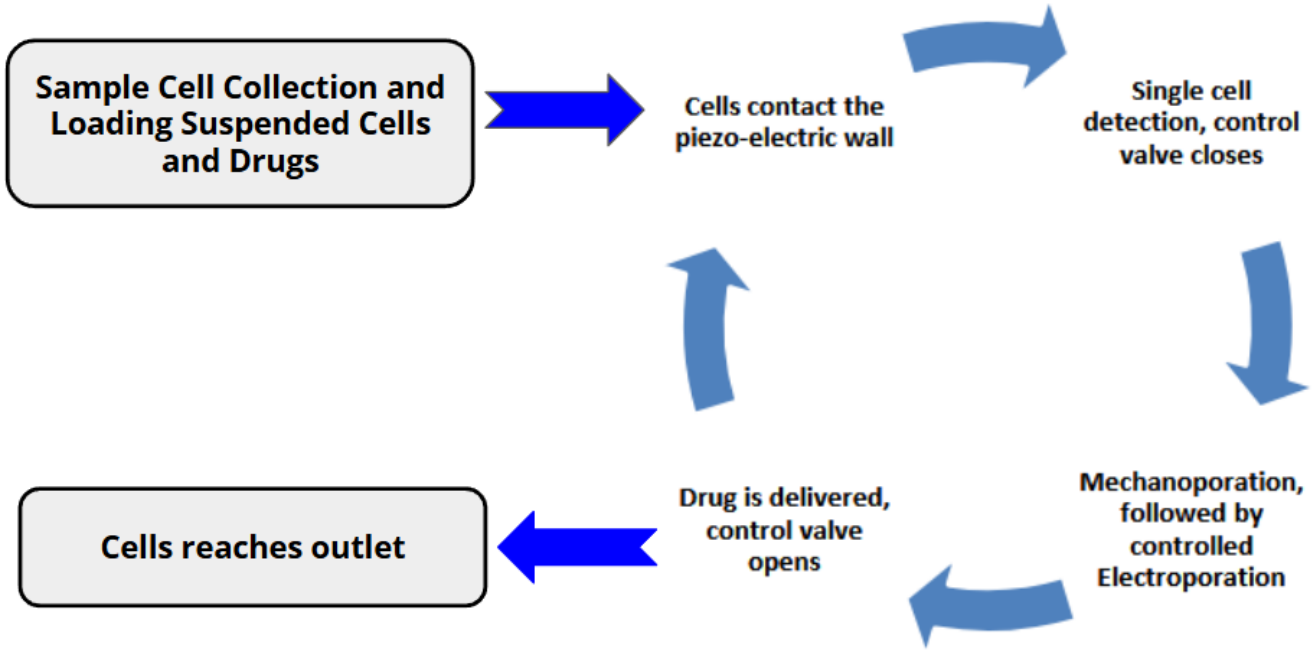

Figure 2: Flow diagram illustrating the sequence of steps in the single-cell drug delivery process.

4.1 Quake style pneumatic microvalves

The use of Quake-style pneumatic microvalves (Figure 3) is central to achieving precise and real-time control over fluid flow within the microfluidic device. These elastomeric valves, fabricated using multilayer soft lithography, consist of a control channel that runs perpendicular to the flow channel and is separated by a thin, flexible PDMS membrane. When pneumatic pressure is applied to the control channel, the membrane deflects into the flow channel, effectively sealing it off. Releasing the pressure causes the membrane to retract, thereby reopening the flow path. This mechanism allows for rapid and reversible modulation of fluid movement with high temporal resolution.

In the context of the single-cell drug delivery system, these valves play a critical role in cell isolation, synchronization of treatment steps, and precise metering of reagents. For example, as a single cell enters the constriction zone for mechanoporation, the valves upstream and downstream can be promptly actuated to trap the cell in place, ensuring precise electroporation. After the electric pulse is applied, the valves are released to allow the treated cell to flow toward the outlet. This level of fluidic control is essential not only for maintaining single-cell resolution but also for minimizing cell loss, cross-contamination, and ensuring reproducibility across cycles. Moreover, the integration of these valves supports automation and scalability, making the system suitable for high-throughput applications in biomedical research and therapeutic screening.

