

Optimization and Microfabrication of an Electrosonic Ejector Microarray for Intracellular Nanomaterial Delivery

Allison Connolly

Biomedical Engineering, Johns Hopkins University

NNIN REU Site: Microelectronics Research Center, Georgia Institute of Technology, Atlanta, GA

NNIN REU Principal Investigator(s): Dr. Andrei G. Fedorov, Mechanical Engineering, Georgia Institute of Technology

NNIN REU Mentor(s): Dr. J. Mark Meacham, Mechanical Engineering, Georgia Institute of Technology

Contact: aconnol2@jhu.edu, andrei.fedorov@me.gatech.edu, jmarkmeacham@gmail.com

Abstract:

This REU project focused on optimization of the electrosonic ejector microarray (EEM) to enable efficient intracellular nanomaterial delivery. The EEM ejects biological cells through microscopic nozzles with incorporated electroporation electrodes, thereby opening pores in the cell membrane for uptake of drugs and/or genes/nucleic acids. The device assembly consists of a piezoelectric transducer for ultrasound generation, a polydimethylsiloxane (PDMS) chamber for holding the biomolecule and cell solution, and a 20×20 array of pyramidal-shaped nozzles etched in silicon and coated with gold. The piezoelectric transducer drives the sono/mechanoporation of the cells, while the gold-coated nozzles are used to generate an electric field, which allows for electroporation. The PDMS serves as both a fluid reservoir and a microfluidic device. The water in the reservoir transfers the generated acoustic waves from the piezoelectric to the cell mixture, and fluidic channels that are cast into the PDMS allow separate groups of nozzles to be accessed for simultaneous ejection of multiple samples.

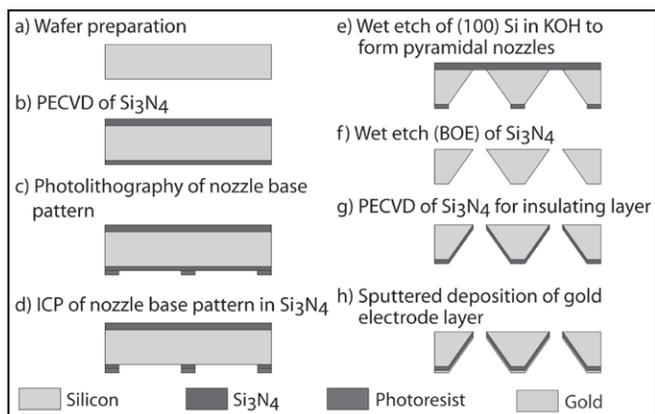


Figure 1: Electrosonic ejector microarray fabrication process.

Device Fabrication:

The fabrication process for the EEM is shown in Figure 1. An array of square openings for the nozzle bases was patterned in Microposit SC 1827 photoresist. This pattern was then transferred into a silicon nitride (Si_3N_4) layer on the backside of a [100]-oriented silicon wafer using inductively coupled plasma (ICP) etching. The pyramidal nozzles were formed by wet etching through the silicon wafer in a potassium hydroxide (KOH) solution (see Figure 1e). The KOH solution etched the [100] orientation (normal to the wafer face) significantly faster than the [111] orientation (54.74° to the wafer face). The size of the nozzle apertures was controlled by varying the etch time. Following the KOH etch, a 500 nm silicon nitride

layer was deposited to insulate the silicon nozzles from the gold electrode layer. For better adhesion, a titanium seed layer was sputtered onto the nitride before depositing the gold.

PDMS was selected for its mechanical properties (e.g., high flexibility and compressibility), biocompatibility, and low cost. The PDMS spacer separates the disposable EEM from the remainder of the assembly, maintaining a sterile environment for the cell mixture. The fluidic channels are designed to isolate small groups of nozzles, minimizing sample volumes while maximizing throughput. A mold for the channels is patterned in SU-8 10 on a silicon wafer [1].

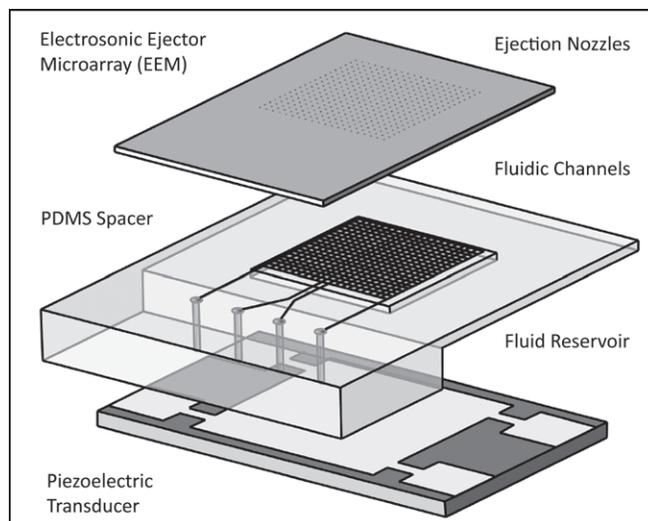


Figure 2: Expanded device assembly.

