CONTINUOUS LOCAL EXPOSURE TO CHEMICAL SUBSTANCES OF SINGLE CELL

Kyohei Terao[1], Murat Gel[2], Ariko Fuke[1], Atsuhito Okonogi[1], Teru Okitsu[1], Takashi Tada[1], Takaaki Suzuki[1], Masao Washizu[1], Hidetoshi Kotera[1]

[1] Kagawa University, Japan, [2] The University of Tokyo, Japan, [3] Kyoto University, Japan

ABSTRACT

We propose a novel single cell analysis method that allows an imaging of intracellular changes induced by continuous exposure to chemical substances that are delivered to limited area of cell surface. Cellular responses to such continuous and partial exposure have been totally unknown, due to the lack of a technique for a stable spatially-confined delivery of chemical substances. We developed a novel microfluidic device with stable and facile flow control, demonstrated continuous local delivery to single pancreatic β cell, and discovered a new phenomenon: Local glucose exposure biases spatial distribution of insulin granules in a pancreatic β cell.

KEYWORDS

Single cell analysis, Cell trapping, Pancreatic β cell, Cell stimulation.

INTRODUCTION

In living tissue, cells are exposed to chemicals on their limited surface area because of steric hindrances resulted from their cytoarchitecture (Figure 1). We reproduce the situation in vitro to elucidate the responses to local exposure by delivering chemical substances continuously with constant concentration to limited area of single cell. Conventional microfluidic devices, including our previous one [1], have such drawbacks as diffusion to the other cell area and stability of a flow. Thus, we developed a microfluidic technique shown in Figure 2. Single cell is first trapped at the microorifice located between two microchannels (ch1 and ch2) by pressure difference between them, where it was kept in a incubator to adhere to the device and to seal the orifice. Chemical substances are introduced to ch1 to expose the cell surface partially. This method allows a constant concentration over time without diffusion of chemicals and disturbance of the boundary between ch1 and ch2 solution because of the solid wall between them. The microfluidic channels were designed for robust and facile flow control (Figure. 3), which spontaneously induces a pressure difference at the orifices by aspiration from the single outlet. The pressure difference can be linearly controlled by the aspiration rate, enabling us to trap cells, to keep them at the orifices and to prevent a leakage of chemical substances to ch2.

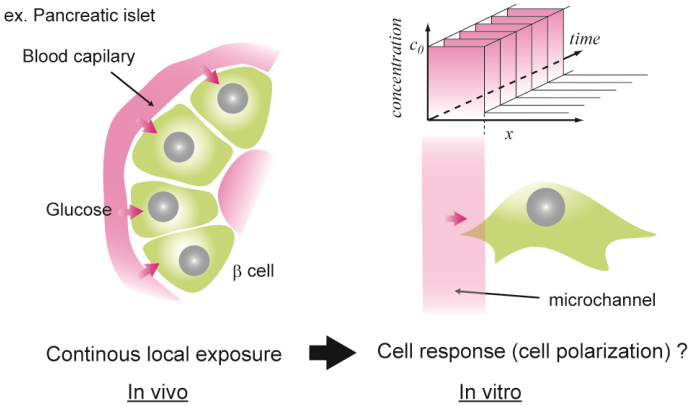

Figure 1. Continuous local exposure to chemical substances in living body (left) and in vitro reproduction of the environment (right).

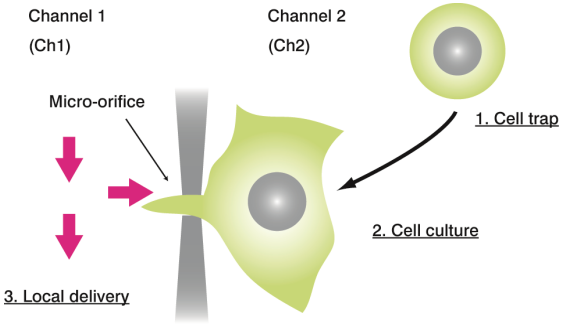

Figure 2. Microfluidic local substance delivery to single cell

EXPERIMENT

Our microfluidic device was fabricated by soft lithography technique as described in the literature [2]. A flow field in the device was evaluated by fluorescent microbeads and molecules. Stream lines were visualized by traces of fluorescent beads of 1μm in diameter. We also simulated the flow field by using COMSOL software. After trapping and incubation of pancreatic β cells for 8 h in 37°C 5 % CO_2 incubator, fluorescent solution (2 μM calcein-AM red orange/PBS) was introduced to ch1, where we checked the stability of the local delivery to a cell by observing a fluorescence intensity in ch1 and ch2 around the orifice for 4 h.

As a demonstration of the continuous local delivery, a pancreatic β cell (MIN6m9) was exposed partially to high glucose solution (25 mM glucose/ DMEM) for 4 h (Figure 5). The intracellular response was evaluated by a change of the spatial distribution of insulin granules tagged by GFP molecules. We also performed the assay of whole cell exposure (25 mM glucose in both channels) and no exposure (0 mM) as control experiments.

