MICROFLUIDICS FOR CONTROLLED SINGLE CELL MICROINJECTION

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Abstract

We report a microfluidics method for microinjection of cells. Cells are lined up in a microfluidic channel by geometry control and brought to the needle by fluid flow. The flow rate of the cell to the needle, the acceleration to pierce the cell by the needle, and the subsequent release of the injected cell are accomplished by manipulating pressure drops in the fluid channels with poly(dimethylsiloxane (PDMS) pneumatic valves. Fluorescently labeled Dextran demonstrates microinjection and cell viability is explored with vital dyes.

Keywords: microfluidics, cell microinjection, polymer microfabrication

1. Introduction

The delivery of nucleic acids, proteins and small molecules across cell membranes into living cells is a key step in many biological experiments. Methods to introduce material into cells include chemical methods (synthetic vectors), biological vectors (viral vectors) and physical methods. Synthetic vectors are relatively cheap and efficient on selected cell types but they are associated with toxicity and produce non-quantitative delivery (*i.e.*, it is not possible to determine how much DNA is delivered into each cell). Biological vectors are more effective than synthetic vectors, but they are expensive and time consuming. Moreover, biological vectors can deliver only relatively short DNA sequences and, as in the case of synthetic vectors, they are rarely quantitative and can have toxic side effects. At present, manual microinjection is considered the most reproducible method, but it is expensive, slow and requires highly specialized operators [1]. Fujitsu has recently developed an automated microinjection robot that automates the capture of cells on a microchip pattern and the corresponding movement of the needle [2]. To our knowledge, the present microfluidic device is the first example of single cell manipulation and injection.

2. Device principle

In traditional microinjection a moving needle is manually positioned on the cell membrane to be pierced. In the present microfluidic device (Figures 1 and 2), cells are lined up in a micro channel by geometric control and brought to the needle by fluid flow control. The flow rate of the cell to the needle, the acceleration to force the cell onto the needle and piercing the membrane, and the subsequent release of the injected cell are accomplished by manipulating pressure drops in the fluid channels with poly(dimethylsiloxane (PDMS) pneumatic valves. Each cell is injected with a micro

needle placed in the device with known volume of a desired solution. After injection, each cell flows to a collection reservoir, and the system is ready to inject the next cell.

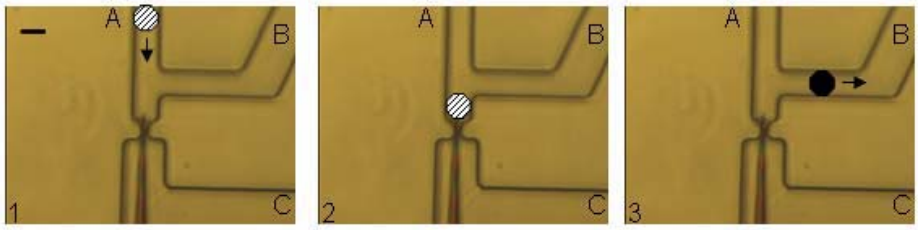

Figure 1: Schematic of microinjection device operation. Frame 1: a solution with suspended cells flows into channel A and the suspension fluid is drained thru channel C (the valve in channel C is opened and the valve in channel B is closed). Frame 2: a cell reaches the needle, it is pierced and injected. Frame 3: The valve in channel C in closed and channel B is opened so that the injected cell is carried away by the fluid.

