# FLOW-THROUGH MICROFLUIDIC CHIP FOR CELL TRANSFECTION BY ELECTROPERMEABILIZATION

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## ABSTRACT

A new flow-through microfluidic chip is presented for the study of cell transfection by electroporation. A silicon-glass chip enables the immobilization of cells and real time monitoring of the electroporation process. Successful electroporation is shown by a propidium iodide (PI) assay and loading nanobeads.

**Keywords: Cell transfection, electroporation, flow-through, single cell manipulation.**

## 1. INTRODUCTION

Transfection of DNA molecules into mammalian cells with electric pulsations, electroporation, is a powerful and widely used method that can be directly applied to gene therapy. Other polar substances, such as dyes, drugs, proteins, peptides, and amino acids, can also be introduced into cells by electroporation. We have developed a flow-through chip that can immobilize, electroporate and transfect individual cells. Different layouts for on-chip electroporation were reported [1-3]. These devices either address flow-through operation or single cell capture, but all lack the ability to microfluidically and electrically address an individual cell from a pre-defined cell batch. With these new chips we demonstrate transfection of fluorescent dye (PI) and polystyrene nanobeads (60nm) in HL60 cells by electropermeabilization.

## 2. EXPERIMENTAL

Figure 1a depicts the microfluidic cell trap device. Figure 1b shows the two channels of asymmetric width (50µm/20µm) and a detail of the trapping feature. All channels are 15µm deep, dry etched in silicon and covered by glass. To initiate the experiments, 100µl of cell sample is placed in inlet (1). Cells are transported through the channel by means of pressure driven flow. To trap single cells a negative pressure was applied to the suction reservoir (4) via a pump. Once the cells have been trapped, the pump is switched off and cells are localized at the traps (Fig 1c). Gold wires immersed in the reservoirs of the chip were used to make electrical connections to a high-voltage supply unit.

## 3. RESULTS AND DISCUSSION

For the electric field mediated cell manipulation, PI-dye was added to the medium and a voltage of 50V was applied to reservoirs $R_1$ and $R_2$ for 5 seconds, while reservoirs $R_3$ and $R_4$ were kept at 0V. The result of this pulse is propidium iodide negative, PI (-), because the cells remain dark on fluorescent images. When this voltage scheme was applied again after 10 minutes, the same non-fluorescent result was obtained: PI(-).Increasing the voltage to 100V on $R_1$ and $R_2$, the PI assay was still negative, even after 45 minutes of operating with the same cell batch.



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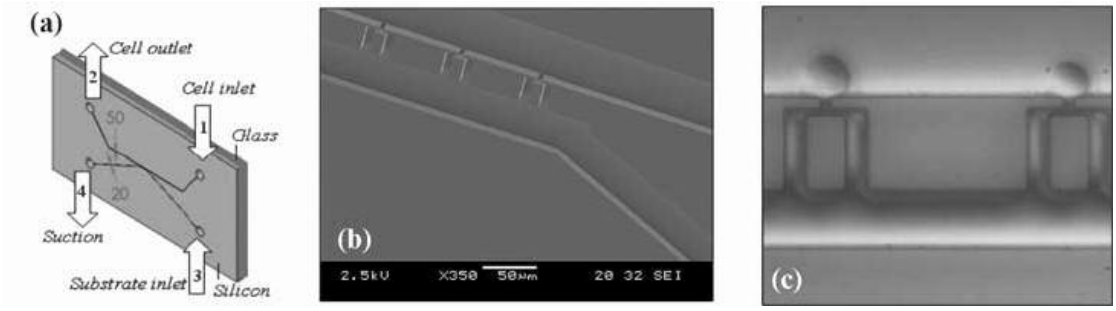
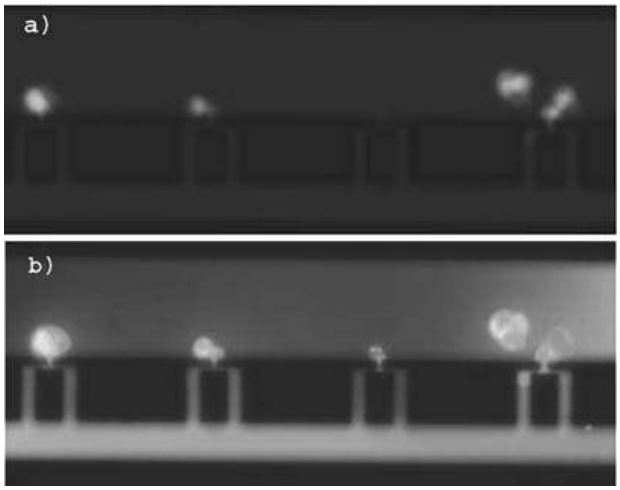