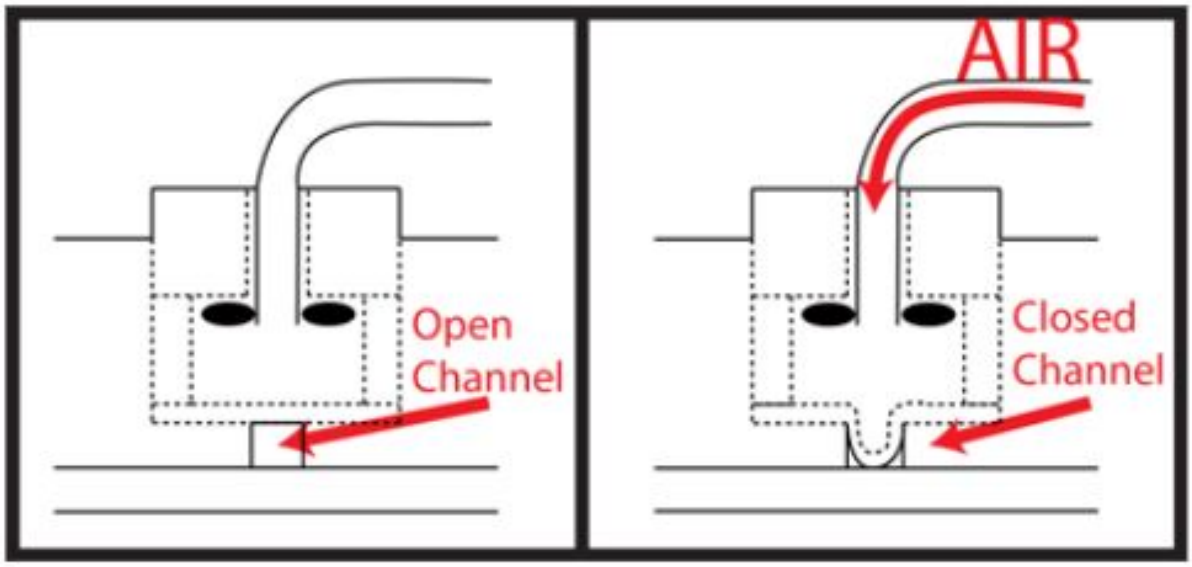

Figure 3: Cross-sectional illustration of a Quake microvalve used for flow regulation.

4.2 Square wave pulse generator

The square wave pulse required for electroporation is generated using a custom-built pulse generation circuit, as illustrated in Figure 4. This circuit is designed to produce short-duration, high-voltage square wave pulses with precise control over amplitude, frequency, and pulse width—parameters that are critical for successful and safe electroporation at the single-cell level.

Square wave pulses are ideal for electroporation because they enable a rapid rise and fall in voltage, which promotes the transient formation of nanopores in the cell membrane without prolonged exposure to the electric field. In this device, the circuit drives the patterned ITO electrodes embedded in the constriction zone, ensuring that the electric field is localized exactly at the site of mechanical deformation, where the membrane is already primed for poration. By tailoring the pulse width (typically in the microsecond to millisecond range) and voltage amplitude (typically below 1 kV), the system can induce reversible poration that allows drug or gene molecules to diffuse into the cytosol while preserving cellular viability. Additionally, the efficient design of the pulse circuit minimizes power consumption and thermal stress, which is especially important for continuous or high-throughput operation. The ability to precisely control and synchronize pulse delivery with cell positioning also enhances reproducibility and makes the platform adaptable to various cell types and molecular payloads.

