SORTING SINGLE CELLS BASED ON DYNAMIC ASSESSMENT OF SIGNALING

Swee Jin Tan[1+], Michelle Z.L Kee[2+], Ajay Sriram Mathuru[3], Stephen R. Quake[4,5], Suresh J. Jesuthasan[2,3], William F. Burkholder[1*]

*[1]Institute of Materials Research and Engineering, A*STAR, Singapore, [2]Duke-NUS Graduate Medical School, Singapore, [3]A*STAR-Duke-NUS Graduate Medical School Neuroscience Research Partnership, NRP, Singapore, [4]Stanford University and Howard Hughes Medical Institute, USA, [5]Visiting Investigator, Institute of Materials Research and Engineering, A*STAR, Singapore*

ABSTRACT

This paper describes a PDMS-based microfluidic device to probe and sort cells based on their response to an external stimulus. The device is capable of interrogating cellular events ranging over broad temporal windows (milliseconds to minutes) at the level of a single cell and then sorting and collecting cells for further analysis, such as mRNA expression profiling by reverse transcription real-time quantitative PCR (qRT-PCR).

KEYWORDS

Single cell sorter, Single cell dynamics, Zebrafish olfaction response

INTRODUCTION

We present a microfluidic device based on micro-valve technology [1] to achieve single cell manipulation and stimulation to characterize the dynamic changes of neuronal cells extracted from the zebrafish olfactory epithelium. Current methodologies for cell sorting included the fluorescence activated cell sorter (FACS) [2] and laser scanning cytometry [3], which are incapable of performing active monitoring of dynamic changes. Thus these methods cannot be used to sort based on transient or time-dependent cellular responses. Furthermore, a relatively large amount of starting material is required which is prohibitive for primary cells available in limited in quantity.

EXPERIMENT

Proof of concept experiments were performed to sort zebrafish olfactory sensory neurons (OSN) based on their response properties. Fig. 1 shows a schematic of the device and setup. The microdevice was created using soft lithography [4] and controlled semi-automatically via custom Labview scripts (Fig. 1b). Micro-valves that control fluid movement within the device [5] enabled single cell interrogation and sorting. An on-chip peristaltic pump (Fig. 1a) provided a gentle and precise means to direct single cells from the inlet to a cell trap. This was confirmed by tracking a single cell throughout the process (Fig. 2). In the cell trap, the stimulus was introduced from a secondary port (Fig. 3).

As changes in intracellular calcium levels is an indicator of signaling responses to a variety of stimuli in many cells [6,7] we used Fluo4-AM or Rhod2-AM (2μM; Life Technologies, USA), fluorescence intracellular calcium indicators to monitor neuronal activity. First, we performed bulk stimulation with potassium chloride (KCl, 100mM). An increase in calcium level is expected due to the activation of voltage-gated calcium channels [8]. As shown in Fig. 3b, a 2-fold increase in fluorescence intensity was observed.

Next, we loaded the device with neurons from a transgenic zebrafish expressing the fluorescent marker protein YFP (Venus) in a subset of olfactory neurons responsive to amino acids. These neurons were identified in the device by marker protein expression in one optical channel (yellow) and their calcium levels in a second channel (red). In response to L-lysine, calcium signals were observed among a subset of cells expressing YFP while negative cells showed no significant changes in fluorescence intensities (Fig. 4a). We then sorted the cells based on YFP expression and performed qRT-PCR on the mRNA extracted from each cell for known molecular markers of positive cells. TrpC2, one such marker was expressed in all positive cells as shown in Fig. 4b.

CONCLUSION

Through the use of calcium imaging, the device allowed single cell interrogation in an active manner unlike conventional cell sorting techniques. The ability of the system to examine the dynamic responses in single cells was demonstrated by accurately identifying and recovering neurons that sense attractive odours from a mixed population of cells isolated from the olfactory epithelium of zebrafish. The universality and flexibility of the device means that it can be easily adapted for other areas in biology where cells need to be sorted and analyzed according to their signaling responses.

+ contributed equally

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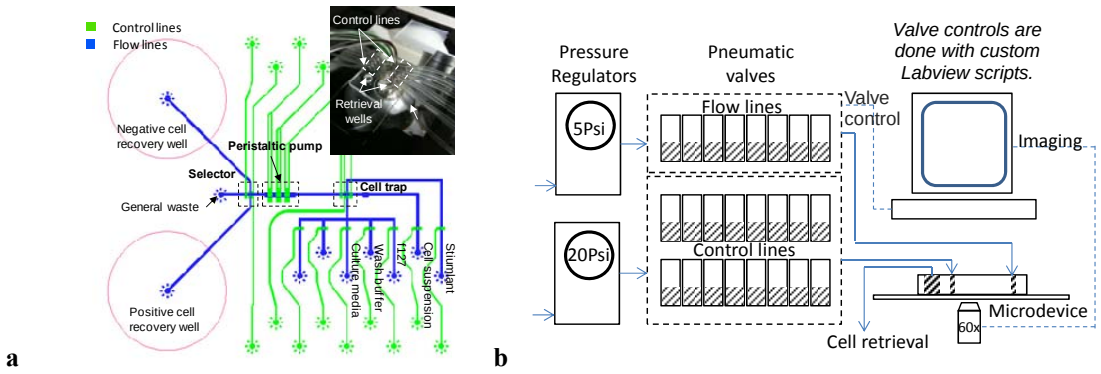

Fig. 1. Device layout and system overview. (a) Schematic of the device made of PDMS and mounted on an inverted microscope for visualization. (b) System setup to operate the device.

