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研究課題名（和文）Control of Laminar flow in open space for subcellular operation

研究課題名（英文）Control of Laminar flow in open space for subcellular operation

研究代表者
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研究成果の概要（和文）：単一細胞刺激と乳酸のリアルタイム電気化学的モニタリングのためのプッシュプルノズルシステムを開発しました。グルコース溶液が細胞に継続的に供給され、細胞から分泌された乳酸が収集され、電気化学的検出のために吸引チャネルに輸送された。 単一細胞からの乳酸変化の迅速かつ高感度な検出が達成されました。成果は分析化学で公表されました。
細胞の一部を正確に操作することで細胞間のコミュニケーションを探求する5ノズルの化学ペンを開発しました。有効領域の流体は安定しており、他の部分に影響を与えることなく単一セルの限られた領域を刺激して操作するのに十分な高い空間分解能を持っていました。

研究成果の学術的意義や社会的意義
We developed new technology for cell analysis. It will be a powerful tool for scientists to carry our single cell researches and cell molecular science.
And the achieved single cell analysis will benefit for disease diagnosis, especially for cancer diagnosis.

研究成果の概要（英文）：Firstly, we developed a push-pull nozzle system for in situ single-cell stimulation and the real-time electrochemical monitoring of lactate secreted from the cell. Glucose solution was continuously supplied to the cell and the lactate secreted from the cell was collected and transport to the aspiration channel for electrochemical detection. Rapid and sensitive detection of lactate changes from a single cell was achieved by amperometry. The results was pubilished on Analytical Chemistry.
Then, we developted a five-nozzle chemical pen to explore the communication between cells by precise manipulation of a part of the cell. By controlling the fluid velocity at each nozzle, a thin effective region was formed in the central region. The fluid in the effective region was stable and had high spatial resolutions enough to stimulate and operate a limited region of a single cell without affecting the other parts.

研究分野：分析化学

キーワード：Microfluidics, Microchemical Pen, Laminar flow, Single cell, Lactate, Subcellular

１．研究開始当初の背景

Single-cell analysis provides critical information to understanding key disease processes and disease diagnosis. In conventional methods for biochemical analyses, samples were prepared from a large number of cells in certain condition in order to get sufficient amounts of molecules. In fact, the results were “average” ones. However, we frequently found that the behaviors of isogenic cells were not identical even in the same culture dish. Under consideration of individual cell behaviors, the “averaged” results might be insufficient and sometimes incorrect.

Information from single cells will be more accurate to uncover the mechanisms of cell behaviors and cell heterogeneity. Substantial methods on single-cell array, single-cell droplet were combined with fluorescence analysis, electrophoresis and mass spectrometry. However, nearly all of the current methods carry out single-cell measurement in suspension, which not only destroy extracellular context but also may perturb the intracellular metabolites. It is essential to develop new methods to meet the requirements of understanding individual cell behaviors and their relations in adherent tissue culture. How to realize precise operation of single cells and subcellular molecules infusion in adherent cell culture is a critical question that researchers face.

２．研究の目的

Our purpose is to develop new tool for surgery on single cell to understand transport of organelles and cell would and repair mechanism. We believe this approach will be a new avenue for sinlge cell operations and subcellular operations. Few approaches are able to realize operation of partial cell. In this proposal, we will control laminar flow in open space. Thus, the flows could be used to treat cells adherent culture.

３．研究の方法

1)Design and fabrication of the probe. The device was fabricated by a capillary stretching technique with a heat puller.

2)Operation system. The operation system contained a positioning system, an XY motion control system and a flow injection and aspiration system.

3) Finite element analysis. Comsol Multiphysics 5.3b (Comsol) was used to carry out 3D simulations. The geometry of the probe was set as same as that in the experiment.

4)Characterization of the probe. The shape and the bottom surface of the probe were examined by a scanning electron microscope.

Investigation of the interface between two injected flows. Solution containing 1 μg/ml fluorescein for visualization, was used to verify the zone of central injected solution and the interface between the two adjacent solutions.

