Cell Patch and Membrane Sandwich Electroporation

Current drug and gene delivery methods have limitations, such as safety issues and low efficiency *in vivo*. A nanofluidic "cell patch" device, which integrates layers of polymeric nanochannels into a single microfluidic device, has been developed in our laboratory. When an electric field is applied, the nanochannels provide a focused electric field that can accelerate the transport of species (such as DNA) or drugs into the cells close to the outlet of the nanochannels. It has great potential to deliver genes or drugs efficiently and safely at cell or tissue levels.

Ordered Pore Array Membrane Fabrication

Track-etched membranes used in the cell patch device provide a random pore distribution through the surface. The randomness of the pores allows cells at different locations to experience a different electric field, some higher and some lower than average. An electric field too high can damage cells and reduce efficiency. We have fabricated membranes with an ordered pore array to minimize the randomness associated with the track-etched membrane. These new membranes will allow each cell to experience the same electric field. A uniform experience among cells is expected to translate into better transfection efficiency.

We have been successful with two fabrication techniques to make our ordered pore array membranes. The first technique is a cleanroom process based on soft lithography methods. Photo lighography is used to create a pattern in SU-8 coated silicon. This silicon is then used as a mold to create a PDMS stamp pillar array. The PDMS stamp is finally used in a polymer spin coating process to create the final membranes. The final membranes have a pore size of 3 or 5 microns and are 8 microns thick. The second membrane fabrication process is a non-cleanroom process using a femtosecond laser. A solid polymer film (8-12 microns) is exposed to a femtosecond laser. The laser power is adjusted to control the pore size, and the laser position is adjusted to control the pore spacing. Current membranes have a pore size of 4.5 microns (Fig. 1).

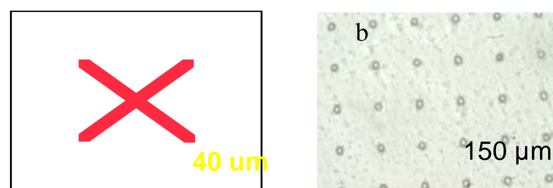

Figure 1. a) Track-etched membrane; b) laser machined pore array

Microfluidic platform for visualizing cell interaction with membranes

We developed a microfluidic platform to interface the cell patch device with our high-speed spin disk confocal microscope. This chip will allow the evaluation of static and dynamic interactions of cells during membrane-based electroporation. The platform allows users to mount membranes and apply suction or vacuum and carry out electroporation events, all under the observation of a high-speed spin disk confocal microscope (Fig. 2).

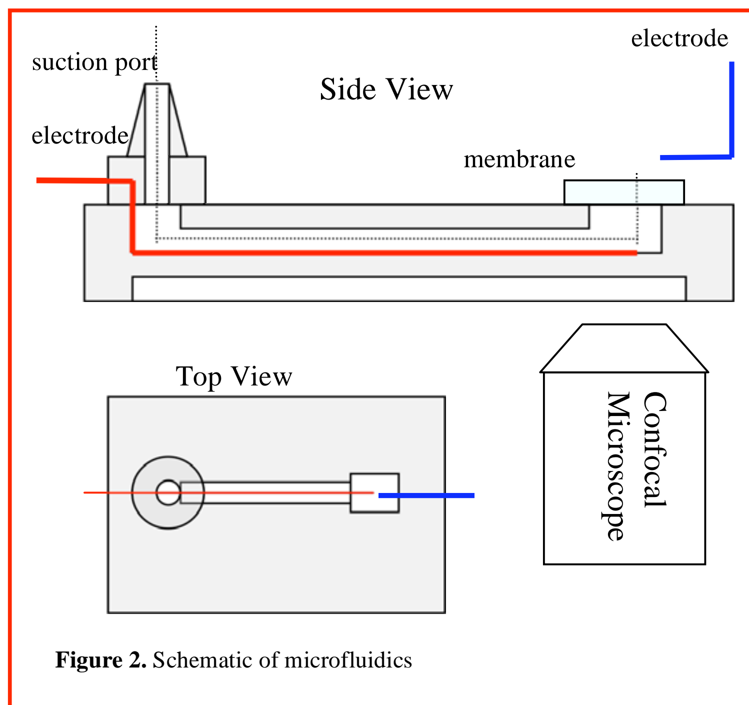

Figure 2. Schematic of microfluidics

Visualization of Electroporation

Using the microfluidic platform just described, we have been able to trap cells to a membrane and carry out a single membrane electroporation event. First, we trapped the cells onto a membrane pore array using suction. Next, we carried out electroporation with YoYo-1 stained plasmid DNA. Three-dimensional confocal images were captured before and after electroporation. From the images below (Fig. 3), we are able to see cells that exhibit fluorescence received the plasmids, and we can investigate each cell's interaction with the membrane to see if there are any differences to account for cells that exhibit higher fluorescence or no fluorescence.