Figure 1. a) Schematic drawing of the microfluidic chip; b) SEM picture: two parallel microfluidic channels are connected by microholes of 4µm width, which act as trapping sites for real-time monitoring of individual cells; c) a close-up of a trapping feature showing two cells being trapped.

After one hour, the live stain AO was added to the chip to check the cell viability: the cells turned green, as expected for viable cells (Fig. 2a). After obtaining this information, a pulse of 100V was applied for 10 seconds to $R_1$ and $R_2$. The cells changed color from green to yellow/red, indicating PI uptake (PI (+), Fig. 2b), thus cell electropermeabilization (EP). The reliability of this procedure, versatile transfection by electropermeabilization, is confirmed by experiments using fluorescent nanobeads instead of PI-dye (Fig 3).




Green intensity 93a.u.
Red intensity 70a.u.

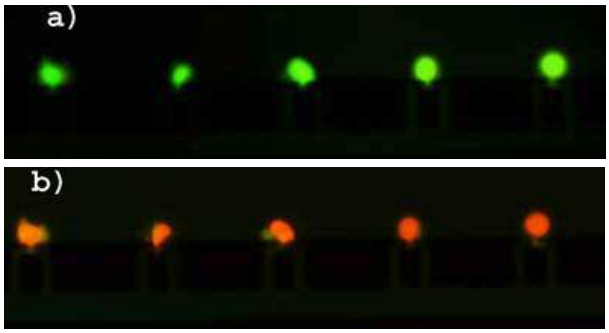
Red intensity 94a.u.
Green intensity 28a.u.

Figure 2. Fluorescence image of cells sitting at the trap after AO was added to the chip. The green fluorescence of the cells in image a) indicates that cells are still alive. After the voltage scheme was applied cells show PI uptake, as can be seen in b): yellow/red colored cells.

Figure 3. Fluorescence image of cells located at the traps and being electroporated and loaded with green fluorescent nanobeads (60nm).Right top, light image of the cells.

Since this new EP device is aimed for performing transfection experiments reversibility of the electropermeabilization is desired. A control experiment was carried out with AO

stained cells treated according to the successful cell permeabilization procedure ($VR_1$, $R_2$ = 100V and $VR_3$, $R_4$ = 0V for 10 sec). One hour after electropermeabilization, PI was added to the chip. The cells showed uptake of PI (Fig. 4). Together with the result of the AO viability test, this means that the cells are still addressable for transfection experiments.




Green intensity 110a.u
Red intensity 37a.u.

Red intensity 116a.u
Green intensity 48a.u.

Figure 4. Fluorescence image of AO stained cells; a) before the voltage was applied (green cells), b) after an hour, PI is added and cells showed PI uptake (PI +; red cells).

Fine tuning of the cell membrane permeabilization process can be achieved by introducing electrically addressable cell trap sites as depicted in the design of our next generation chip (Fig. 5).

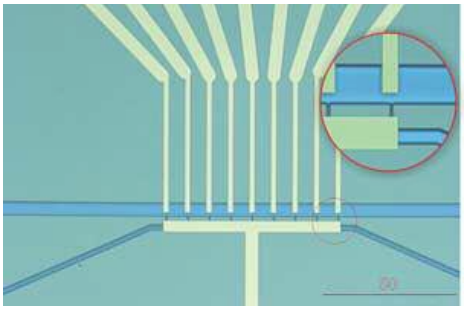


Figure 5. Next generation chip with integrated electrodes

## 4. CONCLUSIONS

We have shown successful cell membrane electropermeabilization and uploading of nanobeads into the cells in a new flow-through chip. This makes it a powerful tool for gene therapy and drug screening on a single cellular level. Additional experiments need to confirm the full viability of treated cells using on-chip culture strategies.

## ACKNOWLEDGEMENTS

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