5) Opening the cell plasma and staining of partial cell. RIPA non-denatured tissue lysis buffer was used in cell cutting experiments for lysing portion of cell.

４．研究成果

1) We used a push-pull nozzle system for *in situ* single-cell stimulation and the real-time electrochemical monitoring of lactate secreted from the cell (Figure 1). The system involved a steady flow in the cellular microenvironment by injecting and then aspirating a solution. Glucose solution was continuously supplied to the cell and the lactate secreted from the cell was collected and transport to the aspiration channel for electrochemical detection. Glass beads modified with LOx and a gold electrode modified with osmium ($Os[byp]_2^{2+/3+}$)-horseradish peroxidase (HRP) conjugated redox polymer were assembled inside the aspiration channel. Lactate in the aspirated flow decomposed at the surface of glass beads and then detected by gold electrode. Rapid and sensitive detection of lactate changes from a single cell was achieved by amperometry. This precise detection represents a powerful tool for single cell diagnosis and drug screening for cancer.

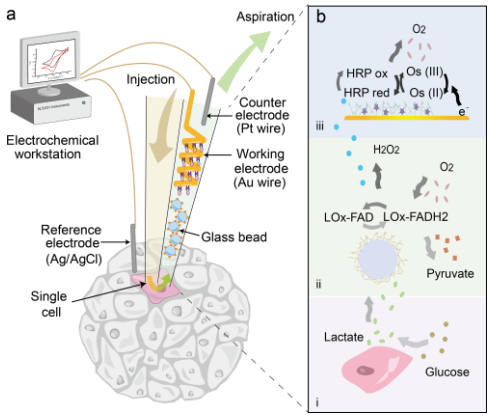

Figure 1. Electrochemical detection of lactate from a single cell by a push-pull nozzle system. (a) Scheme of the push-pull electrochemical nozzle system. (b) Lactate collection, bienzyme reaction and electrode reaction in the detection process.

2) **Detection of lactate at single cell level.**

The concentration of released lactate continuously increased within 47 min (Figure. 2a). The current signal increased with the increasing released lactate. After using PBS instead of glucose at 47 min, the signal rapidly returned to the normal level. The results suggested that the probe was suitable as a tool for real-time, in situ monitoring lactate due to its rapid response to the change of lactate signal. The current signal for individual cells to the application of glucose is shown in Figure. 3b. The effect of glucose dissolved in PBS solution (pH 7.3) on single cell was then investigated to examine whether different cells provided different responses to glucose or not (Figure. 3c). The release of lactate from a single cell either without treatment (Control group, red) or with glucose treatment (Treatment group, green) was examined by the push-pull nozzle system.

