ODOR-SENSITIVE FIELD EFFECT TRANSISTOR (OSFET) BASED ON INSECT CELLS EXPRESSING INSECT ODORANT RECEPTORS

Daigo Terutsuki[1], Hidefumi Mitsuno[2], Yuki Okamoto[3], Takeshi Sakurai[2], Agnès Tixier-Mita[2], Hiroshi Toshiyoshi[2, 3], Yoshio Mita[3], and Ryohei Kanzaki[2]

[1] Department of Advanced Interdisciplinary Studies, The University of Tokyo, Tokyo, JAPAN

[2] RCAST, The University of Tokyo, Tokyo, JAPAN

[3] Department of Electrical Engineering and Information Systems, The University of Tokyo, JAPAN

ABSTRACT

We here propose a completely novel bio-hybrid electronic odorant sensor, termed odor-sensitive field effect transistor (OSFET), which was developed based on insect cells expressing insect odorant receptors (ORs). Living cells of the fall armyworm (*Spodoptera frugiperda*: Sf21 cells) were integrated over a CMOS post-processed Al_2O_3 layer on extended gate terminals of a foundry-made MOSFETs. Exposure of the living cells expressing insect ORs to a specific odorant induced ion influxes, which modulated the drain current of the FET. The electrical output discriminated amongst nearly identical odorants ($\Delta w/w = 0.85\%$) with high sensitivity and selectivity.

INTRODUCTION

Portable, highly sensitive, and selective odorant sensors have drawn attention for a wide variety of applications ranging from security to medical use. Artificial odorant sensors based on polymer composites, surface acoustic waves, or quartz crystal microbalances have been reported and applied in practice [1]; however, their response time, sensitivity, and selectivity are still insufficient. In contrast to existing odorant sensors that are based on artificial sensor elements, those based on odorant receptors (ORs) have also been recently developed, and show high sensitivity and selectivity. These OR-based sensors can discriminate odorants by recognizing slight differences in their molecular structure. For example, mammalian ORs have been used or reconstructed for detecting odorant compounds [2, 3].

Moreover, insect ORs have been applied to sensor elements because of their notable characteristics. Insect ORs along with OR co-receptors (Orco) are ligand-gated ion channels and therefore exhibit simple signal transduction [4, 5]. Specifically, insect ORs cause inward non-selective cation influxes (Na^+, Ca^{2+}) when bound to odorant molecules [6]. An odorant sensor was developed from frog eggs (*Xenopus* oocytes) expressing silkmoth pheromone receptors, and was shown to detect cation influxes as electrical signals with ppb-level sensitivity and good portability [7]. However, the life span of frog eggs is very limited, and can be easily damaged by the invasive electrodes. To overcome this limitation, we previously demonstrated that calcium imaging could visualize Ca^{2+} ions passing through insect ORs for odor detection [8, 9]. Specifically, Sf21 cells derived from the *Spodoptera frugiperda* expressing insect ORs, Orco with fluorescent calcium indicator proteins (GCaMP3 or GCaMP6s) were observed under a fluorescence microscope. These methods could achieve ppb-level sensitivity and discriminated target odorants as patterns of increased fluorescence intensity in different areas. Despite these advantages, a more convenient and portable non-invasive electrical measurement method is desired for the development of compact sensor devices without fluorescence microscopes, and that are easy to integrate with other electrical devices.

***Table 1**: Comparison of odorant sensor characteristics based on insect odorant receptors*

	Two electrode voltage clamp [7]	Calcium imaging [8, 9]	OSFET (This paper)
Sensor element	Frog egg (*Xenopus* oocyte)	**Insect cell (Sf21)**	**Insect cell (Sf21)**
Signal type	**Electrical signals**	Fluorescent intensity	**Electrical signals**
Method	Invasive	**Non-invasive**	**Non-invasive**
Life span	Poor	**Excellent**	**Excellent**
High density integration	Poor	Good	**Excellent**

In this paper, we introduce our newly developed odor-sensitive field effect transistor (OSFET), and demonstrate its good ability to discriminate between two structurally similar odorants by recognizing very slight differences in functional groups as electrical signals, without requiring complex electrophysiological methods. Moreover, the OSFET has excellent reusability and shows great potential for discriminating amongst various odorant targets simply by replacing the insect ORs, and developing CMOS high-density odorant sensors. The key characteristics of odorant sensors based on insect ORs are compared in **Table 1**.

