GENE TRANSFER AND CHARACTERIZATION OF PROTEIN DYNAMCIS IN STEM CELLS USING SINGLE CELL ELECTROPORATION IN A MICROFLUIDIC DEVICE

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Abstract

We have developed a flow-through cell trapping device for the study of gene transfection by electroporation and characterization of protein dynamics in individual stem cells.

Keywords: Gene transfection, single cell manipulation, protein translocation, electroporation, stems cells.

1. Introduction

Transfer of DNA molecules into mammalian cells using electric field pulses, electroporation, is a powerful and widely used method that can be directly applied to gene therapy. Different layouts for on-chip electroporation have been reported [1-2]. These devices either address flow-through operation or single cell capture, but all lack the ability to address an individual cell from a pre-defined cell batch by microfluidic or electronic means. We have developed a flow-through chip that can immobilize, electroporate and transfect individual cells while being imaged using an inverted fluorescent microscope. With these chips we demonstrate transfection by electroporation of a green fluorescent-erk1 fusion protein and subsequent erk1 protein tracking in mouse myoblastic cells (C2C12) and in human mesenchymal stem cells (hMSCs). Our silicon-glass chip enables, to our best knowledge for the first time, the immobilization, electroporation and real-time monitoring of protein dynamics in single cells.

2. Experimental

The microfluidic cell trap device consists of two channels of asymmetric width (50μm/20μm) and details of the trapping feature are shown in figure 1b. 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 is applied to the suction reservoir (4) using a pump. Once the cells have been trapped, the pump is

switched off and cells are localized at the traps (Figure 1c). Using strategically positioned electrodes, a DNA construct encoding green fluorescent protein fused to erk1, pegfp-erk1 was electroporated into C2C12 cells. Extracellular signal-regulated kinase, ERK1, is a protein that is important for transducing signals from the cells' environment to the cell nucleus [3]. Binding of growth factors to receptors on the cell membrane results in ERK1 activation and subsequent nuclear translocation.

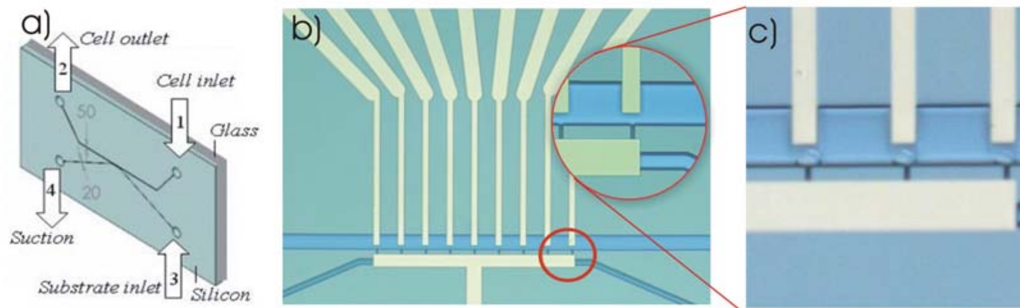

Figure 1: a) Schematic drawing of the microfluidic chip; b) image of the microfluidic chip with electrodes integrated and a zoom of the trapping sites (4µm width) c) a close-up of a trapping feature showing three cells being trapped and awaited to be electroporated.

3. Results and discussion

Twenty-four hours after electroporation, eGFP-ERK1 expression is visible mainly in the cytoplasm of the cells. The cells were then starved by removing all growth factors from the medium, resulting in localization of eGFP-ERK1 protein to the cytoplasm.

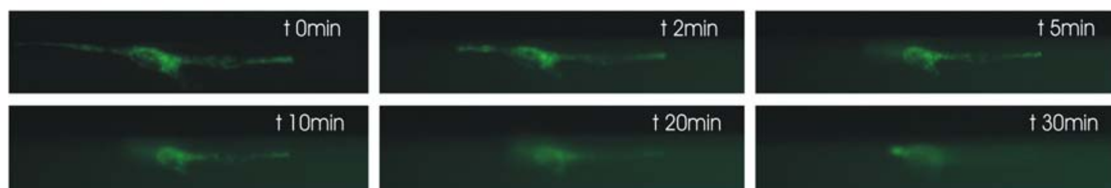

Figure 2: Nuclear translocation of eGFP-ERK after bFGF stimulation in C2C12 cells. eGFP-ERK1 is visible in cytoplasm while the nucleus remains dark (ERK1 is excluded from the nucleus, t = 0 min). After adding growth factors to the microfluidic chip, nuclear translocation of GFP-ERK1 is observed (t = 2 min to 30 min).

Forty-eight hours after electroporation, the medium was replaced for medium with serum (10%) and 10 ng ml-1 bFGF to stimulate ERK phosphorylation and nuclear translocation. Addition of these growth-factors significantly increased the nuclear localization of ERK1 (Figure 2 and 3). Immediately after the addition of the growth factors, track images were taken every 5 minutes using a wide field fluorescence microscope with 488nm excitation and a 527/30 emission filter. Fluorescence intensity changes in either the cytoplasm or nucleus of the cells was determined using ImageJ software [4]. The maximum intensity of eGFP-ERK1 in the nucleus was reached 30 minutes after addition of growth factors, which corresponds with nuclear translocation in response to activation of extracellular signaling in a wide variety of cell types [3].

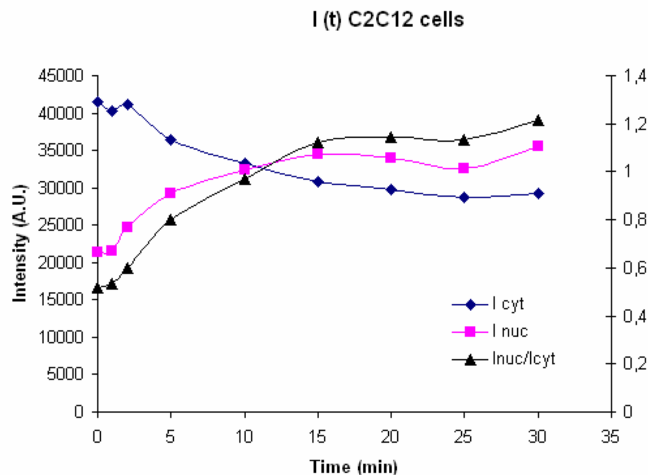

Figure 3: In the graph time courses for GFP-ERK1 translocation following growth factor stimulation of C2C12 cells are plotted: fluorescence intensity of the cytoplasm (♦), the nucleus (■) and the ratio between eGFP intensity in nucleus and cytoplasmic (▲) was measured in time after adding growth factors. It can be observed that the maximum nuclear translocation was reached 30 min after addition of growth factors.

4. Conclusions

To our best knowledge, in this work for the first time the use of microfluidics devices is shown to efficiently transfer genes by single cell electroporation and protein expression in single cells. In addition, the dynamics of the introduced protein can be followed in real-time for each individual cell. The long-term survival and responsiveness of the cells to external stimuli demonstrates the bio-compatibility of the device. We are currently applying this technique for transfection of genes into adult stem cells. Because of the limited availability of adult stem cells, this method is a powerful tool for personalized gene therapy, tissue engineering and regenerative medicine.

Acknowledgements

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