

SOFT-STATE BIOMICROFLUIDIC PULSE GENERATOR FOR FAST ENDOCYTIC RECYCLING ANALYSIS OF SINGLE CELLS

Poorya Sabounchi, Cristian Ionescu-Zanetti, Navid Ghorashian,
and Luke P. Lee

Berkeley Sensor & Actuator Center, University of California, Berkeley, USA

Abstract

We present the design and characterizations of a soft-state biomicrofluidic pulse generator device where biochemicals are pulsed around single trapped cells to study the kinetic response of cell membrane proteins. Hydrodynamic cell trapping via lateral microfluidic junctions allows the trapping of single cells from a bulk suspension. Microfluidic injection site adjacent to the cell-trapping channels enable the pulsed delivery of nanoliter volumes of biochemical reagent. Fast endocytic recycling of cells is studied under 0.5 Hz pulsed flow using a FM fluorescent dye and intensity results were compared to a simple compartment model.

Keywords: Soft-state Devices, Single Cell Analysis, Biochemical Pulse

1. Introduction

Analyzing individual cells will inherently be more accurate than any other cell-based sensor that relies on ensemble-averaged experiments. However, only few studies have carried out experiments on individual living cells using various microfluidic devices [1]. We recently introduced a microfluidic platform for high-throughput patch-clamp electrophysiology in which lateral cell trapping junctions enable hydrodynamic trapping of cells from a bulk cell suspension and electrophysiological recording [2]. We then developed a platform for rapid fluid exchange across the surface of a microfluidic channel at frequencies of up to 10 Hz [3]. We now present the design and characterizations of a soft-state biomicrofluidic pulse generator device where biochemicals are pulsed around single cells to study the fast endocytic recycling in cell membrane. Our method is simpler than existing methods of biochemical pulse generation based on scanning patch pipettes because no mechanical movement is required [4].

2. Design and Fabrication

The soft-state biomicrofluidic pulse generator device is fabricated using a two-layer SU-8 and soft-lithography of PDMS. The main channel and lateral fluidic channels are 50 and 3 μm in height respectively (Figure 1). Central to our setup is a fluidic injection system that uses a solenoid valve. Pulsed chemical reagents flow

through this injection system allowing characteristic response of single cell array to be performed in rapid succession.

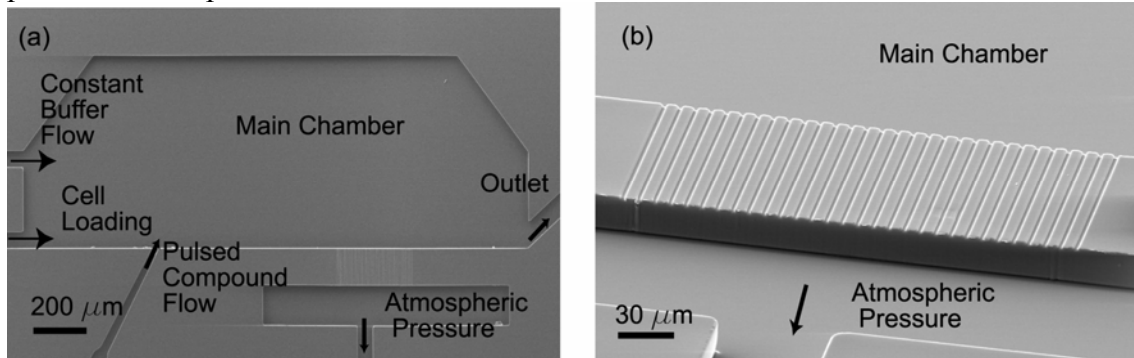


Figure 1. Device Design (a) An SEM image of the microfluidic device before bonding. A constant flow (left to right) is imposed in the main chamber, while a side capillary is used as an injection port. The main fluidic channel is $50\ \mu\text{m}$ in height. (b) Close-up view of the trapping region. Each side channel had a width of $3\ \mu\text{m}$ and height of $3\ \mu\text{m}$.

3. Experimental methodology

Suspended Hella cells were introduced from a syringe connected to cell loading port. As the cells entered the device, the pulse compound port and outlet were closed and the cell trapping port was opened to atmospheric pressure. This directed the flow of cells into the trapping sites (Figure 2 a-c). After opening outlet, closing cell loading port and flushing the cells with constant sterile buffer, Trypan blue was pulsed through the injection channel (Figure 2d). Chronological sequence of application and removal of Trypan blue around trapped single cells for 1 Hz pulsed flow is shown (Figure 2 e-d). After several pulses majority of the cells are still healthy and have not been stained.

