STUDYING REPROGRAMMING OF SOMATIC CELLS VIA FUSION WITH EMBRYONIC STEM CELLS: A MASSIVELY PARALLEL DEVICE FOR CELL FUSION

Alison M. Skelley[1,2], Oktay Kirak[3], Rudolf Jaenisch[3], and Joel Voldman[1,2,4]

[1]Research Laboratory of Electronics, MIT, USA
[2]Microsystems Technology Laboratories, MIT, USA
[3]Whitehead Institute, MIT, USA
[4]Electrical Engineering and Computer Science, MIT, USA

ABSTRACT

Controlled fusion between an embryonic stem cell and a somatic cell is an important strategy for cellular reprogramming. We have developed a microfluidic system specifically to address the main limitation of current fusion technologies: the positioning and pairing of cells prior to fusion. We present a PDMS device designed to pair cells prior to fusion. Thousands of properly paired cells are obtainable within a mm-sized area, and the device is compatible with both chemical and electrical fusion. Fusion efficiencies obtained with our device demonstrate a significant improvement over current technologies.

Keywords: Embryonic stem cell, cell fusion, cell reprogramming, hydrodynamic trap

1. INTRODUCTION

Researchers have recently demonstrated that somatic cells can be reprogrammed back to an embryonic, pluripotent state. Several reprogramming methods have been demonstrated in the literature, including nuclear transfer, fusion with an oocyte, and, recently, fusion with an embryonic stem cell [1]. While these advances hold great promise, the reality is that little is known about cellular reprogramming, primarily due to the limitations of current technologies. State-of-the-art protocols use polyethylene glycol (PEG) or electric fields to disrupt the cell membranes and induce fusion, but cannot control the cell pairing. In addition, the cells must remain immobilized in cytotoxic fusion buffer during the delicate fusion process, necessitating a trade-off between successful fusions and viability. As a result, current fusion techniques yield at best ~5% successfully fused somatic and embryonic stem cells in a high background of non- and incorrectly fused cells, necessitating days of culturing and colony picking to isolate the desired fusions, and preventing the use of assays on pooled populations.

2. RESULTS

The PDMS device for cell pairing contains thousands of capture combs accessed by microfluidic channels (Figure 1). Modeled after previously published designs [2], the PDMS combs utilize a novel double-well structure to enable efficient trapping and pairing of two different types of cells. Cells are loaded sequentially, first capturing one cell of type A per comb (Figure 2, step 1). The flow is then reversed, and all cells of type A are shifted simultaneously to larger wells (Figure 2, step 2). This is a fast (<1 second) and massively-parallel recapture that utilizes the laminar flow attainable in the microfluidic system. The second type of cell is then loaded, and the larger wells already containing cell A now also capture one cell of type B (Figure 2, step 3), precisely positioning the two cells next to each other and in tight contact in preparation for the fusion protocol. Efficiencies of >50 % red-green pairs are capable over the entire device, with >70 % efficiencies in the bottom half of the device (Figure 3), a

978-0-9798064-0-7/μTAS2007/$20©2007CBMS

dramatic increase over existing methods.

Initiation of fusion is accomplished by exposing the cells to either a chemical fusion buffer or an electric field. In Figure 4 we show the time course of PEG-induced cell-cell fusion. Opening of pores in the membrane and exchange of cellular contents, demonstrated by the dye within one cell moving into the other cell, takes place over a 20-minute period. An additional advantage of our device is that by physically constraining the cells, additional doses of PEG can be applied to further increase fusion efficiency while still maintaining proper cell contact. Using a 5-minute dose of PEG, the fusion efficiency doubles when second and third doses are applied.

3. CONCLUSIONS

The ability to immobilize and observe thousands of paired cells during fusion while precisely controlling the exposure of the cells to harmful fusion buffers now allows detailed studies of cellular fusion while increasing the number of viable fused cells for further experiments. With this system we now aim to examine the short-timescale dynamics of reprogramming as well as perform gene expression assays on pooled populations.

REFERENCES/ACKNOWLEDGEMENTS

[1] "Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells," C. A. Cowan, J. Atienza, D. A. Melton and K. Eggan, Science, 5739, 1369-1373 (2005).
[2] "Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays," D. Di Carlo, N. Aghdam and L. P. Lee, Anal. Chem., 14, 4925-4930 (2006).
This work was supported in part by the NIH (EB007278).

