A MICROFLUIDIC DEVICE TO STUDY SINGLE-CELL CALCIUM DYNAMICS UNDER TIME-VARYING STIMULI

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ABSTRACT

We present an integrated microfluidic device to study single-cell calcium signaling dynamics. Our device produces oscillating chemical signals, from which we can control their frequency and amplitude. The microfluidic device consists of a linear concentration gradient generator (CGG) that feeds into nine independent μchambers. Each μchamber comprises 492 μwells that trap individual cells. Intracellular calcium dynamics were measured on Human Embryonic Kidney cells 293 (HEK-293) to test the capability of the device. Our platform could be used to study any single-cell signaling dynamics that relay on a fluorescent signal for its analysis.

KEYWORDS: Calcium, Single-Cell, Time-Varying Stimuli, Concentration Gradient Generator

INTRODUCTION

Changes in calcium concentrations inside the cell and its dynamics can control a wide variety of cellular mechanisms, such as secretion, gene expression, and apoptosis [1]. These dynamics depend on the activation of different pathways that operate at different frequencies and time scales. Thus, there is a special interest on unveiling how those different pathways work together to maintain cell homeostasis. Furthermore, single cells are studied to analyze their individual response, and to compare this response to the population average, which can help reveal different phenotypes inside a population with the same genotype.

Microfluidic devices that study calcium signaling have been reported, including the response to time-varying stimuli at different concentrations [2]. However, to perform these experiments, separate devices are used to generate time-varying signals at different doses. In addition, cells are usually exposed to high shear-stresses in these devices. We designed, fabricated, and tested a microfluidic device to stimulate hundreds of single-cells with time-dependent signals of different concentrations. Cells are subjected to a negligible shear-stress inside the μwells.

THEORY

The microfluidic device comprises three layers: a control, a flow and a μwells-CGG layer (Figure 1a-c). The device was fabricated by multilayer soft lithography. It consists of a CGG, whose nine outputs fed directly into nine chambers; each chamber contains 492 μwells (diameter 20 μm, height 20 μm) to capture single-cells, shown in Figure 1d-e. The CGG contains two inlets and a network of microchannels to create a linear gradient. It was designed based on a previously reported device [3], with microchannels long enough to mix molecules of at least 40 kDa.

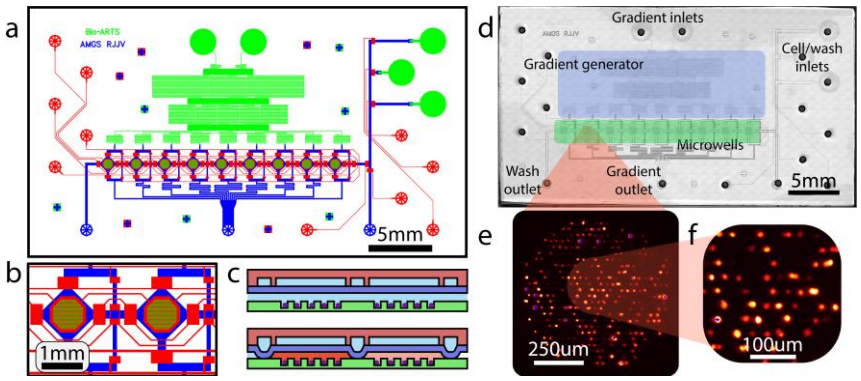

Figure 1: (a) Design of microfluidic device showing flow (blue), control (red) and μwells-CGG (green) layers. (b) Zoom-in to chamber area. (c) Transversal view of (b) that show valve activation for stimulation. (d) Micrograph of the microfluidic device. (e) Fluorescence image of single-cells trapped inside microwells, and a close up of the middle area (f).

Inside the μchambers, single-cells can be stimulated with nine different concentrations through the CGG, and then washed through an horizontal channel using four micromechanical valves. By modulating the time the stimuli is flowed through the chamber and also the washing buffer we can creates pulses of different amplitude (concentration) and frequency.

