‘SNIFFER-PATCH ASSAY’ ON A MICROFLUIDIC CHIP FOR HIGH-THROUGHPUT SCREENING OF DRUGS TO CONTROL NEUROTRANSMITTER RELEASE

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ABSTRACT

The analysis of neurotransmitter in single cell level is essential because drug screening of many fatal neuronal diseases can be discovered. However, current biological experimental methods have difficulty to increase the throughput of drug screening due to lack of sophisticated fluidic control or cell handling. In order to provide an effective experimental tool for the analysis of neurotransmitter release, we developed a microfluidic device for sniffer patch assay using selective trapping of cell-pairs. Two different cells were positioned using an array of trapping channels with a cross-section of 3 μm by 2 μm. It was designed to be compatible with conventional sniffer patch assay setup. We could verify that the operation of the device and we could obtain comparable result about Ca^{2+} response of glioblastoma U-87 and HEK293 with NMDA receptor. This result shows the feasibility of microfluidic devices for sniffer patch assay as a new tools for physiological study of glial cells.

KEYWORDS: microfluidic, cell pairing, drug screening, neurotransmitter

INTRODUCTION

Several techniques has been proposed to detect neurotransmitters. Enzymatic electrochemical detection provides sensitive measurement method when released transmitter can be readily oxidized or reduced. The enzymatic method, however, cannot be used to detect fast dynamics of neurotransmitter release from a cell since the speed of enzymatic conversion is too slow. ‘Sniffer patch’ detection was suggested as a fast responding detection method since it exploits the natural fast response of ligand-gated ion channels to their native neurotransmitter [1]. When we want to detect the glutamate released from a glial cell, calcium imaging of a reporter cell with a glutamate-sensitive calcium ion channel can tell how much glutamate exist around it. The genes of calcium ion channels are expressed in the membrane of reporter cells by gene transfection, and the cell is positioned close to glial cells that releases glutamate. The close positioning of reporter cell to releasing cell is a random seeding process and the random search for appropriate pair of cells is time consuming and laborious to be inappropriate for high throughput screening of drugs that controls the neurotransmitter release. Hence, this paper proposes a microfluidic chip to locate tens of pairs of cells in trapping locations for measuring the secretion of a neurotransmitter from a cell. The individual glutamate-secretion cells were trapped hydrodynamically with equal spacing (20~50 μm) in a row [2] and the reporter cells were positioned at the opposite side of secretion cells. The calcium channel of the reporter cells respond to the glutamate released by the secretion cells.

EXPERIMENTAL

Microfluidic devices were designed to locate a pair of two cells, HEK293 and glioblastoma U-87, within a close distance. SU-8 negative photoresist was used as a master mold for main channel and cell trapping ports on silicon substrate where the patterns for 3 μm deep mirochannels for capturing cells had been etched by deep reactive ion etching. For easy detachment of polydimethylsiloxane (PDMS) mold from SU-8 pattern, the master was coated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane. After mixing with curing agent, PDMS was poured up to 2 mm thickness on the master and was cured at 80 ℃ for 1 hour. The cured PDMS slab was bonded to a slide glass by plasma cleaner and the devices for sniffer patch assay was completed with two levels of channels. The cross section of main channel was 50 μm by 50 μm, and that of cell trapping channels was only 3 μm by 2 μm (Fig. 1a). The channel was filled with poly-L-lysine(PLL) to enhance cell attachment. PLL solution was added in the entire microfluidic channel with pipette and the device was placed overnight in a humid incubator at 37℃. After about 12 hour, the solution was removed and washed twice with deionized water. Then the device was filled with media and put in the incubator

