Extended Abstracts of the 2009 International Conference on Solid State Devices and Materials, Sendai, 2009, pp1340-1341

Biohybrid Chemical Sensor Composed of Microfluidic Device and Cell

Nobuo Misawa[1, 2], Hidefumi Mitsuno[3], Ryohei Kanzaki[3], and Shoji Takeuchi[1, 2]

[1]Institute of Industrial Science, The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan
Phone: +81-3-5452-6650 E-mail: misawa@iis.u-tokyo.ac.jp
[2]Bio Electrochemical Autonomous Nano Systems, Life BEANS Center, The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan
[3]Research Center for Advanced Science and Technology, The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

1. Introduction

Current chemical sensors are based on several technologies; a metal-oxide-semiconductor [1], a cantilever array [2], a quartz crystal microbalance [3, 4], a nanostructured material [5], and a living cell's or biological response [6-9]. There is no doubt that all chemical sensors quest for a high sensitivity and a high selectivity for their detection. Particularly, in terms of selectivity, there must be nothing better than organisms' responses (e.g. immune system, olfactory and gustatory senses) that have been obtained in evolutionary history over many generations. Creatures' specific responses for chemicals are due partially to unique structures of several receptors such as membrane proteins.

Nowadays, we can produce and use some chemical receptors through a genetic engineering. In particular, using recombinant cells as sensing element of a chemical sensor gains much attention. *Xenopus laevis* oocyte (*X. laevis* oocyte), among others, has high versatility as a host cell for such a system since expression systems of many membrane proteins have been constructed with *X. laevis* oocytes.

X. laevis oocyte is often used as test cell in drug screening [10, 11]. Here, we describe a microfluidic device for measuring the electrophysiological activity of an oocyte that expresses artificially pheromone receptors of silkmoth. The receptors are called BmOR1 and BmOR3 which are involved in regulation of ion flows by recognizing selectively pheromones bombykol and bombykal, respectively. In our proposed system, the fluidic channels have a cell-trap region and two electrodes for measurement of a cell's response to a pheromone by two electrode voltage clamp method as shown in Figure 1.

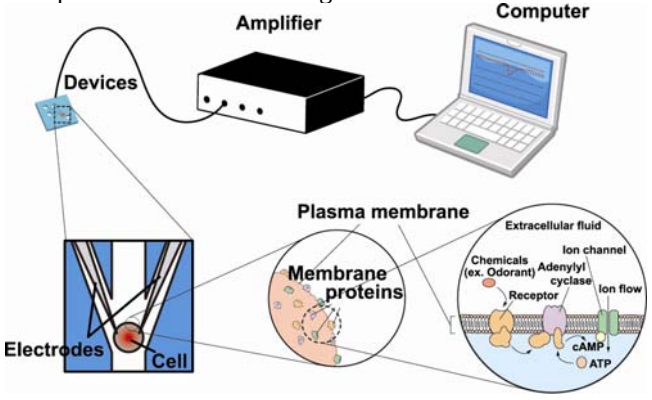

Fig. 1 Conceptual image of the chemical sensor using a cell expressing chemical receptors.

In this report, we show the device that can distinguish two similar chemicals, bombykol and bombykal, due to the receptors' high specificity.

2. Experimental

As shown the illustration in Figure 2, two Ag/AgCl electrodes are inserted into the two capillaries for measurements of oocyte's responses. The distance of two capillaries tips is less than about 1 mm since diameter of *X. laevis* oocyte is generally 1-1.5mm. The capillaries are filled with 3 M KCl solution preliminarily. Furthermore, the center channel is filled with 5 mM HEPES/NaOH buffer solution pH 7.5 containing 96 mM NaCl, 2mM KCl, 1.8 mM CaCl2, and 1.6 mM MgCl2. The buffer solution is perfused constantly using a peristaltic pump.

Pictures in Figure 2 show the device and *X. laevis* oocyte. The buffer solution flows in center channel from near side (inlet) to far side (outlet) with 10-20 ml/min flow rate as seen in Figure 2(a) and 2(b). Oocyte can be trapped by two capillaries on the stream as shown in Figure 2(e) and 2(f).

