A Ca^{2+} ION-SELECTIVE ELECTRODE BIOSENSOR IN MICROFLUIDICS TO MONITOR HEPATOCYTE'S ACTIVITIES

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ABSTRACT

Ca^{2+} plays a crucial role in cell communication. For example, periodical changes in Ca^{2+} concentration act as cell signals. Intracellular Ca^{2+} signals have been measured by Ca^{2+} sensitive fluorescent dye [1]. In this study, extracellular Ca^{2+} variation was monitored by a potentiometry using a solid-state type of Ca^{2+} Ion Selective Electrode (ISE). Ca^{2+} ISE had high sensitivity detecting down to 10^{-10}M of calcium concentration, as well as high selectivity against K^{+}. After KCl stimulus of cells in a microchannel, we observed an oscillating increase in Ca^{2+} concentration, associated with a periodic oscillation of cell activity (Hepatocyte, Hep2G).

KEYWORDS: Ion-selective electrode, Potentiometry, Extracellular, Hep2G

INTRODUCTION

Ca^{2+} ISE has been studied to measure extremely low ionic concentration for chemistry and biology over a few decade [2]. However, in a biological application, it has been difficult to observe cells' behavior because a conventional type of ISE needed large amount of solution for two kinds of electrodes, working and reference electrodes, dipped in certain electrolyte encapsulated in a glass tube. In order to solve the problem, Yoon et al. reported a solid-type of ISE integrated in a PDMS microchannel [3]. Using this method, the volume of culturing solution can be controlled and we can get higher concentration even for the small amount of secreted ion from cells. This ISE integrated in a microchannel can be used as a tools for monitoring the activity of cells.

THEORY

Potentiometry with ISM (Ion Selective Membrane) is the method to measure *Electromotive Force (emf)* associated with the difference in ion concentration between a working electrode and a reference electrode. Theoretically, the *emf* is governed by *Nernstian* equation that predicts 30mV increase in *emf* when ion concentration increases 10 times at room temperature. ISM consists on a polymer-membrane, Polyvinyl Chloride (PVC) or Room Temperature Vulcanized silicon rubber (RTV), including an ionophore through which only certain ions can be passed. In addition, there are additive to plasticize the membrane and to make a low resistance of the membrane.

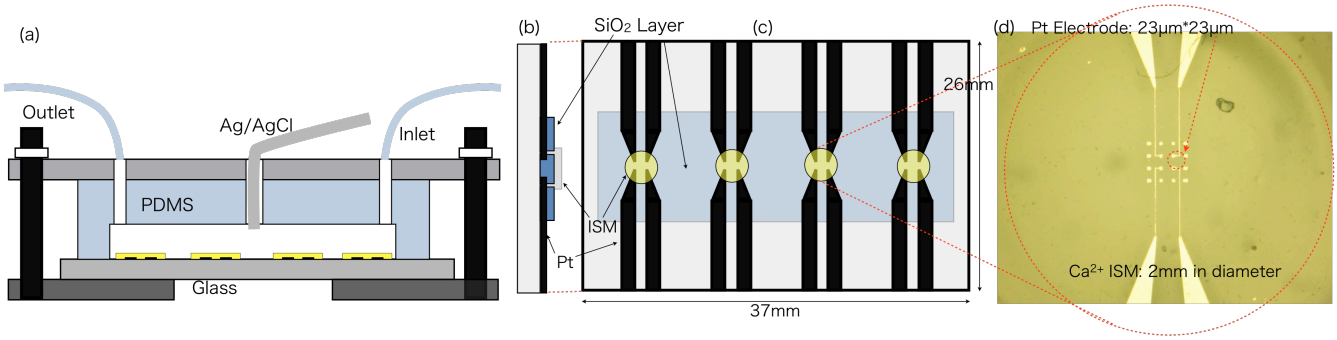

Fig. 1 Structure of Ca^{2+} ISE device. A potentiometer measures the emf between Pt and Ag/AgCl. (a) Cross sectional view of ISE and PDMS channel in length. (b) Cross sectional view of ISE in width; Insulation layer covers over Pt electrodes except for sensing parts. (c) Top view of the ISE device. (d) optical micrograph of the Ca^{2+} ISM and Pt electrodes at the center.