MATERIALS AND METHODS

OSFET definition

The OSFET is composed of Sf21 cells expressing insect ORs and extended-gate field effect transistors (EG-FETs). Upon exposure to target odorants, the Sf21 cells expressing insect ORs induce ion influxes, thereby modulating the FET's drain current. The electrical outputs allow for discrimination of target odorants so that the OSFET can act as an odorant sensor.

Insect cells expressing ORs

Sf21 cells were purchased from Invitrogen (Carlsbad, CA, USA) and maintained as adherent cultures in cell culture flasks. They were cultured in Grace's insect medium (Invitrogen, Carlsbad, CA, USA) at 27 °C. Two different Sf21 cell lines were used in this study. Expression vector construction was performed similarly to the protocols described in the previous study on fluorescent measurement [8]. The diameter of an Sf21 cell is 10–20 μm. We expressed the silkmoth OR BmOR3 and the

Drosophila OR Or13a in each Sf21 cell line with Orco. BmOR3 specifically responds to bombykal (BAL), which is a silkmoth pheromone component, (*E,Z*)-10,12-hexadecadienal, and Or13a strongly responds to 1-octen-3-ol (one of the major moldy odors) [8, 9].

Fabrication of the measurement device

We designed and fabricated EG-FETs with an n-type electronic channel to comprise a part of the OSFET. The schematic of the OSFET design is shown in **Figure 1 (a)**. The gate length and width of the FETs are 2 µm and 5 µm, respectively. The gate electrodes were extended from the FET gates, and their sensing area sizes are 50 × 50 µm^2 and 100 × 100 µm^2 (**Figure 1 (b)**). The surface of the EG-FET chip was covered with an SiO_2 passivation layer except for the sensing areas. An approximately 40–80 nm Al_2O_3 thin film layer was deposited on top of the extended-gate electrodes by sputtering. Al_2O_3 is often used as an ion-sensitive insulator of an ion-sensitive field effect transistor owing to its good pH response and excellent stability [10]. The use of EG-FETs facilitated seeding the Sf21 cells on the sensing areas and washing them off after the end of the experiments. To develop a continuous-perfusion system and the culture of Sf21 cells, we constructed a chamber containing the EG-FET chip. A commercial Ag/AgCl reference electrode (ALS Co. Ltd., Tokyo, Japan) was inserted into the assay buffer solution (140 mM NaCl, 5.6 mM KCl, 4.5 mM $CaCl_2$, 11.26 mM $MgCl_2$, 11.32 mM $MgSO_4$, 9.4 mM D-glucose, 5 mM HEPES, pH 7.2) in a chamber. To detect the slight electrical signal corresponding to the odorant responses, the drain and the source of the OSFET were biased to 2.5 V and −2.5 V, respectively, and the reference electrode was set to 0 V to the extended-gate electrodes through the assay buffer solution. The odorant responses were measured as the drain current modulations using a precision source/measuring unit (SMU; B2902A, Keysight Technologies, Santa Clara, CA, USA).

