

Microfabrication of Membrane Sandwich Electroporation Devices for Drug/Gene Delivery

The cell patch approach has been used to control the injection dosage of genes or drugs into a large number of cells, while simultaneously minimizing cell damage and maintaining a high delivery efficiency. However, safety issues, such as immune response and toxicity, have caused concern about their clinical applications. A much less invasive and more efficient gene delivery method is demonstrated: membrane sandwich electroporation (MSE). First, a 5mm by 5mm polystyrene (PS) membrane with an average pore size of 3~5µm is used as the support membrane and placed in the middle of a 1 cm diameter reservoir located at the center of the microfluidic device. The reservoir is connected to both the inlet (top) and the outlet (bottom) channels, with a channel size of 500 µm in width and depth. A vacuum is used to trap the cells on the support membrane. Immobilizing the cell on a porous surface allows electroporation to be localized with a low applied voltage, achieving dielectric breakdown of the cell membrane. Next, another polystyrene (PS) membrane of the same size with an average pore size of 3~5µm is placed over the immobilized cells with a spacer of approximately 10 µm between the two membranes. A cross-section of the Membrane Sandwich Electroporation device is shown in Figure 1.

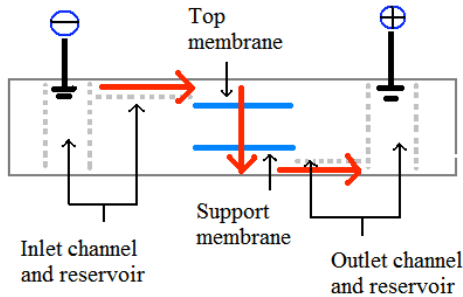


Figure 1. Cross-section structure of the Membrane Sandwich Electroporation device.

Membrane film of Microfabricaiton process

The PS membrane films were fabricated using two approaches: SU-8 photolithography and etching of a silicon pillar mold by an inductively coupled plasma reaction ion etching (ICP-RIE) system to create a master mold with hollow arrays for replication. The hollow circle array in SU-8 was 10~15 µm in height and was transferred to a PDMS film by soft lithography. The PDMS film with circular pillar patterns transferred was used as the mold for another replication. The circular pillar PDMS mold with a height of 10µm is shown in Figure 2.

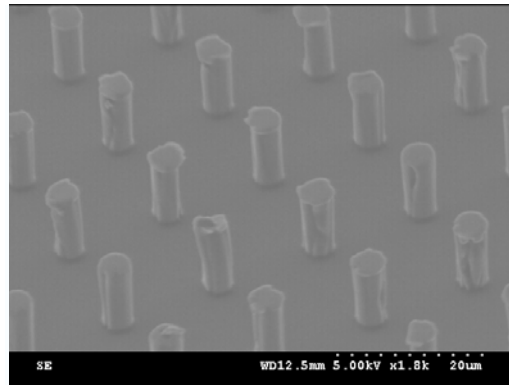


Figure 2. SEM micrographs of circle pillar structures with 10 µm thickness and 3µm diameter in PDMS mold at a viewing angle of 45 degrees.

Finally, uniform polystyrene or poly (ε-caprolacton) (PCL) thin polymer films with a thickness of 5 to 8 µm was applied on the PDMS mold for replication.

Another approach for fabricating master molds is using ICP etching in SF₆/O₂ plasma to create pillar arrays with a high aspect ratio on a silicon substrate. The feature profiles are obtained at various pressures, rf power, ICP power, and O₂ concentration. The etching experiments were performed in a low-pressure (~5–25 mTorr), high-density, inductively coupled plasma etching reactor with a planar coil. The effects of pressure, rf power and SF₆-to-O₂ gas ratio on the etch rate, selectivity, and the feature profile of Si and SiO₂ etching masks have been characterized. Furthermore, the replica molding method was used to get a PDMS mold with a hole array, and then the molding was cast to get PVA sacrificial template with pillar array again. Finally, the coating on the mold is spun

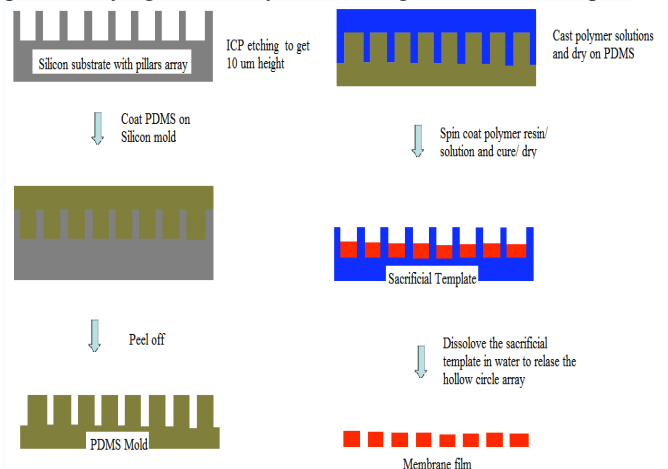


Figure 3. Microfabrication process of thin membrane film

to get a thin membrane film. The basic process is illustrated in Figure 3.