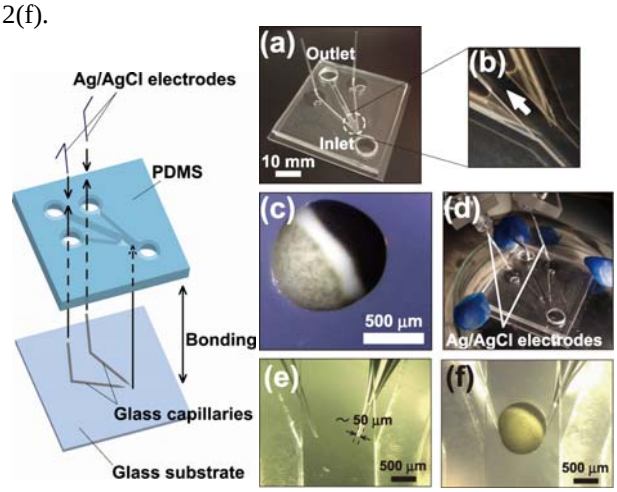

Fig. 2 Pictures of the device and frog egg (*Xenopus laevis* oocyte). (a) Whole image of the device. (b) Close-up picture of cell trap region. (c) Picture of *X. laevis* oocyte. (d) Picture of the actual measurement system. (e) Picture of cell trap region without oocyte and (f) with oocyte.

We used pheromone receptors of silkmoth for (E,Z)-10,12-hexadecadien-1-ol (bombykol) and (E,Z)-10,12-hexadecadien-1-al (bombykal). The receptors are called BmOR1 and BmOR3, respectively.

3. Results and discussion

Figure 3(a) as a negative control shows an evidence of our system is activated by the oocyte. No peak appears in any case of applying buffer solution, bombykol and bombykal in Figure 3(a). Figure 3(b) and 3(c) are results of selective responses of the oocyte for bombykol and bombykal, respectively. Although intensities of responses depend on oocyte's condition, we find that our suggested device can detect odorant-like chemicals obviously. These results are in good agreement with results taken by conventional measuring system.

This system does not require microscopes and micromanipulators to insert electrodes into target cells since the device can trap the cell with inserting electrodes spontaneously. Furthermore, these two different responses are recorded at the same time with independent device as shown in an inset picture of Figure 3. It is possible to record four different oocyte responses in this system due to a multichannel amplifier we use.

In our system, there is no need any shield boxes and any vibration isolated tables in contrast with conventional electrophysiological recording systems. In addition, the amplifier is very compact. So, this system needs only three elements: computer, amplifier and microfluidic device with oocytes.

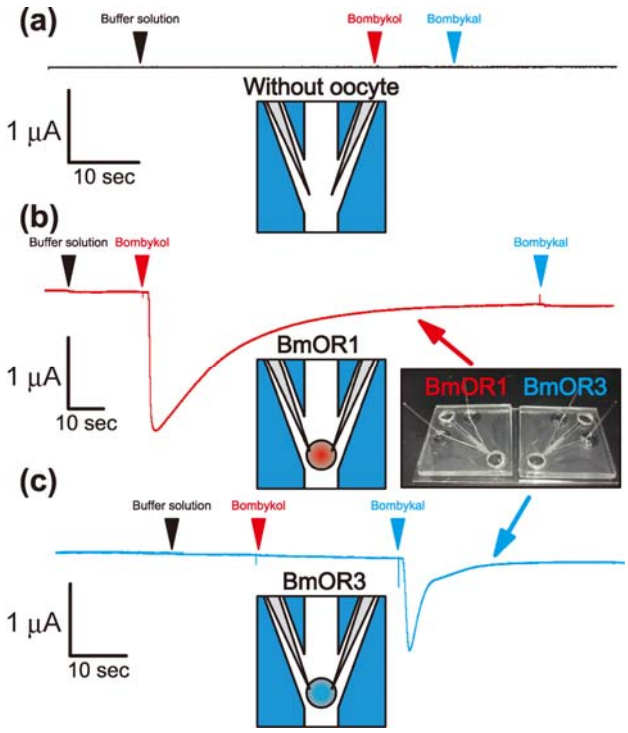

Fig. 3 (a) Current trace without *Xenopus* oocyte as a negative control. Buffer solution 20 μl, 10 μM bombykol 20 μl and 10 μM bombykal 20 μl were applied at the time indicated by arrowheads. (b) Responses of oocyte expressing BmOR1. Buffer solution 20 μl, 10 μM bombykol 20 μl and 10 μM bombykal 20 μl were applied at the time indicated by arrowheads. (c) Responses of oocyte expressing BmOR3. Buffer solution 20 μl, 0.5 μM bombykol 20 μl and 0.5 μM bombykal 20 μl were applied at the time indicated by arrowheads. (b) and (c) were measured in parallel.