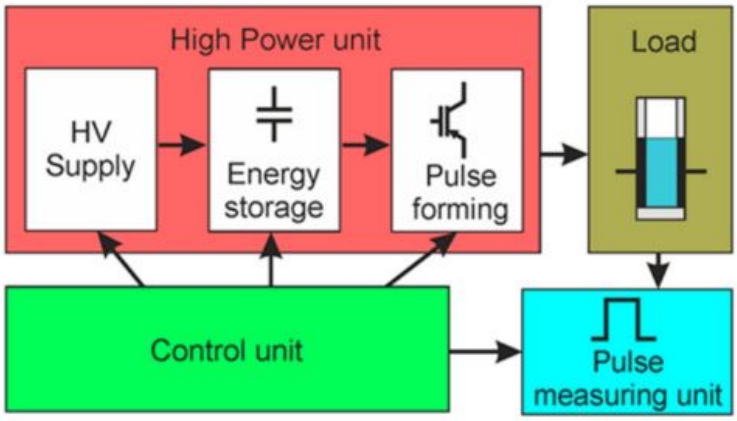

Figure 4: Square wave pulse generator circuit used to drive electroporation across ITO electrodes.

5 Device Fabrication

The device is fabricated using a combination of deposition techniques and soft lithography. A silicon wafer is coated with a 50 nm silicon nitride layer. Backside etching is performed using KOH, and a thermal oxide layer is grown to protect against overetching. Tetramethylammonium hydroxide (TMAH) etching is used to define the membrane area. Indium tin oxide (ITO) electrodes are deposited and patterned using focused ion beam (FIB) lithography. A passivating oxide layer is deposited via PECVD to protect active areas. A piezoelectric thin film of PZT is then deposited using RF magnetron sputtering. Microchannels and valves are fabricated using soft lithography in PDMS and aligned onto the silicon wafer. The device is sealed with transparent glass layers. The fabrication process is illustrated in Figure 5.

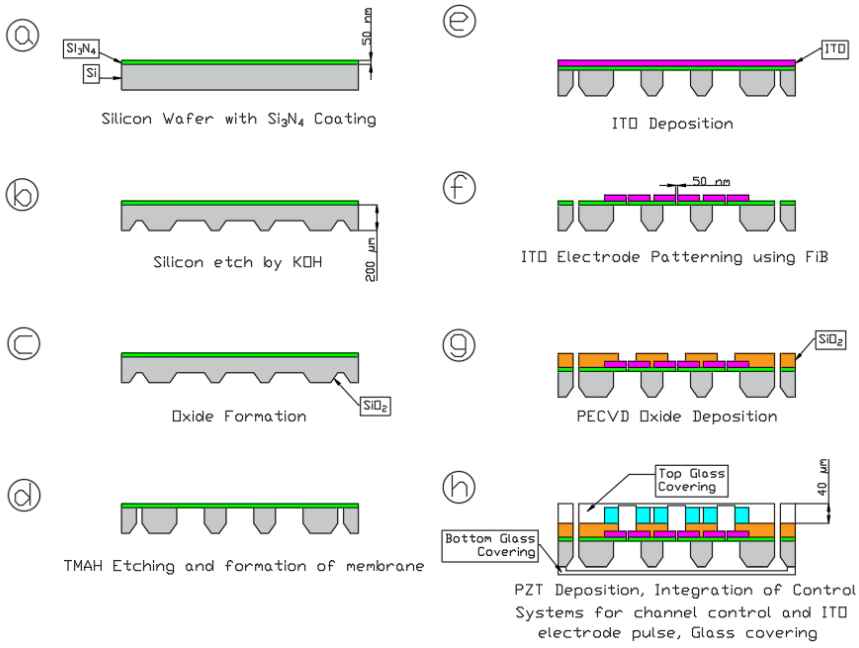

Figure 5: Fabrication steps showing electrode patterning, membrane formation, and microfluidic channel integration.

The completed device is shown in Figures 6, 7, and 8, highlighting the final chip design and the microchannel network.