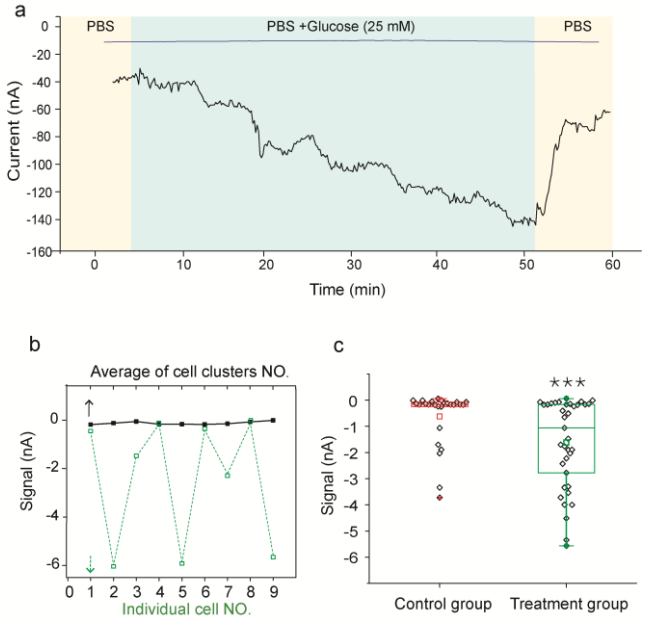

Figure 2. Current response derived from lactate at each measurement. (a) (Black line) Typical i/t recording for lactate analysed with the push-pull nozzle system from cell clusters at 0.27 V (vs Ag/AgCl, aspiration only: 60 μL/h). The distance between nozzle and cells was 20 μm. 25 mM glucose was added during the time shown in the green background. Number of cells were 1×10^5 cells. (Blue line) Current observed without cells. (b) Cell heterogeneity analysis. Signals were measured after 20 min from glucose addition. Black: Average of the signals of cell clusters. Green: Signals of the single cell. (c) Single cell scatter plot showing the distribution of the difference of amperometric response of a single cell at 20 min after addition of PBS (red: control group) or addition of glucose (green: treatment group). A two-tailed Student's t-test was performed in c. ***P <0.001.

3) In situ subcellular processing program for single cell analysis by open microfluidic probe

A new open microfluidic approach, was developed to explore the communication within and/or between single cells based on precise manipulation of partial cell (Figure 3). The method is based on the structure of three-phase laminar flow, the fluid in the effective region is stable and has accurate spatial resolution, which can be used to stimulate and operate part of a single cell without affecting the function of other parts. This device has been successfully used for subcellular structure labeling, cutting and calcium signal tracking. This design provides a new method for cell-to-cell signaling studies and disease treatment.

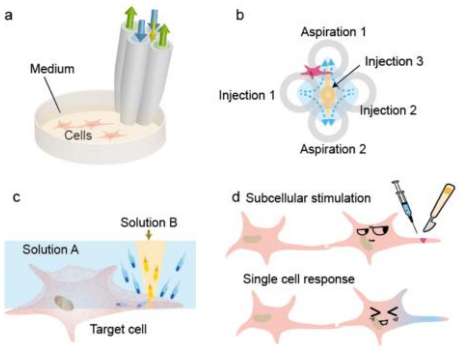

Figure 3. Microfluidic probe for precise processing on single cells. (a) Design and operation of the probe. (b) The principle of fluid control for partial treatment by thin effective region. (c) Subcellular molecular infusion of a single cell using the probe. (d) Illustration of precise processing, stimulation or dissection, on a single cell.

4) Characteristics of fluid dynamics

The change of laminar flow structure and the width of the thin effective region in the figure were shown in Fig. 4a. The results showed that with the increased of flow rate ratio of aspiration 2, the width of fluorescent region decreased down to 8 μm (Fig. 4b). It is worth noting that the fluorescent images come from the superposition of different fluorescent layers, and the actual size (5 μm in simulation) of the bottom fluorescent region can be smaller than 8 μm.

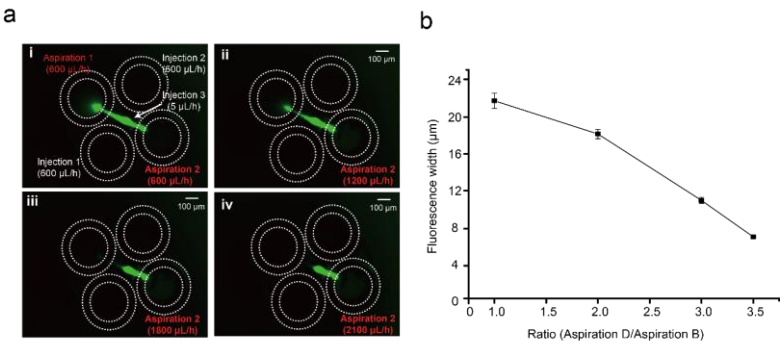

Figure 4 The influence of different aspiration flow rate ratio on laminar flow structure. Fluorescent images of the laminar flow structure (a). The flow velocity of other channels remains unchanged, and the aspiration flow rate of aspiration 2 is adjusted to (i) 600, (ii) 1200, (iii) 1800 and (iv) 2100 μL/h in turn. (b) The line chart is the statistical result of the width of the fluorescent signal of the thin effective region formed between injection 3 and aspiration 1.