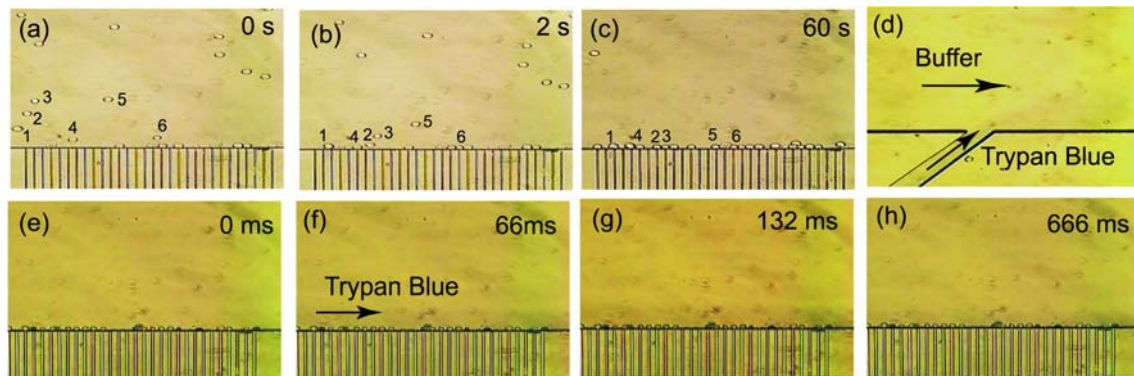


Figure 2. Viability Assay (a-c) Chronological sequence of sequential cell trapping. (d) Close-up view of the injection channel (e-h) Chronological sequence of application and removal of Trypan blue around trapped single cells for 1 Hz pulsed flow. After several pulses majority of the cells are still healthy and have not been stained.

3. Results and Discussion

Fast endocytic recycling of Hella cells cells is studied under 0.5 Hz pulsed flow using an amphiphilic fluorescent dye (FM 2-10) which reversibly partition into cell

membranes reversible (Figure 3 a-c) [5]. For compartment modeling transport from bulk to surface with mass transfer coefficient of $h = 6 \times 10^{-5}$ m/s (Figure 3d) and then a three step kinetic model was used based membrane binding ($\alpha = 1.71 \text{ s}^{-1}$), dissociation ($\beta = 0.77 \text{ s}^{-1}$), internalization ($\gamma = 0.0008 \text{ s}^{-1}$), and recycling ($\lambda = 0.012 \text{ s}^{-1}$) of fluorescent molecules (Figure 3e). The intensity results and model is shown (Figure 3f) and they agree with accepted values for binding and endocytosis parameters [6].

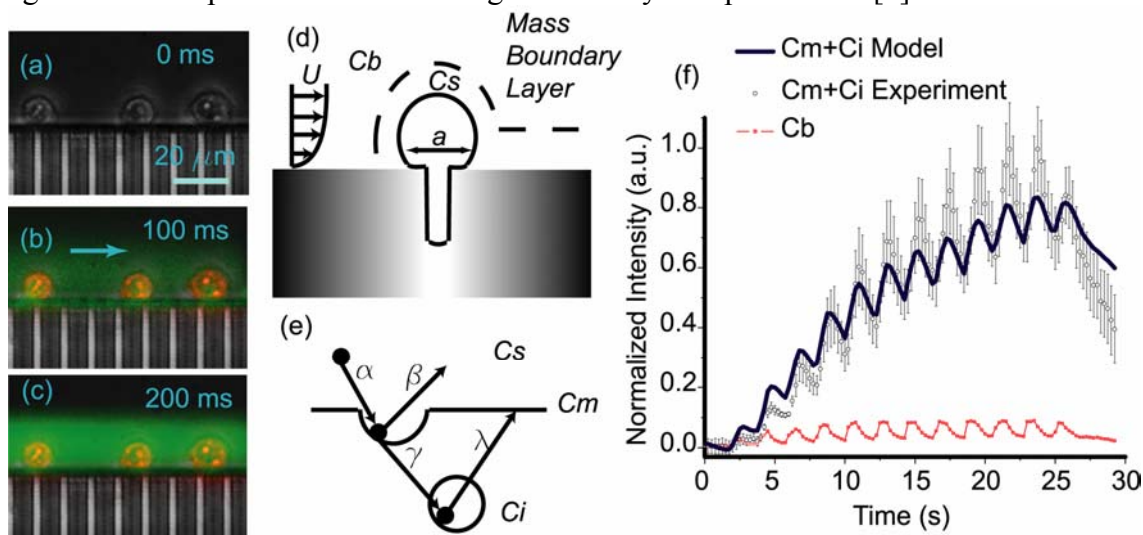


Figure 4. Compartment Modeling of Rapid Membrane Trafficking; (a-c) Chronological sequence of application and removal of amphiphilic fluorescent dye (FM 2-10) which reversibly labels cell membrane was used to characterize reversible binding kinetics of trapped single cells. **(d)** Transport from bulk to surface with mass transfer coefficient of $h = 6 \times 10^{-5}$ m/s **(e)** Schematic of a three step kinetic model based membrane binding ($\alpha = 1.71 \text{ s}^{-1}$), dissociation ($\beta = 0.77 \text{ s}^{-1}$), internalization ($\gamma = 0.0008 \text{ s}^{-1}$), and recycling ($\lambda = 0.012 \text{ s}^{-1}$) **(f)** Fluorescent intensity measurement of FM-210 association to cells fitted to the kinetic model for 0.5 Hz pulse. The error bars represent standard deviation from three different trapped cells.

In summary, soft-state pulse generating biomicrofluidic device is designed and fabricated. Membrane trafficking analysis at single cell level is performed under pulsed flow. The intensity results were compared to simple compartment model.

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References

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