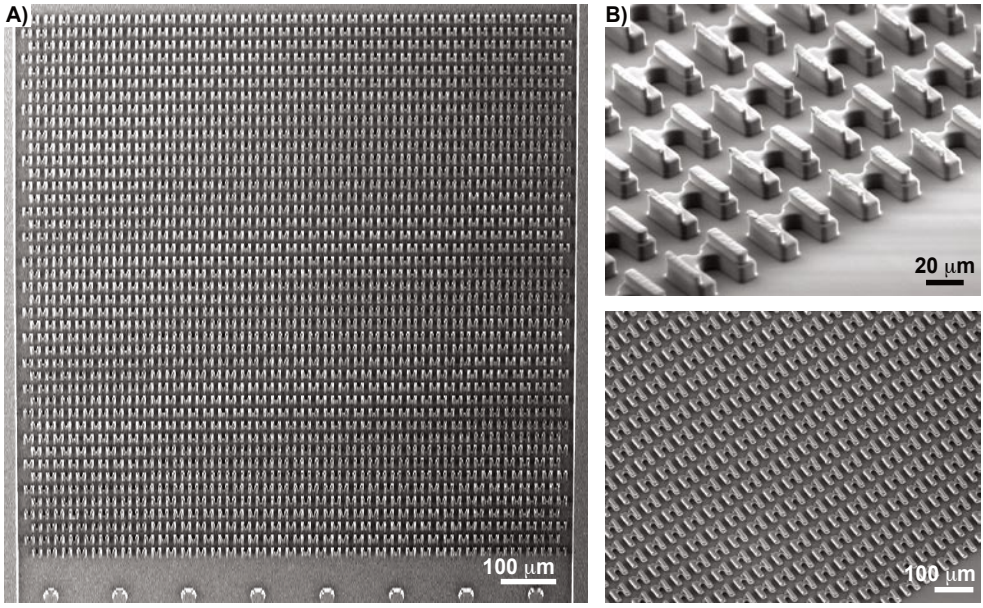

Figure 1. SEM images of the 2-cell capture device. A) Top view showing the 2-mm by 2-mm capture area. B) Close-up SEM photos showing the capture comb and support pillars. The front chamber (top in A) fits 2 cells,while the back chamber (bottom in A) fits one cell.

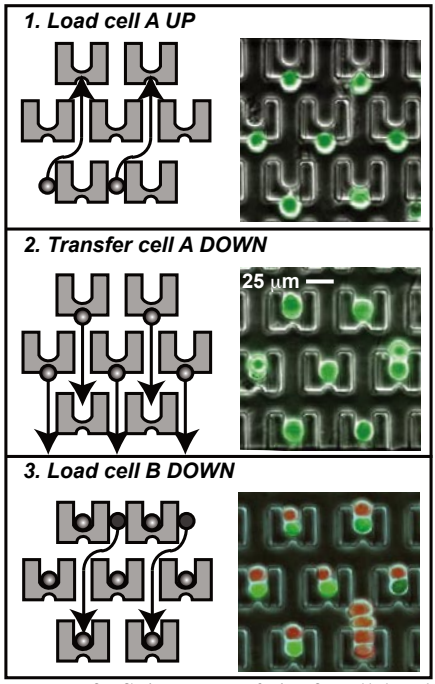

Figure 2. Schematic of the 2-cell loading process.

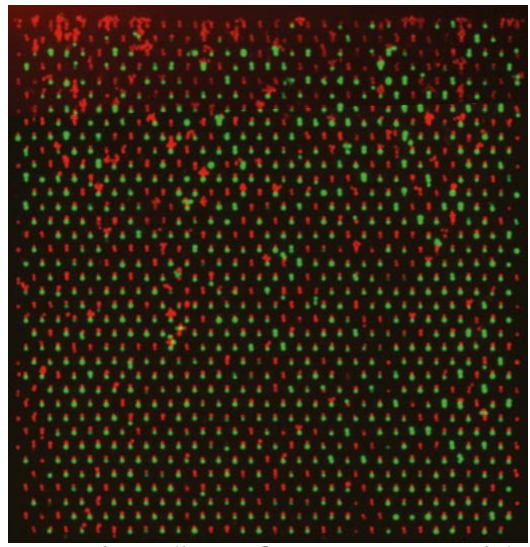

Figure 3. Red/green fluorescent images of the 2-mm by 2-mm capture aray. Green and red 3T3 cells were loaded as described in Figure 2. Efficiencies of >50 % R/G 2-cell capture are possible over the entire device, with >70 % efficiencies in the bottom half of device.

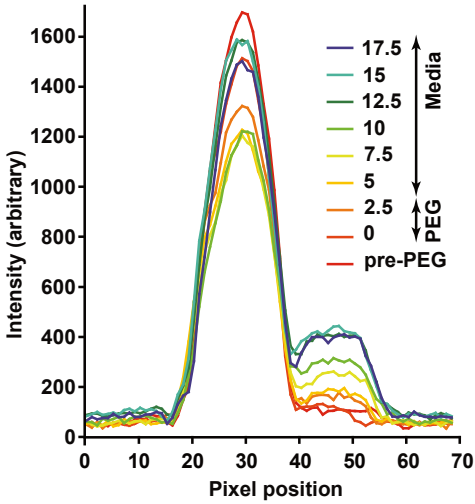

Figure 4. Timecourse for PEG-induced fusion monitored by transfer of dye from one cell to the other. The green fluorescence is initially localized within one cell. After a 3-minute dose of PEG, the green fluorescence is visible within the second cell. The signal stabilizes after ~ 15 minutes.

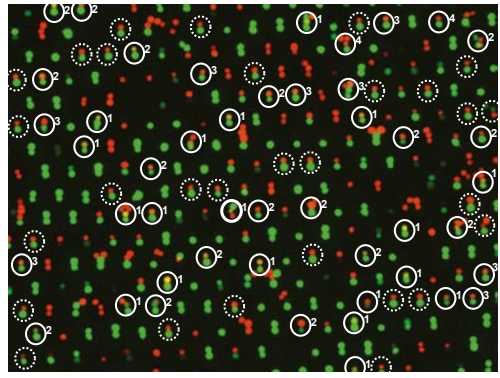

Figure 5. Fusion of 3T3 cells with multiple doses of PEG. Solid circles indicated fused cells along with the dose that initiated fusion (total 35% red/green cells are fused); dashed circles indicate potentially fused cells (57% total confirmed and possible fusions).