To fabricate mold masters with nanoscale structures, we use either electron beam lithography to directly fabricate the mold in soft polymer materials, such as SU-8, or transfer the nanoscale structures in resists or lifted-off oxides to a hard mask using the ICP-RIE process as described above. Utilizing the proximity effect during electron scattering, we can control and create vertical or tapered profiles. SU-8 is primarily a UV negative thick resist with very high sensitivity but poor resolution. By adjusting the photo-reactive component and thinning down the resist, we have been using SU-8 successfully as a high-resolution negative tone electron beam resist. Figure 4 shows the tapered and vertical nanopillars in SU-8 fabricated by electron beam lithography.

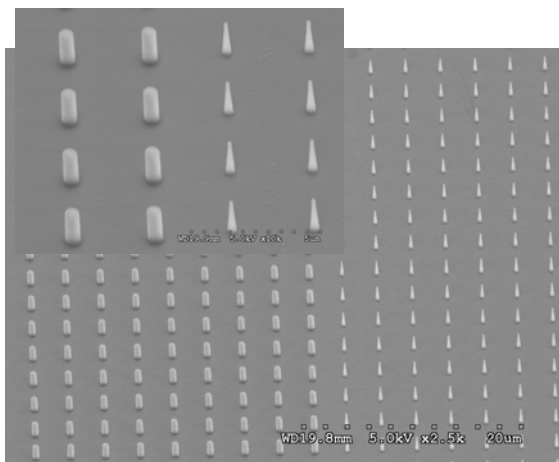


Figure 4. Nanoscale SU-8 pillar arrays with different profiles manufactured by electron beam lithography.

Opti-MEM ITM-reduced serum medium is then loaded into the channels and the center reservoir, and the DNA sample is loaded into the inlet reservoir. Finally, electroporation is carried out, followed by a 24- to 48-hour cell culture before measuring the transfection efficiency. The conically circular pore arrays provide a great potential gradient when an electrical bias is applied. This provides an efficient way to accelerate the rigid carriers to a high momentum. The small size and high momentum would help deliver species inside cells without damage to cell membrane. Figure 5 shows the fluorescence images of NIH 3T3 cells after DNA delivery by electroporation in a PCL MSE device with 5 μm pores array. Evidently, the cells are uniformly trapped on the micro-pores array and each cell experiences the same condition during electroporation. Such devices for local electroporation with well defined array structures have demonstrated great improvement in comparison with devices with random pores.

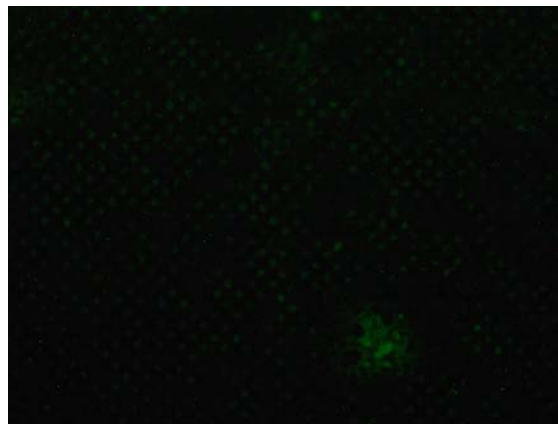


Figure 5. Fluorescence image of NIH-3T3 cells after DNA delivery by electroporation in fabricated PCL membrane with 5 μm pores array.

Publications

1. Brian E. Henslee, Zhengzheng Fei, Orin L. Hemminger, Hae Woon Choi, Kun-Yeh Chiang, Wu Lu, David Farson, L. James Lee, "Optimizing Membrane Sandwich Electroporation Through Microfabrication And High Speed Spin Disk Confocal Imaging", The 2007 Annual Meeting of American Institute of Chemical Engineers.