RESULTS AND DISCUSSION

A flow field in the device was visualized by fluorescent microbeads (Figure 4 a). Stream lines were visualized by traces of the beads, showing a good agreement with those of the simulation. This indicates that the pressure difference is successfully induced between ch1 and ch2. The stability of the flow was evaluated by fluorescent molecules (Figure 4 b, c). The difference of fluorescence intensity between ch1 and ch2 was kept over 4 h, suggesting that the device allows the continuous spatially-confined delivery to single cell without leakage and backflow of the delivered molecules. The flow was control by single syringe pump connected to the outlet of the channel, allowing the simple operation for the local exposure assay.

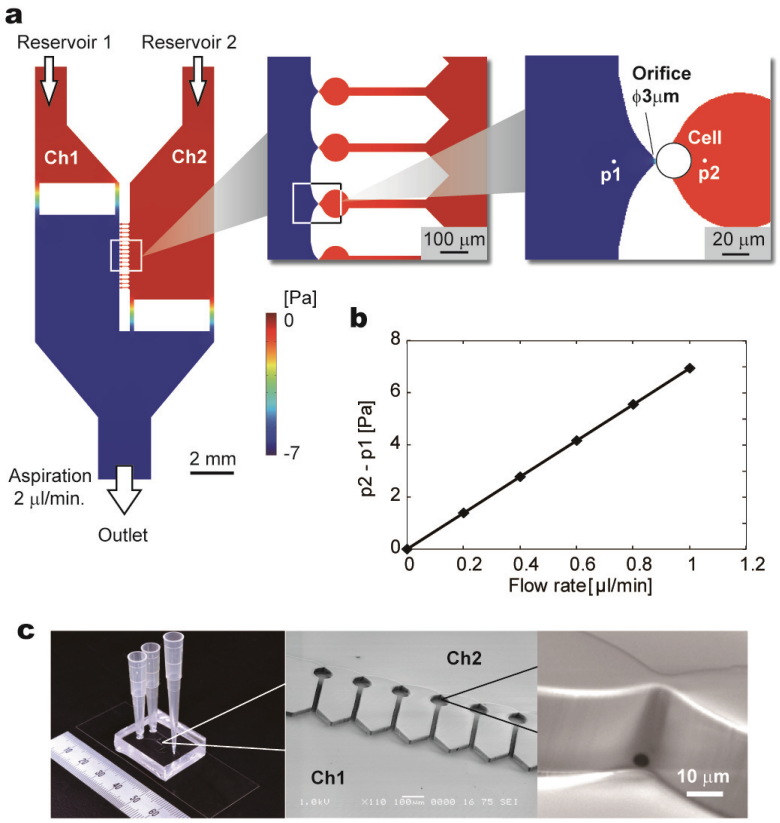

Figure 3: Microfluidic device for continuous local delivery. ***a.*** *Microchannel design with a contour map of pressure in aspiration from the outlet.* ***b.*** *Pressure difference between p2 and p1 in* ***a*** *of different flow rates at outlet.* ***c.*** *PDMS device.*

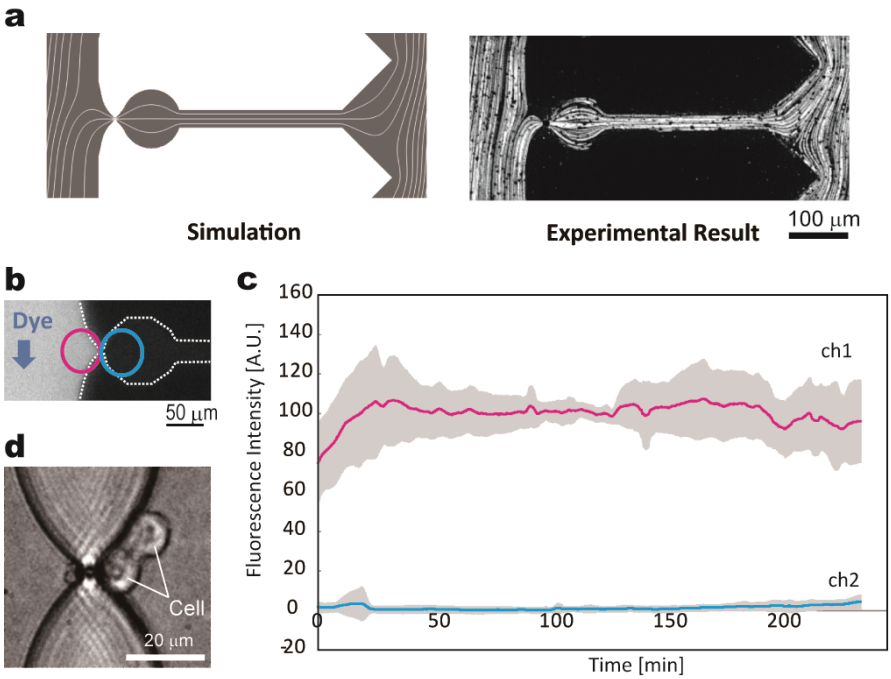

Figure 4. Flow in the device. ***a.*** *Stream lines inside the channel during cell trapping: Simulation and experimental results.* ***b.*** *Fluorescence image in a flow stability test. Fluorescent solution loaded in Ch1 is visualized.* ***c.*** *Time courses of fluorescent intensities in Ch1 and Ch2 (ROIs indicated in b). Gray area shows the standard deviations.* ***d.*** *Result of cell trapping.*