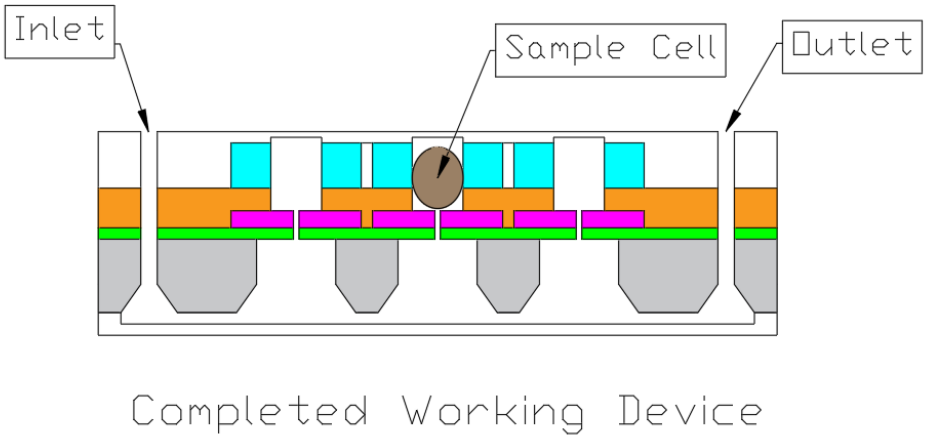

Figure 6: Photograph of the fabricated microfluidic device.