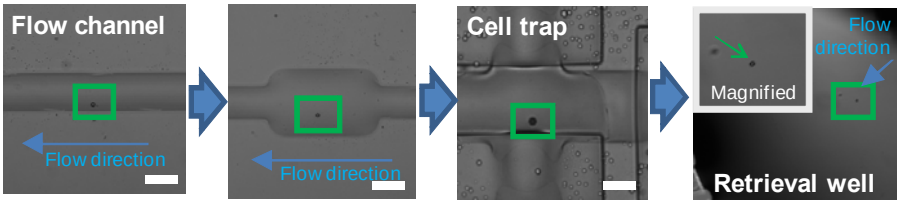

Fig. 2. Real time tracking of cell manipulation, capture and recovery. Scale bar denotes 100µm.

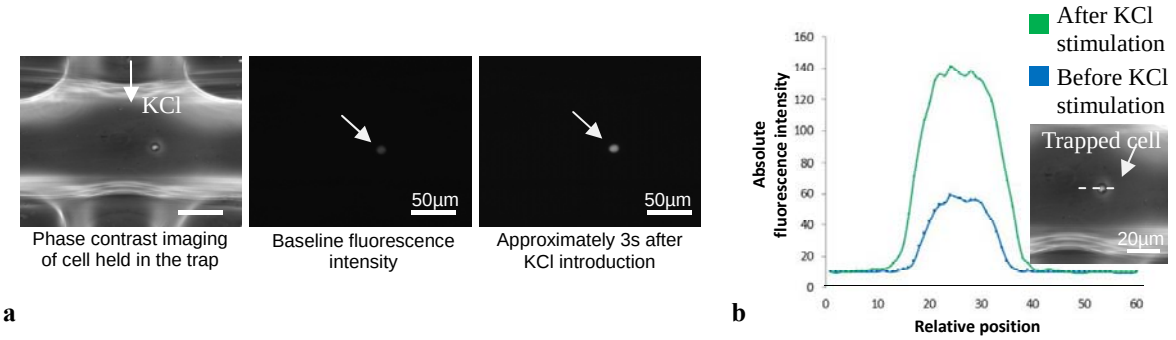

Fig. 3. KCl stimulation of a single cell in the cell trap. (a) Overview of cell trapped and KCl stimulation. Exposure taken at 300ms. (b) Fluorescence profile of the cross section of the stimulated cell.

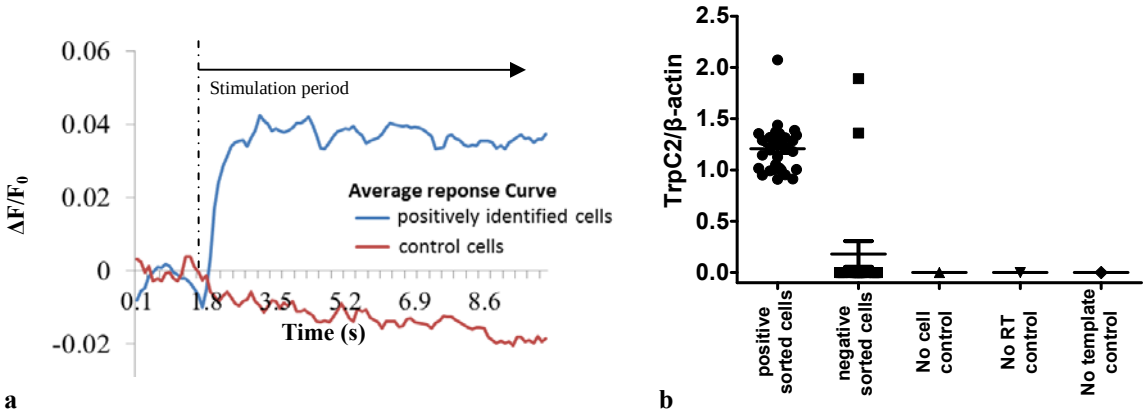

Fig. 4. Responses of single cells. (a) Average fluorescence response profile from 32 YFP positive cells and 18 control cells. (b) mRNA expression profile of YFP-positive and YFP-negative cells recovered in the sorting experiment determined by qRT-PCR.

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CONTACT

William F. Burkholder +65 6586-9840 or wfburkholder@gmail.com