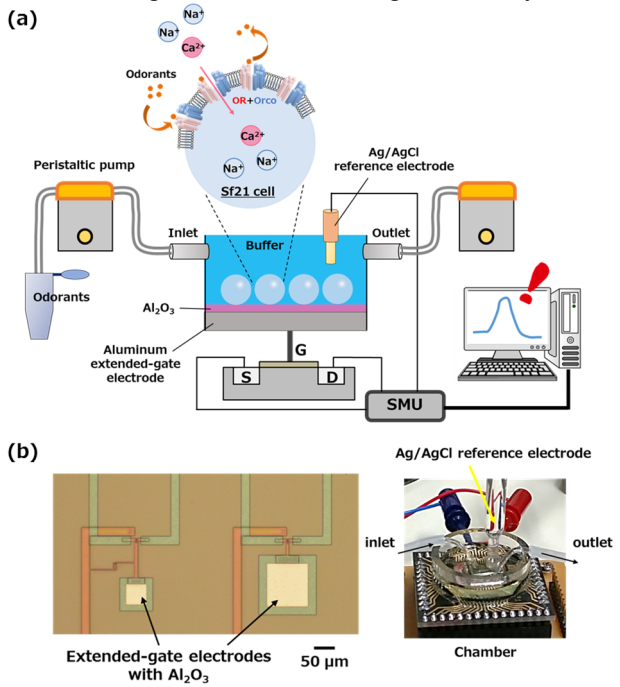

***Figure 1**: (a) Schematic of an odor-sensitive field effect transistor (OSFET). (b) Photographs of the fabricated EG-FET chip and a chamber with a reference electrode. The sizes of the extended-gate electrodes are 50 × 50 µm^2 and 100 × 100 µm^2.*

Perfusion system

A perfusion system was included in the OSFET design to allow for odorants-attached insect ORs to be washed away so that the Sf21 cells can return to their stationary state, and the next odorants can then be perfused in the system. The process for the fabrication of a cell chamber is as follows. An EG-FET chip covered with a sputtered Al_2O_3 layer is mounted and wire-bonded on a custom-made printed circuit board (PCB). An acrylic resin ring is glued onto the PCB, and an epoxy resin (EPOTEK 302-3M, Epoxy Technology Inc., Billerica, MA, USA), which is commonly used for medical and semiconductor applications, is applied to protect the bonding wires and pads. A pyramid-shaped polydimethylsiloxane (PDMS; SILPOT 184, Dow Corning Toray Co., Ltd., Japan) block is placed upside down on the processed chip, and the epoxy resin is poured between the PDMS block and the acrylic resin ring. After solidification of the epoxy resin, the PDMS block is removed, and the epoxy resin acts as a cell chamber. Perfusion was conducted at a rate of 140 µl/min using two peristaltic pumps (MP-2010, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The perfusate is the assay buffer

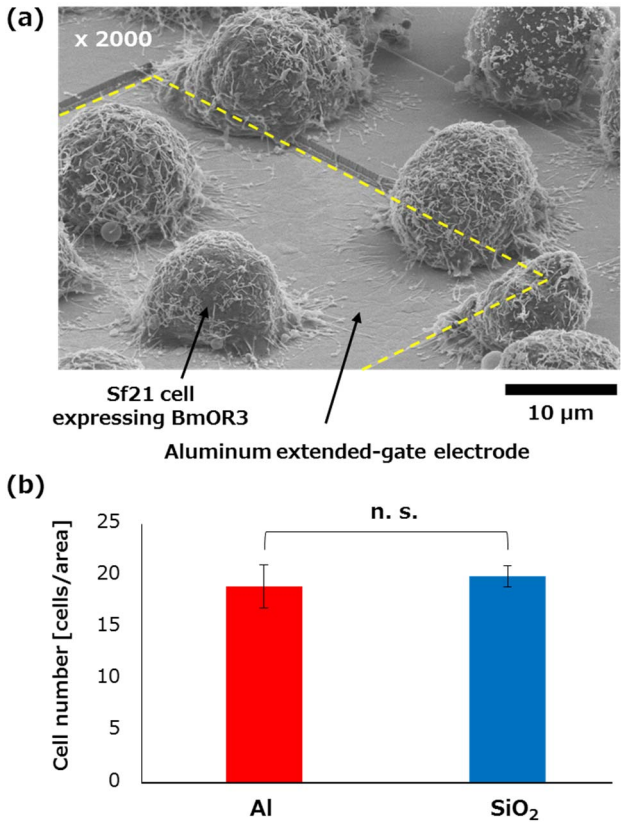

***Figure 2**: (a) Scanning electron microscope image of Sf21 cells on a 50 × 50 µm^2 aluminum extended-gate electrode (within the yellow dotted lines). (b) Cell numbers on 100 × 100 µm^2 aluminum extended-gate electrodes and 100 × 100 µm^2 SiO_2-insulated areas. Data represent the mean ± SEM of cell number (N = 3 individual electrodes and insulator areas, Student's t-test: n. s. = not significant).*

solution with dimethyl sulfoxide (DMSO, <1%). Observation under a bright-field microscopic before and after the experiments confirmed that the number of Sf21 cells on the extended-gate electrode was almost unchanged.