4. Conclusion

We demonstrated a device that can distinguish two similar odorant-like chemicals, bombykol and bombykal, due to receptors' specificity and sensitivity.

This microfluidic device thus is useful as a chemical odorant sensor, easy to use, portable, and is easily multiplexed showing the potential of this odorant detection system as a next generation chemical detection sensor.

Acknowledgements

The authors would like to thank Prof. M. Asashima of The University of Tokyo for kindly giving the *X. laevis* oocytes. We also really appreciate Dr. Y. Tanaka of Tecella LLC for customizing of a multichannel amplifier, Triton, for our experiment.

References

[1] D. Kohl, L. Heinert, J. Bock, Th. Hofmann, P. Schieberle, "Systematic studies on responses of metal-oxide sensor surfaces to straight chain alkanes, alcohols, ketones, acids and esters using the SOMMSA approach", Sensors and Actuators B, vol. 70, pp. 43-50, 2000.

[2] H.P. Lang, M.K. Baller, R. Berger, Ch. Gerber, J.K. Gimzewski, F.M. Battiston, P. Fornaro, J.P. Ramseyer, E. Meyer, H.J. Güntherodt, "An artificial nose based on a micromechanical cantilever array", Analytical Chimica Acta, vol. 393, pp. 59-65, 1999.

[3] K. Yano, U.T. Bornscheuer, R. D. Schmid, H. Yoshitake, H-S. Ji, K. Ikebukuro, Y. Masuda, I. Karube, "Development of an odorant sensor using polymer-coated quartz crystals modified with unusual lipids", Biosensors & Bioelectronics, vol. 13, pp. 397-405, 1998

[4] H-S. Ji, S. McNiven, K. Ikebukuro, I. Karube, "Selective piezoelectric odor sensors using molecularly imprinted polymers", Analytical Chimica Acta, vol. 390, pp. 93-100, 1999.

[5] X-J. Huang, Y-K. Choi, "Chemical sensors based on nanostructured materials", Sensors and Actuators B, vol. 122, pp. 659-671, 2007.

[6] X. Feng, J. Castracane, N. Tokranova, A. Gracias, G. Lnenicka, B.G. Szaro, "A living cell-based biosensor utilizing G-protein coupled receptors: Principles and detection methods", Biosensors and Bioelectronics, vol. 22, pp. 3230-3237, 2007.

[7] V. Radhika, T. Proikas-Cezanne, M. Jayaraman, D. Onesime, J.H. Ha, D.N. Dhanasekaran, "Chemical sensing of DNT by engineered olfactory yeast strain", Nature chemical biology, vol. 3, pp. 325-330, 2007.

[8] Ch. Ziegler, W. Göpel, H. Hämmerle, H. Hatt, G. Jung, L. Laxhuber, H-L. Schmidt, S. Schütz, F. Vögtle, A. Zell, "Bioelectronic noses: a status report. Part II", Biosensors & Bioelectronics, vol. 13, pp. 539-571, 1998

[9] X. Feng, J. Castracane, N. Tokranova, A. Gracias, G. Lnenicka, B.G. Szaro, "A living cell-based biosensor utilizing G-protein coupled receptors: Principles and detection methods", Biosensors and Bioelectronics, vol. 22, pp. 3230-3237, 2007.

[10] P.R. Joshi, A. Suryanayanan, M.K. Schulte, "A vertical flow chamber for *Xenopus* oocyte electrophysiology and automated drug screening", Journal of Neuroscience Methods, vol. 132, pp. 69-79, 2004.

[11] E. Dahan, V. Bize, T. Lehnert, J-D. Horisbeger, M.A.M Gijs, "Integrated Microsystems for no-invasive electrophsiological measurements on *Xenopus* oocytes", Biosensors and Bioelectronics, vol. 22, pp. 3193-3202, 2007.