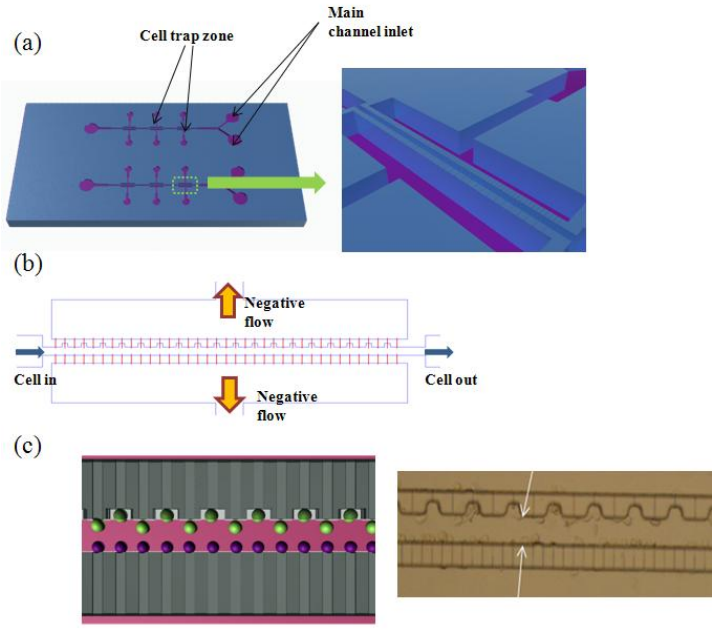

Figure 1: (a) schematic diagram of microfluidic chip to pair 2 types of cells: main channel has two inlet for U-87MG and HEK293 respectively. There are three cell trap-zones (b) principle of cell trapping: upper and lower chambers are used for suction of cells to be trapped (c) magnified schematic and photo of trapped cells: U-87MG cells were fixed first and then HEK293 cells were positioned.

before used for sniffer patch.

Each device has six cell-trap ports connected to a 1 ml disposable syringe. As depicted in Figure 1b, cell suspension were flowed from left inlet to right outlet through main channel, and top and bottom ports were controlled by suction for cell-trapping in a row. Independent control of these ports allowed selective trapping of each kind of cell on a specific side respectively.

As shown in Figure 2, the device was installed on a calcium imaging microscope for sniffer patch. We used two cells; one is a glioblastoma cell (U-87MG) releasing glutamate responding to certain drug and another is human embryonic kidney cell line, HEK293 with calcium ion channels to respond glutamate sensitively. HEK293 was transfected by NMDA (*N*- methyl -*D*- aspartate) receptor gene to detect glutamate. When glutamate is released by U-87G, the calcium channels with glutamate receptor in HEK 293 cell react and become open. The concentration of glutamate was quantified by calcium imaging of fura-2-acetoxymethyl ester (Fura-2AM) reagent to recognize the opening of ion channel. It is a radiometric calcium indicator that can reduce artifacts of manual handling [3]. Before experiment of sniffer patch assay in the chip, two cells were soaked in Fura-2AM for 30 min and then washed by HEPES buffer. We injected 30 μM Selective PAR-1 activating peptide TFLLR-NH_2 (H-Thr-Phe-Leu-Leu-Arg-NH2, TFLLR)for 30 second to observe if it activates protease activated receptor(PAR-1)receptors in U-87MG to release glutamate and we switched the inlet to HEPES buffer for washing. The calcium imaging of more than 16 cells was recorded and quantified by image analysis software. Then we added mixed solution of 100 μM glutamate and 50 μM glycine for 30 second to guarantee the expression of transfected NMDA receptor genes, because NMDA receptor consist of two subunits, NR1 and NR2, which specifically respond to glutamate and glycine respectively [4].