Cell seeding

Sf21 cells were seeded in the chamber and incubated at 25 °C for 30 min. We prepared a dilution of Sf21 cells in the assay buffer solution to reach a concentration of approximately 1.5×10^6 cells/ml. Then, the cells in a 200 µl suspension were seeded in the chamber. The Sf21 cells were seeded sparsely on the extended-gate electrodes in order to perfuse the odorants so that they reached all parts of the attached cells.

RESULT AND DISCUSSION

Interface between Sf21 cells and extended-gate electrodes

First, we observed the interface between the Sf21 cells and aluminum extended-gate electrodes. The conditions of the interface could significantly affect the electrical signal transfer. **Figure 2 (a)** shows the scanning electron micrograph of Sf21 cells expressing BmOR3 attached on a 50×50 µm^2 aluminum electrode, indicating that Sf21 cells were in close contact with the aluminum extended-gate electrode. Several studies have demonstrated the toxicity of aluminum [11, 12], and therefore cell monitoring with CMOS devices typically avoids the use of bare aluminum electrodes [13]; nevertheless, in the present work, there was no difference in the number of attached Sf21 cells on the aluminum extended-gate electrodes and SiO_2 insulators (**Figure 2 (b)**). This result suggests that the general foundry CMOS process can be applied to Sf21 cells without inducing damage, demonstrating its potential to reduce post-processing costs such as the requirement for biocompatible coating.

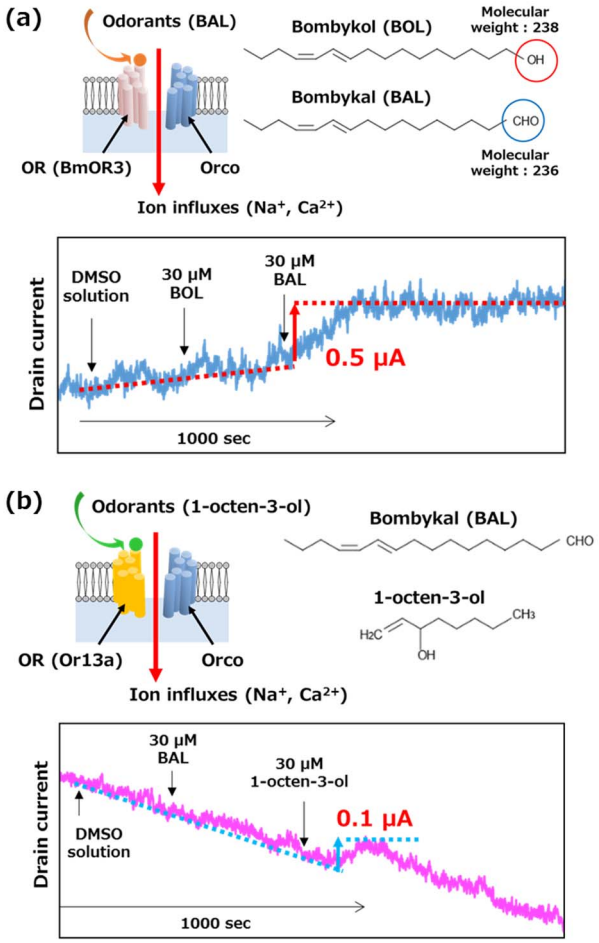

***Figure 3**: Drain-current changes of the OSFET with Sf21 cells expressing BmOR3 or Or13a using 100 × 100 µm^2 Al_2O_3-sputtered extended-gate electrodes. (a) DMSO (<1%) assay buffer solution, including 30 µM BOL and 30 µM BAL. (b) DMSO (<1%) assay buffer solution, including 30 µM BAL and 30 µM 1-octen-3-ol.*

Electrical measurements

Figure 3 (a) shows that the drain current of the OSFET increased when 30 µM BAL was applied to the Sf21 cells expressing BmOR3. By contrast, no such drain current changes were observed when the cells were exposed to only the DMSO (<1%) (the medium of the odorants) assay buffer solution or 30 µM bombykol (BOL; a silkmoth pheromone component, (*E,Z*)-10,12-hexadecadien-1-ol) as negative controls. Consequently, the structurally similar odorants BOL ($C_{16}H_{30}O$) and BAL ($C_{16}H_{28}O$) were effectively discriminated based on distinct drain current modulations of the OSFET.