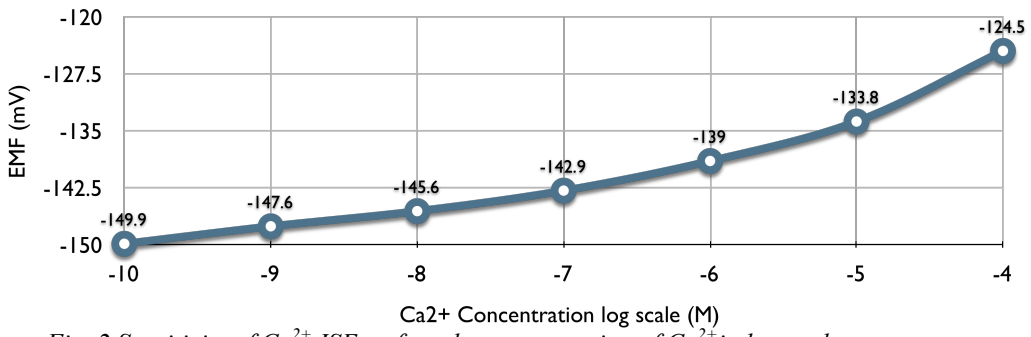

Fig. 2 Sensitivity of Ca^{2+} ISE emf vs. the concentration of Ca^{2+} in log scale.

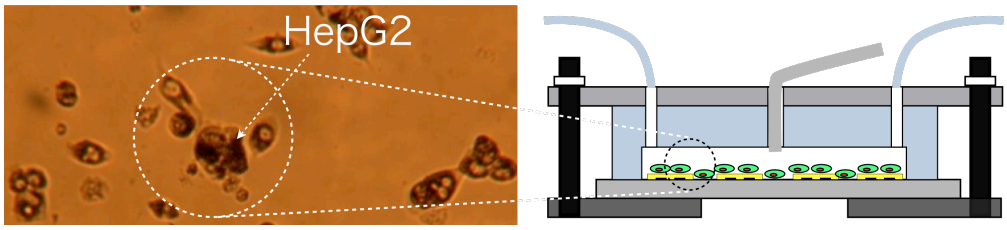

Fig. 3 Microscopic view for Hepatocytes cultured in the microchannel for 3 days.

EXPERIMENTAL

ISE was fabricated by MEMS technology on a glass substrate. The ISE comprised of a Ca^{2+} ISM, a Pt/Ti electrode, and an Ag/AgCl reference electrode (Fig. 1 (a)). The Pt/Ti electrodes, 23μm*23μm in size (Fig. 1 (d)) were sputtered on a glass substrate and 130nm thick SiO_2 layer was deposited on the device except only contact area to ISE and a potentiometer. To make ISM layer, Ca^{2+} ionophore (ETH 1001) was blended in RTV with a tetrahydrofuran (THF) solvent [3]. Then ISM cocktail was deposited on top of the Pt/Ti electrodes and dried in a vacuum chamber for 2 days. Keeping the ISM in standard Ca^{2+} solution of 10^{-3}M for 2 days activated the membrane. A PDMS channel was placed over the ISEs. By using a potentiometric method the *emf* was measured between the Pt/Ti ISE and an Ag/AgCl reference electrode that was pierced into the PDMS channel (Fig. 1 (a)).

We have calibrated the ISEs in sensitivity and selectivity. Calcium standard solutions were prepared by $CaCl_2$ in Tris-CH_3COOH (pH. 7.6, 0.05M) from 10^{-10}M to 10^{-1}M. The calibration result showed the ISEs could discriminate Ca^{2+} concentrations down to 10^{-10}M (Fig. 2). Moreover the *emf* of ISEs depended only on Ca^{2+} concentration against 100 times higher K^+ concentration change.

For cells' experiment, we cultured Hep2G (150cells/μl of cell concentration) in cell-culture medium (DMEM/F-12 + Gluta Max) in the PDMS channel for 3 days (37˚C, 5% of CO_2), expecting the number of cells to double (Fig. 3). Before injecting KCl stimulus, the cell-culture medium was washed and replaced with PBS (Phosphate Buffered Saline) using a peristaltic pump with 400μm/sec of a fluidic speed in the microchannel. We measured the Ca^{2+} ISE *emf* in the PDMS channel for 50 minutes. The initial state was recorded for 4 minutes ((1) of Fig. 4 (a)). In the stimulus state, from 4 to 14 minutes, 20mM of KCl in PBS including 5mM of $CaCl_2$ was injected to activate the cells by a peristaltic pump ((2) of Fig. 4 (a)). Then, we observed the *emf* without flow from 14 minutes ((3) of Fig. 4 (a)).