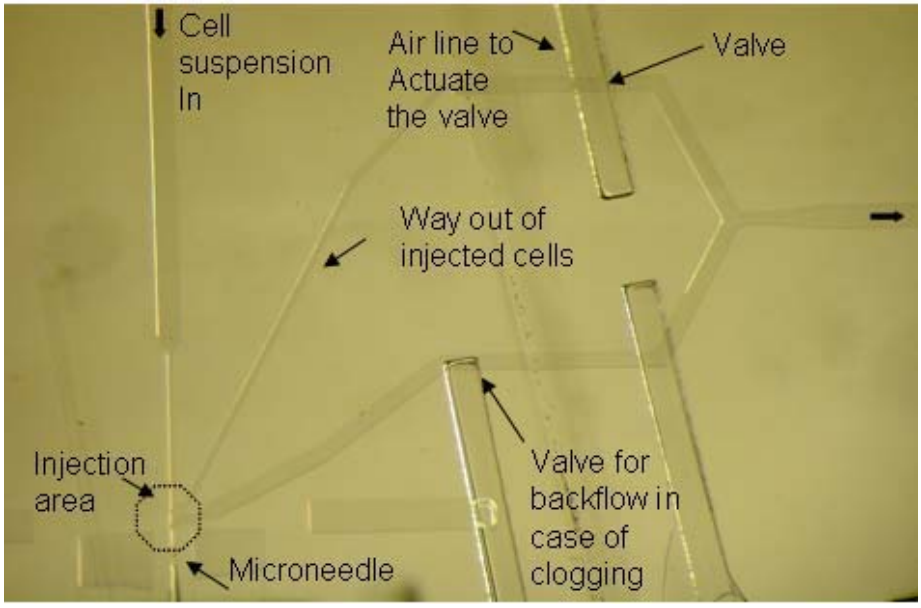

Figure 2: PDMS microfluidic device for single cell microinjection.

2. Experimental

The microinjection system (Figure 2) was constructed in PDMS by standard softlithography techniques. A micro needle made from a pulled glass capillary (tip

internal diameter 0.5 micron) was inserted and positioned in the device with the help of a micrometer stage and a stereo microscope.

In the experiments, a solution of cells was introduced in the microchannels system. Channel geometry and flow positioned the cells on the microinjection needle, one at a time. Cells were pierced, microinjected, released from the needle, and moved into a reservoir. Injection of fluorescent Dextran (tetramethylrhodamine MW 10000, Molecular probes, USA) demonstrated microinjection as the compound was sufficiently large to be unable to cross the cell membrane. The viability of cells flowing through the system without injection as well as cells injected with Dextran was explored by vital dyes. Figure 3 shows a cell (dyed for visualization purposes with orange acridyne, a vital dye) being pierced by the needle. Figure 4 shows the same cell being inject with Dextran, the fluorescence reveals both needle and cell. The viability of injected cells after injection was probed by the absence cell coloration in the presence of Trypan Blue

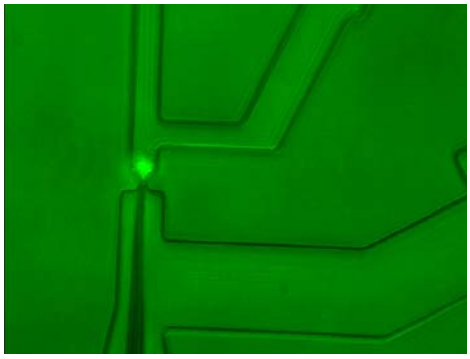

Figure 3: Injection area with a cell dyed with Acridine Orange (vital dye) for visualization purposes

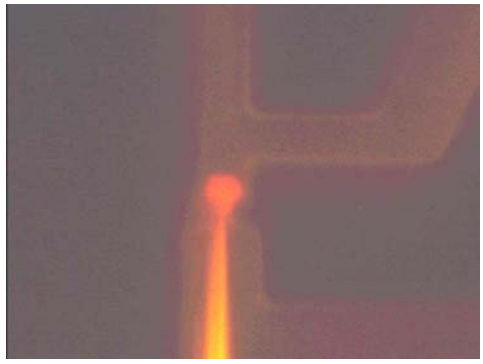

Figure 4: Injection area with a cell injected with red fluorescent dye (dextran, tetramethylrhodamine) Also the filled needle can be recognized

4. Conclusions

We have demonstrated the fabrication and operation of a microfluidic device for microinjection into cells. Further testing with gene transvection is being performed to evaluate the system for specific biological applications.

References

1. Chou TH, Biswas S, Lu S, *Gene delivery using physical methods: an overview*. Methods Mol Biol. 2004;245:147-66
2. http://www.fujitsu.com/us/news/pr/bsg_pr-2005-08-10-ci-release.html