In the case of Or13a (**Figure 3 (b)**), the drain current increased only when 30 µM of 1-octen-3-ol (the compound responsible for a typical moldy odor) was applied to the

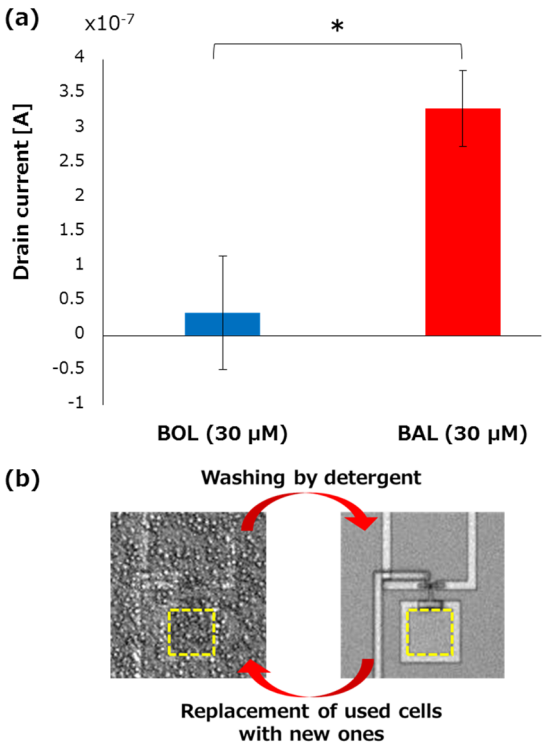

***Figure 4**: (a) Response repeatability of BmOR3-expressing cell lines. Data represent mean ± SEM of OSFET drain-current changes (N = 4 individual tests, Student's t-test value, *P < 0.05). The drain-current changes were normalized by subtracting the mean drain-current changes of the DMSO assay buffer solution. (b) Device reusability by washing with a commercial detergent.*

system, and again no drain current changes were observed when the cells were exposed to the DMSO assay buffer solution or 30 μM BAL as negative controls. These response specificities were consistent with those obtained with the previously reported fluorescent intensity measurements of these cells [8, 9], indicating that the OSFET could effectively detect the odorant responses of insect ORs. A moving-average filter was used for smoothing of the drain current change data.

By replacing the cells with new ones after detergent cleaning, the same extended-gate electrode could detect the odorant responses again from the newly seeded cells. **Figure 4 (a)** shows the repeatability of the OSFET measurements for Sf21 cells expressing BmOR3, and **Figure 4 (b)** indicates that the OSFET has satisfactory reusability with a simple cleaning process.

CONCLUSION

We have proposed a new concept for the design of a bio-hybrid odorant sensor, and successfully developed an OSFET that can discriminate structurally similar odorants with high sensitivity and selectivity as electrical signals. An OSFET does not require a high-skill invasive electrode method such as a patch clamp to detect the odorant responses of insect ORs, and simply attaching Sf21 cells expressing insect ORs allows it to perform its function as an odorant sensor.

The electrical outputs of the OSFET were consistent with those detected in our previous fluorescent measurement, indicating that the OSFET detected the odorant responses of the expressed insect ORs. We also found that Sf21 cells could be attached to the aluminum extended-gate electrodes without causing damage, and the OSFET showed satisfactory reusability. Based on these results, the OSFET proposed herein could be applied for security control in airports, cost-effective disease diagnosis systems that can detect signs of disease in breath, and food risk control, simply by changing the type of insect ORs.

