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SINGLE CELL STIMULATION ASSAY: MICROFLUIDIC SUBSTANCE DELIVERY TO A LATERALLY TRAPPED CELL

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ABSTRACT

We propose a novel cell stimulation device for the analysis of cell responses to chemical stimuli. In order to deliver chemical substances to target single cells, we developed a microfluidic device having microchannels and apertures in the side wall to subject stimuli to laterally trapped cells. The channels were designed to allow simple flow control with single syringe pump. We demonstrated single cell trapping and culturing of pancreatic β cell with the device. To test its feasibility in cell stimulation assay, intracellular response of the cell to glucose stimulation was demonstrated.

INTRODUCTION

Cellular responses to chemical stimulation play a central role in living tissues, such as hormone production, apoptosis and differentiation etc. Pancreatic β cells in a living islet are stimulated by glucose of high concentration in blood capillaries and in response secrete stored insulin[1]. However, its details are not well-understood at single cell level, because there has been no method to apply stimuli to single cell *in vitro* with a controlled manner. To overcome this problem, we developed a microfluidic device that allows delivery of stimuli to single targeted cell, constant concentration of stimuli and observation with high spatial resolution. The device consists of microfluidic channels, having apertures in its sidewall. Single cell is laterally trapped at the aperture and chemical substances are delivered in the channel to stimulate the cell exclusively (Figure 1).

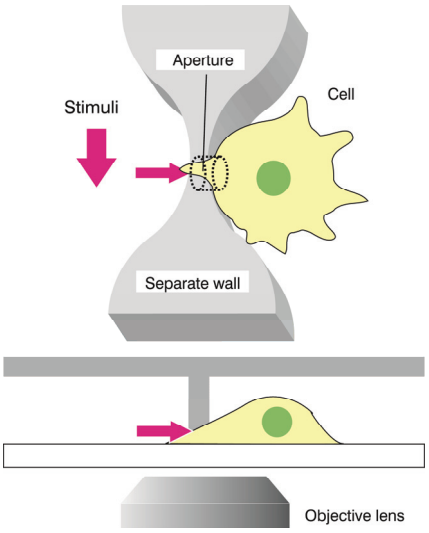

FIGURE 1. SCHEMATICS OF THE SINGLE CELL STIMULATION ASSAY USING MICROFLUIDIC CHANNELS

During the stimulation to the cell, the intracellular signal can be monitored using fluorescence imaging techniques. This scheme allows the observation of intracellular response of single stimulated cell with constant stimulus concentration and also

the propagation of the signals to the surrounding cells, which are not directly stimulated, based on the intercellular communication[2]. This device also enables high-resolution imaging because there exists only glass substrate separating the cell and the objective lens (i.e. high numerical aperture lens can be used). This paper reports the design and the fabrication of the device and the feasibility test by demonstrating the imaging of intracellular response of a pancreatic β cell to glucose stimulation.

EXPERIMENTAL

The major technical hurdle for handling single cells and chemical substances in the device is the stability of the pressure and flow rate, because their fluctuations bring leakage of stimuli to untargeted cells, release of trapped cell or dilution of stimuli. Precise control in each microchannel, however, requires multiple expensive equipments such as flow sensors and pumps. To simplify the external equipments, we designed microfluidic channels that are driven by single syringe pump (Figure 2a). The microfluidic device has 2 microchannels (Ch1 and Ch2 in Figure 2a) that join together towards 1 outlet, and lateral apertures between them, whose size is smaller than the cell. Ch1 and Ch2 have similar structures, but rectangular obstacles at different positions, which give large pressure drop inside the channels. When the channels are aspirated from the single outlet, a flow through the apertures is spontaneously generated due to the asymmetric channel shapes between Ch1 and Ch2 (Figure 2b). Figure 2 a) shows the pressure map (simulation was done by COMSOL Multiphysics [COMSOL Inc.]). This indicates that the pressure in Ch2 is consistently higher than that in Ch1 around the apertures. Thus, there exists the flow from Ch2 to Ch1 through the apertures. When the cells are applied to Ch2, they are carried by the flow and some of them are trapped at the apertures. After that, they are cultured to adhere to the channel surfaces. The pressure difference is maintained by keeping the aspiration from the outlet, holding the cells at the apertures during the process.

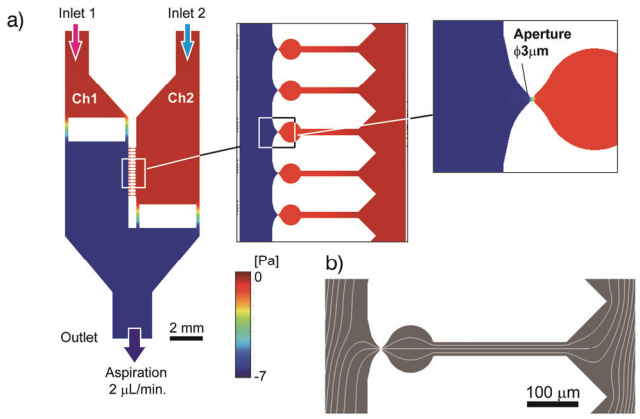

FIGURE 2. DESIGN OF THE MICROFLUIDIC DEVICE. A) PRESSURE MAP IN THE MICRODEVICE. B) STREAMLINE.

