CELLS SMELL ON A CMOS: A PORTABLE ODORANT DETECTION SYSTEM USING CELL-LADEN COLLAGEN PILLARS

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ABSTRACT

We developed a portable odorant detection system composed of collagen micro-pillars with cells expressing olfactory receptors and a CMOS imaging sensor; the CMOS imaging sensor caught fluorescence signals of Ca^{2+} increased by odorant molecules. While low sensitiveness of the CMOS imaging sensor was insufficient to detect the fluorescent signal from a single cell, the odorant detection system achieved supply of sufficient odorant-induced fluorescence intensity to the CMOS imaging sensor by vertical accumulation of the fluorescence from each cells in the pillars. We believe that our portable odorant detection system will be a useful platform for quantitative and selective portable odorant sensor.

INTRODUCTION

Detection of odorants have gathered attentions for applications of medical, environmental and security field because the odorants are strong evidence for the existence of cancers, pesticides and explosives. For the odorant detection, working animals are mainly used as "qualitative odorant sensor" with high sensitivity and selectivity [1]. While mechanical detection systems composed of polymers or metal oxide semiconductors has been widely used for quantitative odorant detection [2], the systems has limitation of sensitivity and selectivity in the odorant detection compared to the animals.

For quantitative odorant detection with high sensitivity and selectivity, many researchers have investigated odorant sensors using proteins (olfactory receptors) derived from the animal nose. The odorant sensor can quantitatively measure signals from the olfactory receptors with high sensitivity and selectivity by transducers [3], [4]. The odorant sensors are classified into two types: protein based sensors and cell based sensors. Although the protein based sensors using only olfactory receptors is portable, it is impossible to discriminate between agonist and antagonist [5]. On the other hand, the cell based sensors use calcium imaging; the signals of cells stimulated by odorant molecules were detected by fluorescence corresponding to Ca^{2+} concentration. The cell based sensors achieve high selectivity by transmission of signals corresponding to recognitions of agonist ligands. However, the cell based sensors do not have portability because the calcium imaging needs fluorescence microscopes due to weakness of the fluorescence intensity.

Here, we propose a portable odorant sensor consisting of a CMOS imaging sensor and cell-laden collagen pillars (Fig. 1). The CMOS imaging sensor is inexpensive and small rather than conventional cooled charged-coupled devices (CCDs) although CMOS has lower sensitivity than CCDs [6]. To compensate for the low sensitivity, the cell-laden collagen pillars facilitate the increment of cellular fluorescence intensity by vertical accumulation of fluorescence emitted from each cells. Therefore, we achieved detection of odorant molecules using our portable odorant detection system.

MATERIAL AND METHOD

Cell culture and transfection

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (D5796, SIGMA), 10 % (v/v) fetal bovine serum (FBS) (FB-1365; Biosera), 100U/ml penicillin and 100 µg/ml streptomycin (P4333, SIGMA) at 37℃ under humidified atmosphere containing 5% CO_2. The HEK293T cells were transfected with 7.2 µg pME18S-Flag-Rho-mOR-EG, 4.8 µg pME18S-Gα15, 2.4 µg pME18S-RTP1, 2.4 µg and pEGFP-N3-Myr-Ric-8A by using lipofectamine 3000 (L3000015, Thermo Fisher) (2 µl per 1 µg DNA), where mOR is a mouse olfactory receptor, Gα is G protein α subunit, RTP is receptor transporter protein to transport protein to membrane and RIC is resistance to inhibitors of cholinesterase to support Gα