ACKNOWLEDGEMENTS

This research was supported by Tateisi Science and Technology Foundation, 2016 Research Grant (C), and VLSI Design and Education Center (VDEC), The University of Tokyo, with the collaboration of Cadence Corporation. Establishment of the Sf21 cell lines was supported by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry. Bombykol and bombykal were kindly provided by Dr. S. Matsuyama (Tsukuba University, Ibaraki, Japan). The authors would like to thank JEOL Ltd. for helpful discussions and for supporting the scanning electron microscopy observations.

REFERENCES

[1] K. Arshak, E. Moore, G. M. Lyons, J. Harris, and S. Clifford, "A review of gas sensors employed in electronic nose applications", *Sensor Review*, Vol. 24, Issue 2, pp. 181–198, 2004.

[2] M. Marrakchi, J. Vidic, N. Jaffrezic-Renault, C. Martelet, and E. Pajot-Augy, "A new concept of olfactory biosensor based on interdigitated microelectrodes and immobilized yeasts expressing the human receptor OR17-40", *European Biophysics Journal*, Vol. 36, Issue 8, pp. 1015–1018, 2007.

[3] J. H. Lim, E. H. Oh, J. Park, S. Hong, and T. H. Park, "Ion-channel-coupled receptor-based platform for a real-time measurement of G‑protein-coupled receptor activities", *ACS Nano*, Vol. 9, No. 2, pp. 1699–1706, 2015.

[4] T. Nakagawa, T. Sakurai, T. Nishioka, and K. Touhara, "Insect sex-pheromone signals mediated by specific combinations of olfactory receptors", *Science*, Vol. 307, pp. 1638–1642, 2005.

[5] T. Sakurai, T. Nakagawa, H. Mitsuno, H. Mori, Y. Endo, S. Tanoue, Y. Yasukochi, K. Touhara, and T. Nishioka, "Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*", *Proceedings of the National Academy of Sciences of the USA*, Vol. 101, pp. 16653–16658, 2004.

[6] K. Sato, M. Pellegrino, T. Nakagawa, T. Nakagawa, L. B. Vosshall, and K. Touhara, "Insect olfactory receptors are heteromeric ligand-gated ion channels", *Nature*, Vol. 452, pp. 1002–1006, 2008.

[7] N. Misawa, H. Mitsuno, R. Kanzaki, and S. Takeuchi, "A highly sensitive and selective odorant sensor using living cells expressing insect olfactory receptors", *Proceedings of the National Academy of Sciences of the USA,* Vol. 107, pp. 15340–15344, 2010.

[8] H. Mitsuno, T. Sakurai, S. Namiki, H. Mitsuhashi, and R. Kanzaki, "Novel cell-based odorant sensor elements based on insect odorant receptors", *Biosensors and Bioelectronics*, Vol. 65, pp. 287–294, 2015.

[9] M. Termtanasombat, H. Mitsuno, N. Misawa, S. Yamahira, T. Sakurai, S. Yamaguchi, T. Nagamune, and R. Kanzaki, "Cell-based odorant sensor array for odor discrimination based on insect odorant receptors", *Journal of Chemical Ecology*, pp. 1–9, 2016.

[10] H. Abe, M. Esashi, and T. Matsuo, "ISFET's using inorganic gate thin films", *IEEE Transactions on Electron Devices*, Vol. 26, Issue 12, pp. 1939–1944, 1979.

[11] B. Platt, G. Fiddler, G. Riedel, and Z. Henderson, "Aluminium toxicity in the rat brain: Histochemical and immunocytochemical evidence", *Brain Research Bulletin*, Vol. 55, No. 2, pp. 257–267, 2001.

[12] A. Campbell, D. Hamai, and S. C. Bondy, "Differential toxicity of aluminum salts in human cell lines of neural origin: Implications for neurodegeneration", *Neurotoxicology*, Vol. 22, pp. 63–71, 2001.

[13] A. H. D. Graham, C. R. Bowen, J. Robbins, and J. Taylor, "Formation of a porous alumina electrode as a low-cost CMOS neuronal interface", *Sensors and Actuators B*, Vol. 138, pp. 296–303, 2009.

CONTACT

* R. Kanzaki, tel: +81- 3-5452-5195;
kanzaki@rcast.u-tokyo.ac.jp