Trimethylchlorosilane (TMCS) is vacuum desiccated onto the mold to ease removal of the PDMS and allow the mold to be reusable. The fluid reservoir lays flush with the piezoelectric (see Figure 2) and transfers the acoustic waves into the cell medium in each of the nozzles. Although the acoustic properties of PDMS are similar to those of water and the cell medium, the PDMS layers dissipate acoustic energy. Thus, a higher amplitude signal is required to achieve ejection.

Cell Treatment:

The three-layer device assembly can be seen in Figure 2. The cell mixture is fed into the nozzles through the inlet holes in the spacer. We first apply a direct current (DC) to the gold layer generating an electric field in the sample within the nozzles. The electric field causes the polarized cell membrane to break down and separate leading to pore formation and growth [2]. We apply an alternating current (AC) signal to the piezoelectric transducer, which converts electrical oscillations to mechanical motion. The pyramidal shape of the nozzles focuses the generated acoustic field at the aperture. Significant velocity gradients occur only within the aperture and not within the remaining cell mixture. Here, pressure and shear act to both increase the size of the membrane pores and to eject the sample from the device [3]. While the pores are open, nanomaterials enter the cell via diffusion. The solution is ejected into a Petri dish where the membranes refuse, capturing the nanomaterials inside the cell.

Conclusions:

We have shown that a multifunctional device can be fabricated. The modified EEM can combine electroporation with sono/mechanoporation for dual treatment of the cell sample (see Figure 3). Cell ejection was performed using the original EEM to prove that mechanoporation occurs. Figure 4 shows cell uptake of calcein (bottom left) and cell death (bottom right). With the multifunctional device, these factors should be optimized. Due to its acoustic properties and biocompatibility, PDMS was an ideal material for the microfluidic spacer.

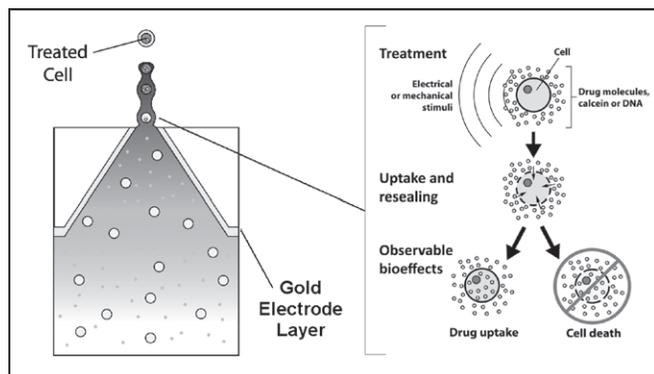


Figure 3: Cell treatment—combining electroporation and mechanoporation.

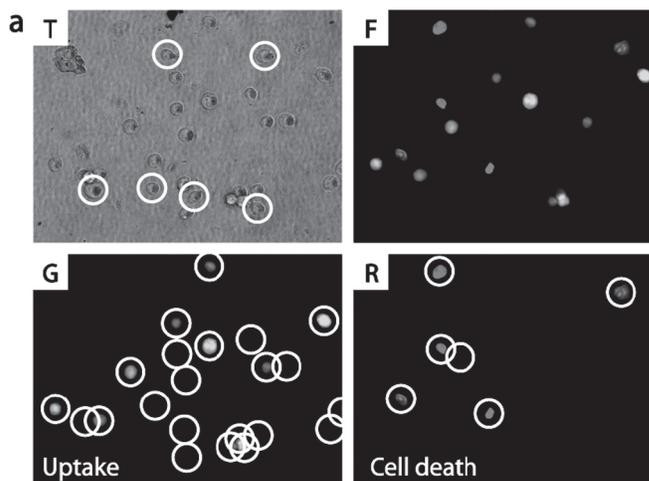


Figure 4: Uptake and viability experiments with calcein (G) and propidium iodide (R) stains.

The spacer reduces cost by minimizing sample sizes and separating disposable parts from reusable ones. Overall, the fabrication of the EEM and spacer is flexible, which allows for parametric optimization for each specific cell line.

Future Work:

In order to characterize the EEM for drug delivery, cell uptake and viability experiments will be conducted with the optimized device. The small fluorescent molecule calcein will be used to evaluate delivery efficiency. Post-ejection staining with propidium iodide will test for cell viability, marking cells whose membranes have not refused. After optimization of ejection parameters (e.g., applied voltage and aperture size), the device will be used for gene transfection with difficult cell lines. We hope to treat glioblastoma cells that are resistant to gene therapy using traditional methods by exploiting the multifunctionality of the EEM.

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