For stimulus delivery, solution containing stimuli is applied to Ch1, and aspirated from the outlet. The stimuli flow in Ch1 and are delivered exclusively to the cells. Because the pressure in Ch1 is consistently lower than that in Ch2, stimuli do not leak into Ch2 and the cells seal the apertures. This secures the delivery with constant stimulus concentration and target cell stimulation. As we mentioned above, the whole processes (cell trap, cell culture and stimulation) require only single pump for aspiration from the single outlet. Thus, the precise fluidic control is simplified with this design.

The device was fabricated by using soft-lithography of PDMS with self-forming polymer bridge that have been previously developed and detailed in the literature [3]. Figure 3 shows the device. The device was placed on an inverted optical microscope (IX-71 Olympus Co.) equipped with a stage top incubator, and incubated at 37°C. The outlet of the device was connected to a syringe pump (KD Scientific).

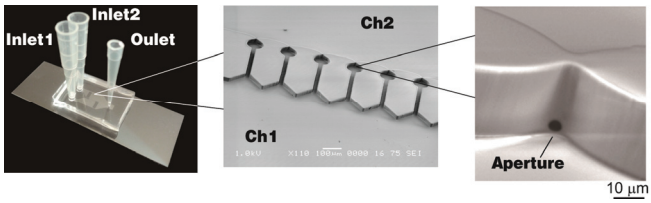

FIGURE 3. FABRICATION RESULTS OF THE MICROFLUIDIC DEVICE.

RESULTS AND DISCUSSION

We demonstrated cell trapping and stimulation using a pancreatic β cell, MIN6m9 [4]. Figure 3 shows the results of cell culture and stimulation. This shows that the cell adhered to the channel surface and proliferated there, indicating cells were successfully cultured in this chip.

To test the stimulation to the cell, we used a fluorescent intracellular [Ca^{2+}] indicator, Fluo-8. MIN6m9 cells were first stained with the dye, and then trapped at the apertures. Intracellular [Ca^{2+}] of MIN6m9 cells increases when the cells are stimulated by 20 mM glucose solution [5]. Figure 3 shows the result of intracellular [Ca^{2+}] change after stimulation. The intracellular [Ca^{2+}] increased when 20 mM glucose was applied, whereas it did not change with 0 mM glucose solution. This indicates the successful stimulation to the targeted single cell with this device.

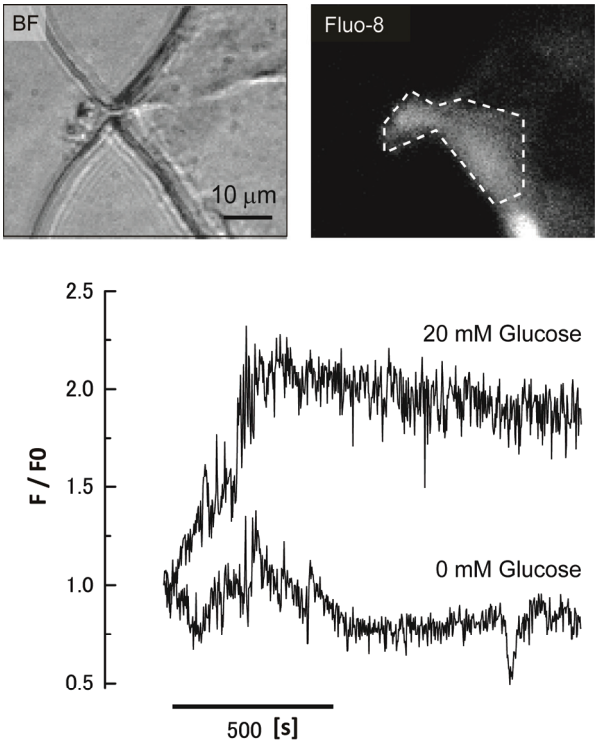

FIGURE 4. TIME COURSE OF INTRACELLULAR [Ca2+] AFTER GLUCOSE STIMULATION. UPPER: BRIGHT FIELD AND FLUORESCENT IMAGES OF THE TRAPPED CELL. LOWER: NOMALIZED FLUORESCENT INTENSITY INSIDE THE CELL AFTER STIMULATION.

CONCLUSION

In conclusion, we developed a method for the single cell stimulation assay that allows the analysis of intracellular responses to target stimulus delivery. The targeted delivery of glucose to a pancreatic β cell, as well as the imaging of intracellular response, were demonstrated. This method is expected to enable the single cell analysis of various types of cells and also the studies of cell-cell interactions at single cell level.

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