5) *In situ* labeling of monocellular and subcellular structures

Single cells were first used to verify the function of the probe before staining the subcellular structure. (Fig. 5) During the labeling process, stimulus solution was

injected from injection 3, and culture medium was injected from injection 1 and 2. As a standard condition, the injection flow rate (Ri) =600 μL/h, the aspiration flow rate (Ra) = 600 μL/ h, and the gap =50 μm. The laminar flow formed under the probe approached the cell along the direction perpendicular to the cell until the target region was completely immersed in calcein solution. Calcein-AM solution formed the thin laminar flow region under the pressure of buffer and diffused into the target region of single cell, while the rest of the cells were protected from interference by the buffer. Calcein-AM was decomposed by esterase after entering cells to form calcein with green fluorescence.

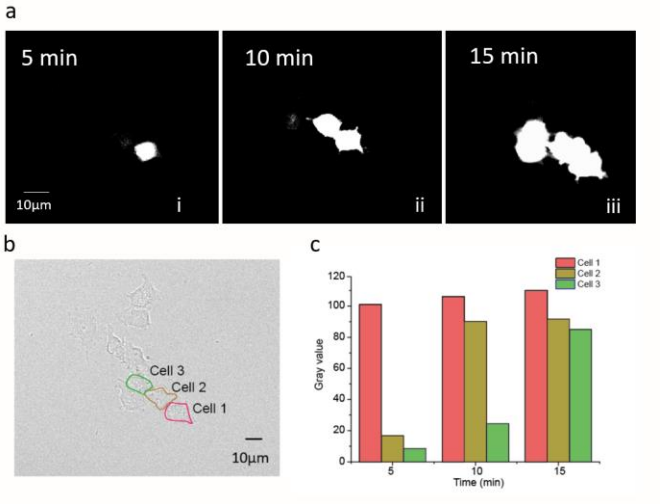

Figure 5 Precise labeling of single cells. (a) Fluorescence field image of cells. i-iii represents cells at three different locations. (b) Schematic diagram of bright field of cells. (c) The average value of cell fluorescence intensity.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Zhou Lin、Kasai Nahoko、Nakajima Hizuru、Kato Shungo、Mao Sifeng、Uchiyama Katsumi	４．巻 93
２．論文標題 In Situ Single-Cell Stimulation and Real-Time Electrochemical Detection of Lactate Response Using a Microfluidic Probe	５．発行年 2021年
３．雑誌名 Analytical Chemistry	６．最初と最後の頁 8680～8686
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.analchem.1c01054	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Nishitani Yuki、Kasai Nahoko、Nakajima Hizuru、Kato Shungo、Mao Sifeng、Uchiyama Katsumi	４．巻 58
２．論文標題 Regioselective fabrication of gold nanowires using open-space laminar flow for attomolar protein detection	５．発行年 2022年
３．雑誌名 Chemical Communications	６．最初と最後の頁 4308～4311
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/d2cc00507g	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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２．発表標題 Local Fabrication of Nanowires with High Aspect-Ratios using a Diffusion Mixing Reaction System for Temperature Sensing
３．学会等名 2021年第68回 応用物理学会春季学術講演会
４．発表年 2021年

１．発表者名 Lin Zhou, Sifeng Mao, Hizuru Nakajima, Shungo Kato, Nahoko Kasai
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３．学会等名 第44回日本神経科学大会／第1回CJK国際会議（国際学会）
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１．発表者名
西谷祐貴，河西奈保子，中嶋秀，加藤俊吾，毛思鋒，　（内山一美）

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１．発表者名
Peng Chenhan，河西　奈保子，中嶋　秀，加藤　俊吾，Mao Sifeng

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３．学会等名
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2022年

〔図書〕　計0件

〔出願〕　計2件

産業財産権の名称 東京都公立大学法人	発明者 内山 一美	権利者 同左
産業財産権の種類、番号 特許、特願2021-137979	出願年 2021年	国内・外国の別 国内

産業財産権の名称 東京都公立大学法人	発明者 毛　思鋒	権利者 同左
産業財産権の種類、番号 特許、特願2021-107375	出願年 2021年	国内・外国の別 国内

〔取得〕　計0件

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関