RESULTS AND DISCUSSION

During KCl injection by a peristaltic pump, there was lots of noise from fluidic motion ((2) of Fig. 4 (a)). After activating the cells with KCl, the *emf* increased gradually to about 44mV that means about 29 times higher Ca^{2+} concentration than initial state, while it oscillated for 30 minutes ((3) of Fig. 4 (a), (b)). Fig. 5 (a) shows that the period of Ca^{2+} oscillation had its oscillation in around 34.9 sec. In addition, the oscillation phases of Ca^{2+} signals were synchronized well among ISEs at different positions nearby an inlet, in the center of the channel and outlet respectively. (Fig. 5 (b), (c), (d))

It has been known that cells of the same kind in a limited space synchronize their Ca^{2+} oscillations [1]. The oscillation cycle of extracellular Ca^{2+} signal that we measured with Ca^{2+} ISE is consistent with intracellular Ca^{2+} oscillation by fluo-

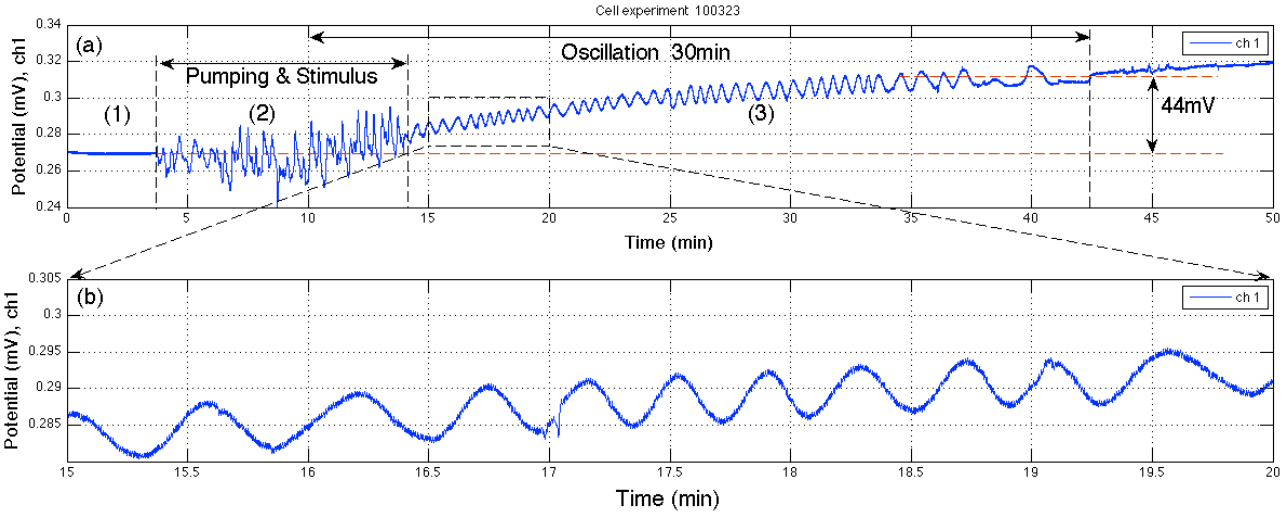

Fig. 4 Graph for increasing the concentration of Ca^{2+}(y) vs. time (x). (a) ①: Initial state, ②: 20mM of KCl stimulus to cells. ③: Measurement without flow, (b). Expanded graph of Ca^{2+} oscillation corresponding the rectangle in (a).

