MICROFLUIDIC ANALYSIS OF NEURODEGENERATIVE IMPACT EVOCKED BY LOCAL STIMULATION-TRIGGERED APOPTOTIC INFORMATION

Yoshikuni Edagawa1, Taku Watanabe2, Masaru Fuji2, Kentaro Kawai3 and Shuichi Shoji4

1Waseda Institute for Advanced Study, Japan, 2Graduate School of Science and Engineering, Waseda University, Japan, 3Graduate School of Engineering, Osaka University, Japan, 4Faculty of Science and Engineering, Waseda University, Japan

ABSTRACT
Microfluidic analysis system for microscopic pharmacology handling single cells was demonstrated in this paper, which included the stimulation only on a local area of the target cell by using the laminar flowing of drug solutions and the systemic monitoring of the effect evoked on the whole cell. By this system, it was first elucidated that the stimulation of the tip of neurite with an apoptosis-inducing drug resulted in the expression of an apoptotic marker in the cell body as a remote position from the stimulated point. This indicates a new paradigm of microscopic pharmacology opens up here.

KEYWORDS: Single cell, Apoptosis, Neurite transmission, Neurodegeneration, Micropharmacology

INTRODUCTION
For the conventional pharmacology approach, where a cause of the neuronal loss was, in the cell body or the neurite, has been difficult to elucidate; whereas losses of brain function due to the neurodegenerative disease are often caused by the breakdown of the neural network with the cell death. However, recent progress for handling single cells and biomedical application [1,2] has enabled approach for the explication of such a phenomenon. And, this paper showed a powerful application for elucidating the neuropathological event(s) and relieving conventional pharmacology distress.

Fig. 1 shows the concept and accessible scheme of this study, which demonstrated stepwise procedure for non-invasive capture and culture of a single cell, differentiation of the cultured cell, local drug stimulation at an arbitrary domain and systemic observation of the cell behavior that focused on apoptotic impact transmitted from the spot to the whole cell.

Fig. 1: Schematic illustration of the concept and procedure of this study. (a) Non-invasive single cell capture at a microchamber of the device. (b) Isolate culture and induction of neurite outgrowth of the captured cell. (c) Local drug exposure at a targeted microdomain by using laminar flow. (d) Systemically microscopic observation of the event(s) evoked by local drug exposure. (e) Elucidation of the neuropathological phenomenon by the microsystem: involvement of subregion controlling whole cell fate and its mechanism.
EXPERIMENTAL

Device Fabrication. Devices were fabricated in poly-dimethylsiloxane (PDMS) by using conventional soft-litho-graphic lift-off and grafting process [2]. Fig. 2 shows the fabrication and design of the present system. The element structure of the microfluidic device in PDMS consisted of two main channels of 100 μm-wide, where cell suspension or culture medium flowed, and a narrow bypass tract of 3 μm connecting them. Microchambers for cell capture and culture were semi-cylinder-shaped of 100 μm in diameter, which were faced and lined on one of main channels.

Preparation of Cells Introduced into the Device. PC12 cells were cultured at 37°C in a humidified atmosphere of 5% CO₂ and 95% air in Dulbecco’s Modified Eagle’s Medium (DMEM, Sigma, USA) supplemented with 10% fetal bovine serum (FBS, biowest, USA), 10% horse serum (HS, ICN biomedicals, USA), 100 U/mL penicillin (Sigma, USA), and 100 μg/mL streptomycin (Sigma, USA). Petridishes of 100 mm in diameter with cells approximately 80% confluent were trypsinized (0.2% Trypsin EDTA, Sigma, USA) under sterile conditions. Typical concentration of the sample cell suspension of 1 x 10^5 cells/mL.

Cell Capture. Single-cell capture was performed in the device by controlling flow rate of two straight channels. Non-invasive cell capture was achieved by the appropriate balance between flow rates of two main channels [2].

Cell Culture in the Device. Cultivation of the cells captured was performed in the device with the culture medium containing serums with reduced concentration (1% FBS and 1% HS) for differentiation of the cells. Culture of cells was continued up to the pharmacological application.

RESULTS AND DISCUSSION

Non-invasive capture of single PC12 cells from cell suspension of 1 x 10^5 cells/mL was achieved in the microchambers of the present device. Each cell was sequentially cultured in each microchamber and differentiated for neurite(s) outgrowth with 100 ng/mL nerve growth factor-containing medium. Fig. 3 shows successive images of culture of single PC12 cell. In most cases, the cultured cell put the soma in a microchamber and increased its neurite(s) into the main channel.

Fig. 4 shows a stepwise exposure of cultured cell on fluorescent dyes in order to demonstrate a model for the application of drug solution at an arbitrary site. Staining was processed using the one cell with two dyes in reversal directions. The cell was first stained with phalloidin for visualizing the actin, a protein of cell structure, in red. The process was performed from the tip of neurite to soma for staining whole cell body (Fig. 4, upper panel). Next trial was challenged in an opposite direction; from soma to the tip of neurite, with which a dye for imaging the MAP-2 protein in green (Fig. 4, lower panel). The cell was dyed with only a part prescribed in the interface of the dye solution and finally whole cell was stained with the dye exposed.
Fig. 5 shows a pharmacological application for the analysis of the cell death. The cell cultured was divided into two cells in the microchamber, and one of them was differentiated with neurite in the case of Fig. 5. Neurite was exposed to 2 uM staurosporine, an apoptosis inducer, at a targeted-local area with the laminar flow. The stimulation only at the neurite tip induced the activation of caspase-3, an effector protein for apoptotic cell death, in the soma as a remote area (red fluorescent). On the other hand, the cell in the same chamber without neurite was not stimulated and not expressed any factor responsible to stress reaction against drug administration. This indicated that the local stress evoked cell death manifestation in the remote part that should result in the whole cell death.

These results demonstrated that the present system enabled the analysis of biomedical issues, and should contribute to elucidate the effect of local stimulation on systemic events associating apoptotic impact determining the whole cell fate.

CONCLUSION
The present device was successful for single-cell capture, culture and the pharmacological application to the cultured cells. This should be beneficial for the micropharmacological analysis to elucidate the influence of the change in a part to the whole system.

This paper demonstrated that a pharmacological stress at the tip of neurite induced the whole cell fate as the observation of an expression of apoptosis-related protein was critical for the cell death. And this suggested that the neurite transmission of "death information" from the tip of neurite to the soma was crucial role in the cell death.

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REFERENCES

CONTACT
Yoshikuni Edagawa, Ph.D., eday@aoni.waseda.jp; Shuichi Shoji, Ph.D., shojis@waseda.jp