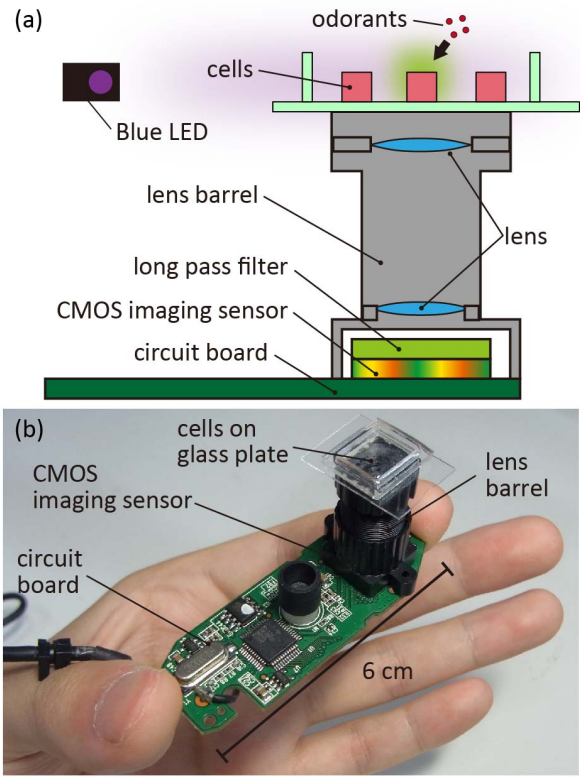

Figure 1. Concept of a portable odorant sensor. (a) Schematic illustration of the portable odorant sensor composed of a CMOS imaging sensor and an array of cell-laden collagen pillars on a glass plate. (b) Image of the portable odorant sensor.

subunit synthesis [7]–[9].

Fabrication of collagen pillar array

For formation of the cell-laden collagen pillars, we first prepared polydimethylsiloxane (PDMS) micro-well array devices by using standard photolithography techniques. We designed micro-wells on a photo mask (CBL2506Bu-AZP, Clean Surface Technology) using a mask exposure machine (DLS50, NanoSystemSolutions). A SU-8 dry film (SU-8 3000CF DFR, Micro Chem) was laminated on a silicon wafer from 50 to 200 μm thickness, and then the film was placed on a mask aligner machine (PEM-800, Union Optical) to be exposed by UV light through the photo-mask. After post-exposure baking, we fabricated a SU-8 mold by development of the SU-8 film using SU-8 developer (Y020100, Micro Chem). We then poured a mixture of elastomers and curing agent (Sylgard 184, Toray) with concentration 10:1 (w/w) into the SU-8 mold and cured it at 75℃ for 90 min for formation of a PDMS replica. Finally, we obtained the PDMS micro-well array device by releasing the PDMS replica from the SU-8 mold.

The transfected cells were cultured 24 h in a 10 cm dish coated with fibronectin (BT-226S, Biomedical technologies). Subsequently, we washed cells using phosphate buffered saline (PBS) (1102P05, SIGMA), and treated them with 2 mM EDTA solution for 5 min at 37℃. After resuspension of the cells in DMEM, we collected the cells by 2g centrifugation for 5 min. We then removed supernatant and resuspended in Hanks balanced salt solution (HBSS) (14170-112 invitrogen). After centrifugation and removal of supernatant, we suspended the cells in collagen solution (CellMatrix, Nitta Gelatin) for preparation of 10^8 cells/ml cell suspended collagen solution. The cell-mixed collagen solution were dropped on the PDMS micro-well array device coated with bovine serum albumin (BSA) (A7906, SIGMA) to prevent cell adhesion [10], [11], and stamped on a glass plate coated with collagen. After gelation of collagen by incubation of the glass plate with the PDMS device at 37℃ for 30 min, we added the culture medium and peeled off the PDMS device. Finally, an array of cell-laden collagen pillars was formed on the glass plate (Fig. 2 (a-c)).