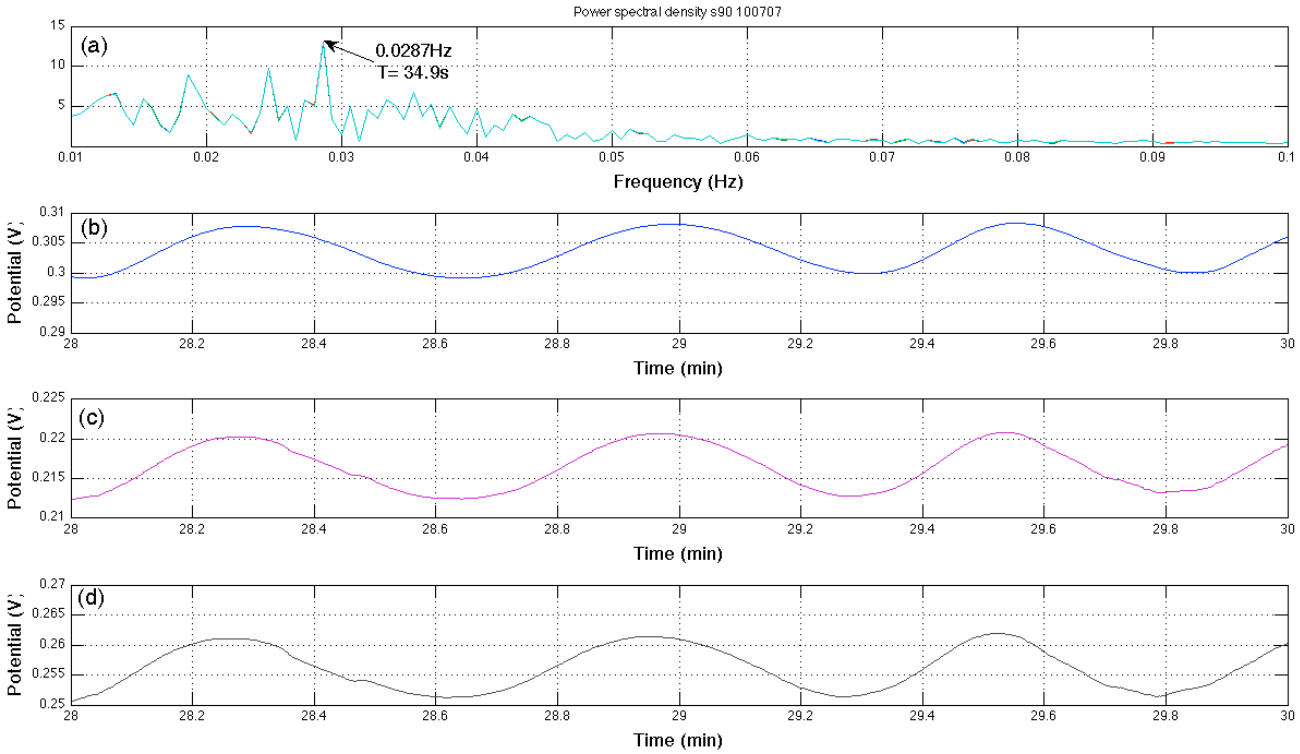

Fig. 5 Analysis of Ca^{2+} signal: (a) Frequency analysis: a period of the Ca^{2+} oscillation was in about 34.9 sec, (b) ~ (d). Phase comparison among ISEs located at different positions, (b) sensor nearby an inlet, (c) sensor in the center, (d) sensor nearby an outlet.

rescent observation for Hepatocytes [4]. Currently, we are working on the calibration of the final value of Ca^{2+} concentration released from cells after stimulus.

CONCLUSION

By means of the Ca^{2+}-sensitive-solid-type ISE, the monitoring of Ca^{2+} secretion from Hep2G was carried out in the microfludic channel. Change in Ca^{2+} ion concentration was observed through the Ca^{2+} ionophore (ETH1001) in the RTV polymer Membrane deposited on Pt/Ti electrode by MEMS fabrication process. During the secretion process by cells, we obtained: (1) 44mV of total *emf* increase corresponding to the increase in calcium ion concentration, (2) the calcium oscillation period of 34.9s, (3) little phase difference by different position of ISEs. We are planning to measure Ca^{2+} signal oscillation for different cell culturing conditions. We expect that system can contribute to further understanding of biological reaction among cells.

ACKNOWLEDGEMENTS

This work was supported by the JSPS International Training Program (ITP) and Core Research for Evolutional Science and Technology (CREST) in Japan. One of the author, J. Park, is supported by GCOE program “Secure-life Electronics” of MEXT.

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