We demonstrated a continuous local exposure to high glucose solution of a pancreatic β cell and observed its insulin granules to visualize the intracellular response (Figure 5). The result indicates that the continuous local glucose exposure biases intracellular insulin granules towards the exposure site. This suggests that the continuous local chemical exposure can polarize a cell, which is expected to lead to the understanding of the cell function and polarity in living body.

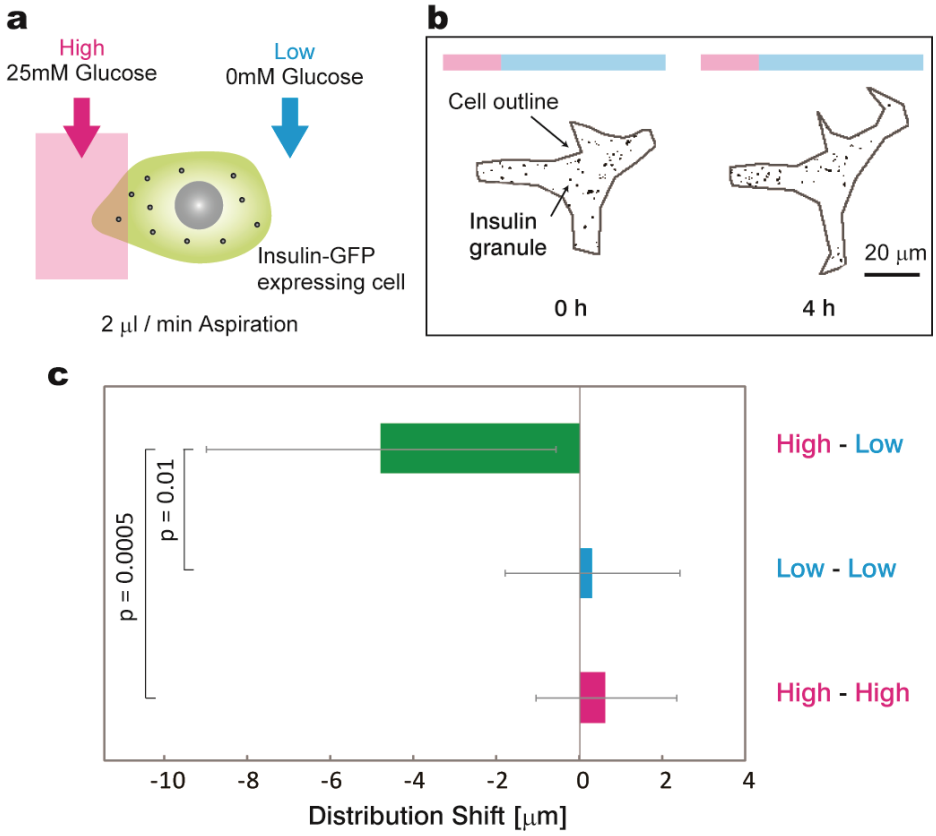

Figure 5. Distribution shift of intracellular insulin granules after 4 h local glucose exposure. ***a****. Experimental design.* ***b****. Insulin granules in a MIN6m9 cell before and after glucose exposure.* ***c****. Distribution shift induced by local glucose exposure.*

CONCLUSION

In conclusion, we developed a method for continuous localized delivery of chemicals to single cell and imaging for the analysis of intracellular responses. The targeted delivery of external substances for 4 h to the surface of a pancreatic β cell, as well as the imaging of intracellular changes, were demonstrated.

ACKNOWLEDGEMENTS

This work was supported in part by the research project 'Development of Bio/Nano Hybrid Platform Technology to-wards Regenerative Medicine', CREST, Japan Science and Technology Agency (JST) and the KAKENHI (21651062) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. Part of this work was conducted in the Kyoto-Advanced Nanotechnology Network, supported by 'Nanotechnology Network' of the MEXT, Japan.

REFERENCES

[1] "Localized substance delivery to single cell and 4D imaging of its uptake using a flow channel with a lateral aperture," K. Terao, et al. Microfluid. Nanofluid., **12**, 423 (2012).

[2] "Fabrication of three-dimensional structure by self-forming meniscus and its application to on-chip cell fusion " M. Gel, et al. Proc. Micro Total Analysis Systems 2008, 1968 (2008).

CONTACT

Kyohei Terao 81-87-864-2346 or terao@eng.kagawa-u.ac.jp