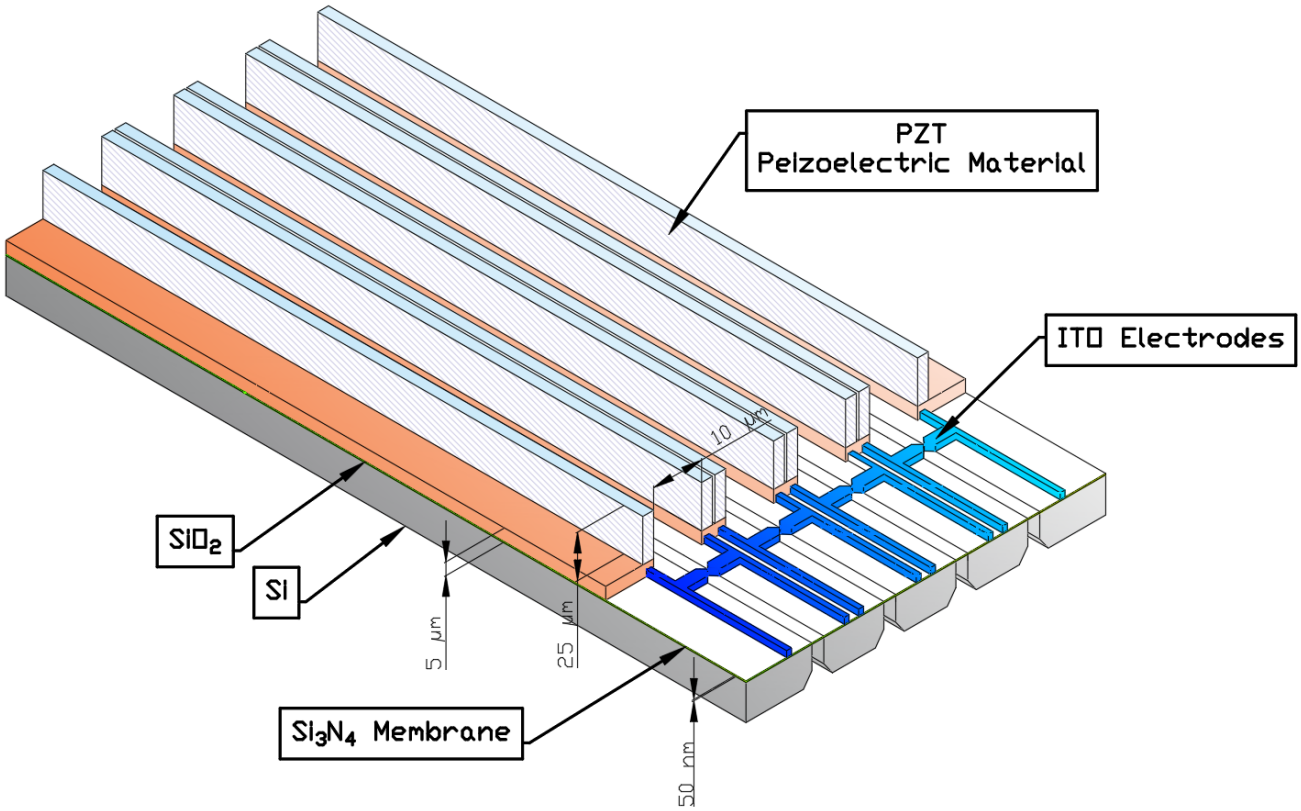

Figure 7: Top-view layout of the microchannel design.

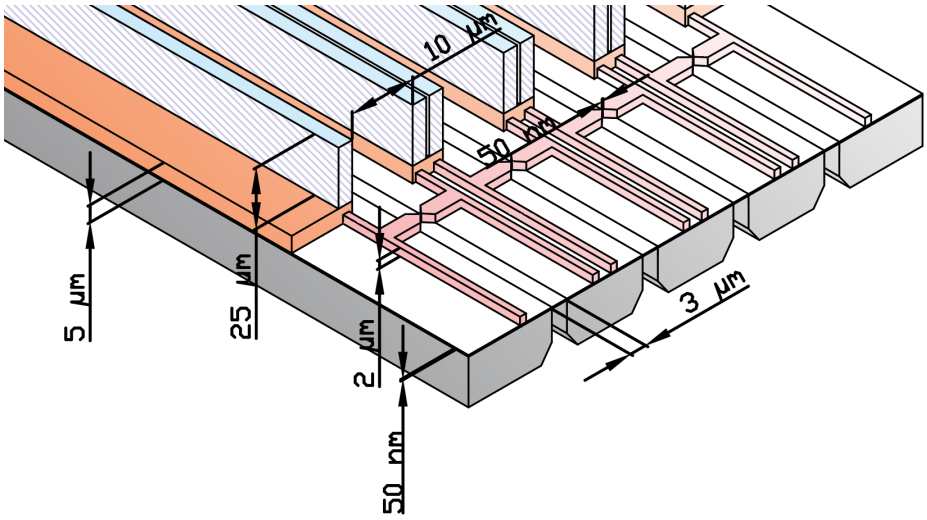

Figure 8: Detailed view of the device layout highlighting dimensions.

6 Expected Results

The proposed system is expected to yield highly localized electroporation at the single-cell level, with a uniform distribution of electric fields and minimized collateral damage. By leveraging the combined effect of mechanical deformation and low-voltage electric pulses, transfection efficiency is anticipated to increase across various cell types including HeLa cells, activated T-cells, and even plant guard cells.

Furthermore, the system should allow delivery of a wide range of cargo—including dyes, proteins, and nucleic acids—with consistent repeatability. Reduced pulse duration and optimized constriction geometries are anticipated to preserve cell viability while maximizing membrane permeability. Real-time control through valve automation and sensor integration will provide reliability and reproducibility at scale. Overall, this approach offers a balance between efficiency and biocompatibility, ideal for sensitive or high-value biological samples.

7 Conclusion

The development of a microfluidic system that integrates mechanoporation and electroporation provides a powerful tool for targeted intracellular delivery. By applying both mechanical and electrical stimuli in a coordinated manner, this platform achieves precise and efficient drug loading at the single-cell level. The combination of piezoelectric actuation, ITO electrode integration, and automated valve control ensures high performance with minimal manual oversight.

Although the system shows significant promise, optimization of operating parameters for different cell types remains a challenge. Nonetheless, its modular and reusable design, together with its compatibility with diverse biological materials, makes it highly attractive for applications in gene editing, regenerative medicine, and diagnostics.

8 References

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