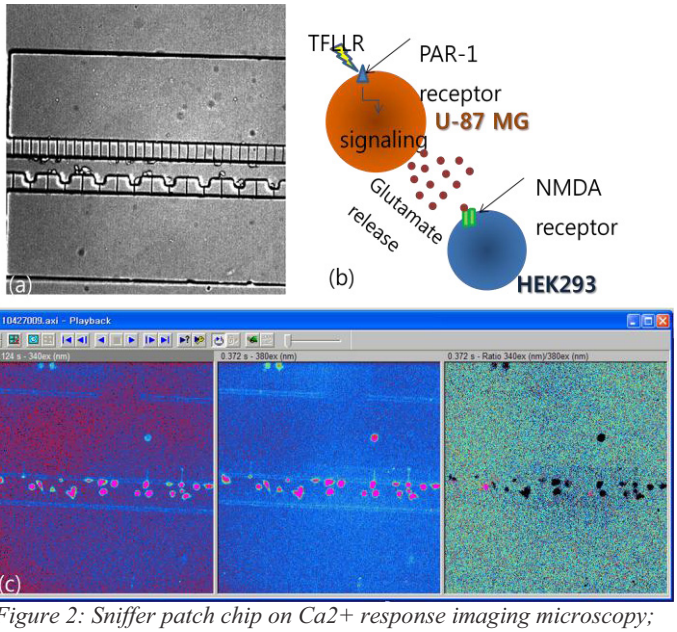

Figure 2: Sniffer patch chip on Ca2+ response imaging microscopy; The calcium imaging of more than 16 cells was recorded and all the quantified signals of the cells (a) optical image of cells in the chip (10X) (b) working principle of sniffer patch technique to detect neurotransmitter such as glutamate (c) Ca^{2+} response image; this program analysis change of cells' optical signal by ion channel opening

RESULTS AND DISCUSSION

The sequential suction of trapping channels allows selective entrapment of each cells. The efficiency of cell trapping in the chip is important for more effective cell handling of microfluidic chip than of conventional random cell seeding. The experimental result showed that trap efficiency was inconsistent depending on the position of trap zones and on the cell loading time. The zone close to inlet has higher efficiency and the longer cell loading time can decreased the efficiency since it can induce the disturbance of main flow due to the attachment of sunken cells at the bottom of inlet. Nevertheless, the trap efficiency was almost over 80% and the number of trapped cells was enough to conduct sniffer patch assay.

After the cells were paired at each side, TFLLR was injected to stimulate calcium channel for the secretion of glutamate from glioblastoma. The calcium imaging of U87MG showed the calcium ion channel with PAR-1 receptor that responds to TFLLR, and that of HEK293 revealed that the NMDA receptor responded to glutamate released by U87MG (Figure 3). The second injection of glutamate and glycine indicates that the transfection into HEK293 cells were imperfect since a few of the HEK 293 cells did not show calcium influx. It is noted that the calcium influx of HEK293 followed that of U87MG with time delay. That means HEK293 cells responded with the glutamate from U87MG. Some HEK293 cells, however, showed simultaneous increase of calcium influx with that of U87MG. It is believed that those cells had PAR-1 receptor to respond TFLLR and we excluded the experimental result in statistical analysis

To observe the effect of location of cells, we selected out a calcium imaging of one pair of cells and plotted the graph in Figure 4. It is clearly observed that the response of HEK293 with NMDA receptor (red line) immediately appears after that of glioblastoma to TFLLR injection (black line). It indicates that the signal of HEK293 was caused by the glutamate release of U-87 that was located near the HEK293 cell. The TFLLR was effective in the activation of the PAR-1 mediated calcium ion channel of glioblastoma in microfluidic chip.

Though these results showed the feasibility of 'sniffer patch' assay in microfluidic chip, we need to consider the effect of advection caused by the flow of TFLLR. The glutamate released by U87MG was transported by main channel flow and the concentration of glutamate was affected by the flow. The response of HEK 293 depends on the flow speed and the trapped position. If the response of single pair of cells are desired, the direction of flow needs to be redesigned. In addition it is important to select reporter cell line carefully. It was observed that some of HEK293 cells responded to TFLLR non-specifically. It might be helpful to construct another control channel to check if drug candidate causes non-specific response.