EXPERIMENTAL

To test the operation of the device, CGG mixing efficiency was characterized using molecules of different molecular weight: FITC (389.4 Da) and Dextran-Rhodamine (40 kDa). Next, we characterized the generation of time-dependent signals by creating pulses of three different frequencies (7.4, 4.2 and 2.9 mHz) using the nine outlets of CGG in tandem with the washing microchannel.

To study calcium dynamics in the device, HEK293 cells loaded off-chip with Fluo-8 AM, were flowed in the device, and trapped inside the μwells. Cells were then stimulated with three pulses of the muscarinic agonist carbachol (10 μM) and washed with Krebs-HEPES (pH 7.4) solution at 4.4 mHz. The fluorescence intensity of the cells was monitored over time, and its pseudo-color map was generated to facilitate the visualization of the heterogeneity in their response.

RESULTS AND DISCUSSION

The CGG produce a linear gradient for both molecules tested, maintaining this profile for a few hours (Figure 2a), this ensures that every chamber would be delivered with the same concentration over the duration of the experiments. We were able to control the pulse duration by opening the valve from the horizontal channel to wash the stimuli in the microchambers. (Figure 2b).

As shown in Figure 2c-d, cells responded in average to every pulse with a similar amplitude and duration (red line), but the response of individual cells is not represented by the mean. Many cells responded with a higher amplitude, while other did not respond at all. Further analysis would be carried out to get specific details, such as subpopulations of cells responding with the same intensity or percentage of cells that doesn't respond at all.

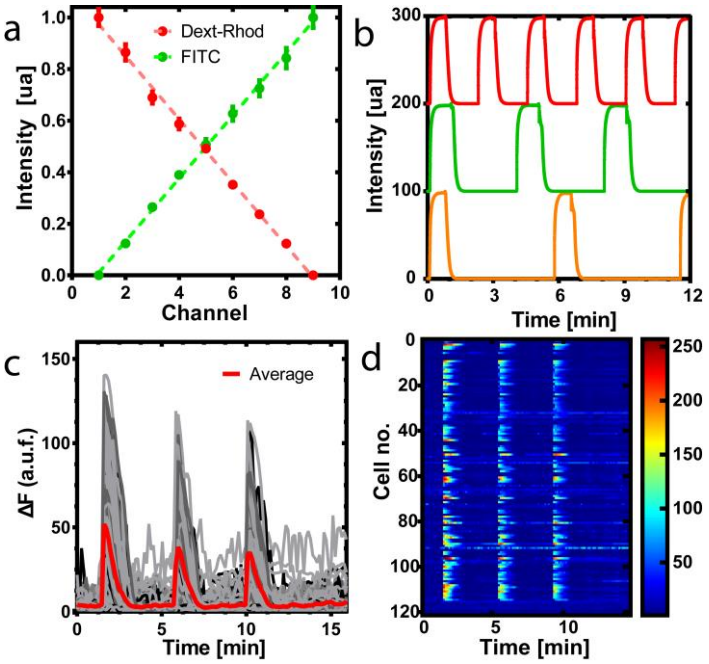

Figure 2: (a) CGG linear profile, showing accurate fit of both channels (r^2 = 0.99 in both cases). (b) Pulses generated inside chambers at different frequencies. (d) Three pulses of carbachol (10 μM) to HEK-293 cells, showing all cells in one chamber and its pseudo-color map (e). Grey lines indicate individual cells (n=120).

CONCLUSION

Our integrated microfluidic device offers the opportunity to gain insight on cellular responses that could be concentration and frequency dependent, simplifying the analysis of single cells in a low-shear stress environment.

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REFERENCES

[1] M.J Berridge, M.D. Bootman and H.L. Roderick, "Calcium signalling: dynamics, homeostasis and remodeling," *Nat. Rev. Mol. Cell Biol.*, 4, 517-529, 2003.

[2] A.S. Kniss-James, C.A. Rivet, L. Chingozha, et al., "Single-cell resolution of intracellular T cell Ca^{2+} dynamics in response to frequency-based H_2O_2 stimulation," *Integr. Biol.*, 9, 238-247, 2017.

[3] K. Campbell and A. Groisman, "Generation of complex concentration profiles in microchannels in a logarithmically small number of steps ," *Lab Chip*, 7, 264-272, 2007.

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