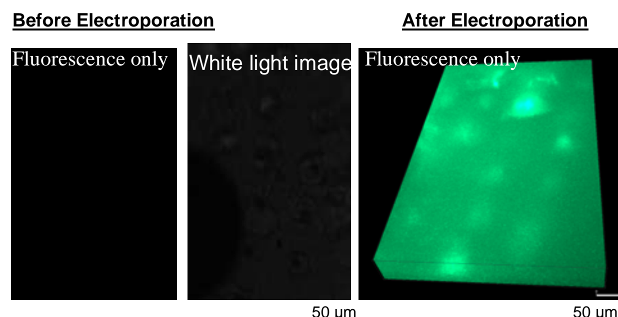

Figure 3. Confocal images of NIH-3T3 cells on the laser-machined membrane array before and after electroporation with YoYo-1 stained plasmid DNA

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In the images below (Fig. 4), the red oval surrounds a group of cells that are not over the pores in the membrane, and in the resulting confocal image they exhibit no fluorescence, as expected. Cells that are trapped on the pores clearly exhibit fluorescence, and it can be seen that some cells, such as the one outlined in blue, exhibit more fluorescence. By analyzing the 3D confocal image volume we can see that there are two cells interacting with this single pore. This technique can be used to study different membrane geometries.

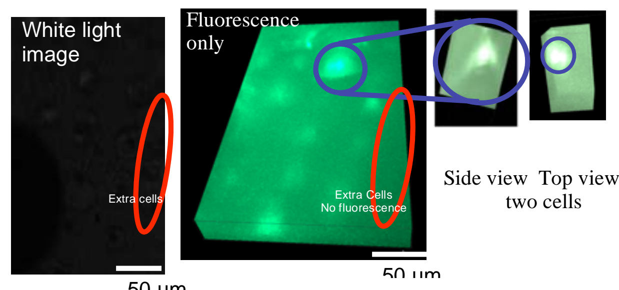


Figure 4. Individual cell analysis of the 3D confocal images

Near Term Plan

Our near term plan is to use this device and previous cell patch microfluidics to study the effects of different membranes on membrane-based electroporation. We currently have the original track-etched membrane, the nanonozzle, soft lighography membranes, and two styles of laser machined membranes to compare. Laser machining is capable of producing straight-walled or conically shaped pores in polymer films, and we will be studying the effects of both geometries on membrane-based electroporation.

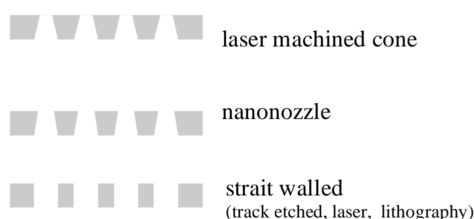
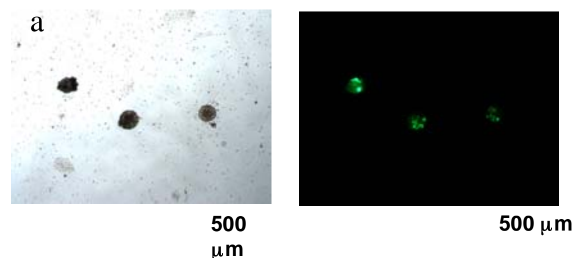


Figure 5. Three membrane geometries to study in membrane-based electroporation

Different membrane geometries can affect membrane-based electroporation because of their influence on the electric field and surface characteristics. Pore size is inversely proportional to electric field. Conically shaped pores have an exponential increase in electric field strength as you travel from large to small end, while straight-walled pores have an step function jump in electric field going from outside the pore to inside the pore. These factors can affect the outcome of electroporation based on how the cell interacts with the membrane surface and the pores.

Electroporation of embryonic bodies

Given the importance of embryonic bodies in the biomedical field and the ongoing collaboration between our proposal team and the research group at Columbus Children's Hospital, we further investigated embryoid bodies (EBs) differentiated from mouse embryonic stem cells (mESCs) using our MSE method. For mESCs differentiation, the microcapsule system is used to generate EBs with controlled size. An analysis of culture one day after MSE revealed successful transfection of ~75% of EBs with GFP, while only a ~42% transfection efficacy was observed using nucleofection (Fig. 6).



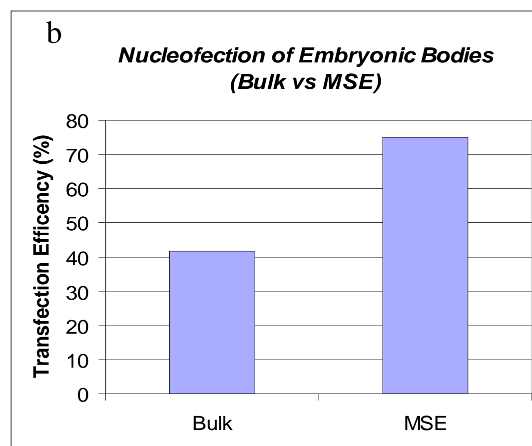


Figure 6. a) GFP expression of EB b) Transfection graph

Publications

1. Zhengzheng Fei, Wang, S., Zie, Y, Henslee, B.E., Koh, C.G., Lee, L.J. Gene Transfection of Mammalian Cells Using Membrane Sandwich Electroporation. Anal. Chem. 2007, 9, 5719-5722.

2. B.E. Henslee, Z. Fei, H. Choi, K. Chiang, L.J.Lee. "Membrane pore geometry in membrane based electroporation" (to be submitted)b

3. B.E. Henslee, O. Hemminger, H. Choi, K. Chiang, L.J. Lee. "Real time imaging of membrane based electroporation" (to be submitted)

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