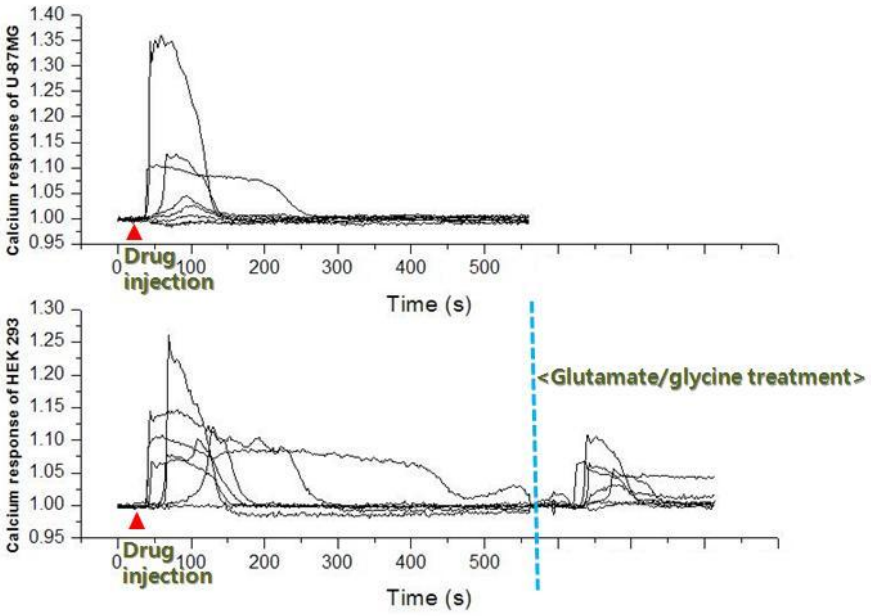

Figure 3: Ca^{2+} response of U-87 and HEK293 with NMDA receptor in one trap zone; U-87MG(the upper graph) and HEK293 with NMDA receptor(the lower graph) ((lower graph' right part of blue dash line indicates response of NMDA receptor by glutamate and glycine)

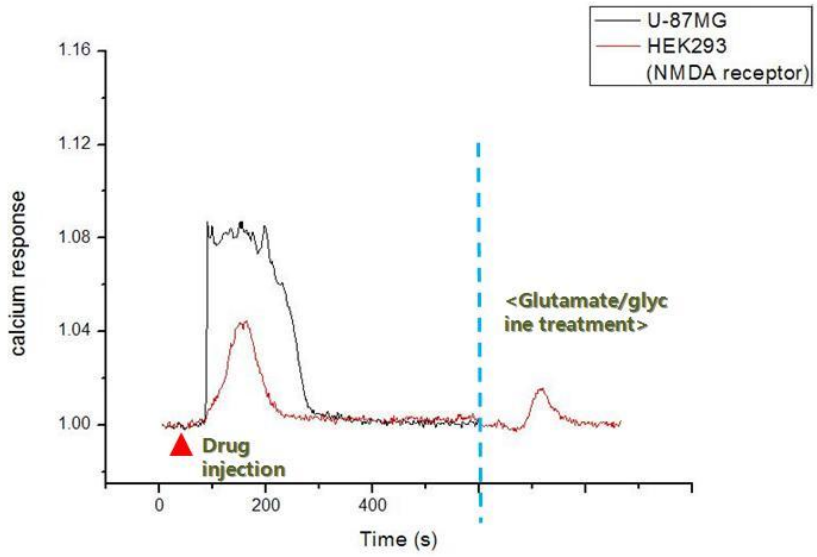

Figure 4: Ca^{2+} response of selected one pair of U-87 and HEK293 with NMDA receptor ;U-87MG-Blue line and HEK293-Red line (Right of blue dash line shows response of NMDA receptor by glutamate and glycine)

CONCLUSION

This work described the characterization and the feasibility of microfluidic devices for sniffer patch assay. This method provides facile method to locate the reporter cells close to glioblastoma cell with small volume of reagent. We demonstrated that the chip is capable of high cell capture rate and reliable pairing rate. Also, without any modification of conventional sniffer patch assay setup, we could conduct an experiment of glutamate detection effectively. It is expected that this device can provide a tool for better and more efficient experimental conditions in cell-based physiological study.

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