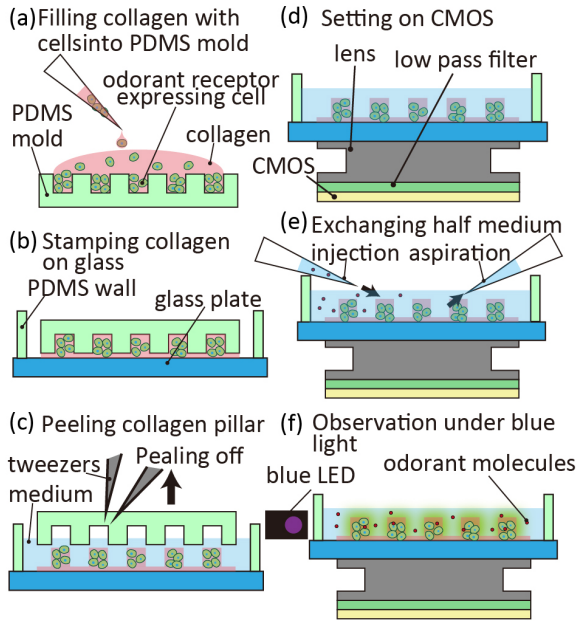

Figure 2. Fabrication flow of the portable odorant sensor with the array of cell-laden collagen pillars. (a) Pouring cell-mixed collagen solution into a PDMS micro-well array device coated with BSA. (b) Stamping the PDMS device on a glass plate coated by fibronectin. (c) Peeling off the PDMS device in culture medium after incubation for collagen gelation. (d) Placing the glass plate with the cell-laden collagen pillars on the device composed of a CMOS imaging sensor, a low pass filter and a lens. (e) Adding odorants with pipettes to the culture medium. (f) Observing fluorescence emitted from the cell-laden collagen pillars under blue light.

Fabrication of portable odorant detection system

We obtained a CMOS imaging sensor from a web camera (C270sBW, Logicool) as a fluorescence detector and mounted lens obtained from another web camera (CMS-V31SETBK, SANWA) on the sensor. We set a blue light source (OEHB220, OptoSupply) at the side of the CMOS device. Finally, we installed the glass plate with collagen pillars on the lens to observe them fluorescently using the CMOS imaging sensor (Fig. 2 (d)).

Measurement of the shapes of pillars

We measured diameters of the cell-laden collagen pillars in pictures taken by a microscope (IX71, Olympus) using image processing software (ImageJ) to pictures taken by a microscope (IX71 olympus). To measure heights of the pillars, we used a 3D laser scanning microscope (VK-X21, Keyence) with water immersion lens (LUM Plan FLN, Olympus) and analyzed heights of pillars using a microscope software (VK analyzer, Keyence).

Detection of odorants

To detect the cell response to odorants, we used calcium imaging enabling us to detect Ca^{2+} concentration by fluorescence. The calcium imaging for the array of cell-laden collagen pillars was performed using a working solution containing 5 μM fluo-8 AM (21082, AAT Bioquest) in HBSS with 0.02% Pluronic F-127 (P2443, SIGMA). After incubation of the cell-laden collagen pillars with the working solution for 30 min, we washed them with HBSS three times. Finally, we added 1 mM eugenol (E51791, SIGMA), odorant molecule from clover, to the HBSS and got the fluorescence intensity of pillars by extraction of a green channel from raw fluorescence image when we exposed blue light (Fig. 2 (e,f)).

RESULT AND DISCUSSION

Uniformity of cell-laden collagen pillars

To evaluate uniformity of the cell-laden collagen pillars, we measured diameter of the pillars (Fig. 3 (a,b)). When we used the PDMS device with 200 μm diamter molds, average diameter of the pillars was about 205 μm, and their cofficient of variation (CV) was 0.027. The results show that our process achieved transcription of the mold shape to the cell-laden collagen pillars in uniform diameter. Moreover, we measured heights of the cell-laden collagen pillars using a 3D scanning microscope. As a

result, average height 189 ± 8 μm was a little smaller than that of the PDMS mold (200 μm) (Fig. 3(c)). The result indicates gelation of collagen induced its shrinkage. It seems that the shirinkage supported to release of the cell-laden collagen pillars from the PDMS mold.

Fluorescence intensity emitted from cell-laden collagen pillars

We measured fluorescence intensity of cell-laden collagen pillars stained by calcein-AM whose height were 50, 100, 150 and 200 μm. Because calcein emits green fluorescence by excitation of blue lights, we extracted a green channel from fluorescent images of the cell-laden collagen pillars by image processing. As a result, we obtained the fluorescent image of green circle on the same position of the cell-laden collagen pillars. This result shows the CMOS imaging sensor detected green fluorescence emitted from cells stained by calcein in the pillars. Moreover, the fluorescence intensity rose according to increasing the height of the pillars (Fig. 4). This result indicated that the vertical accumulation of the green fluorescence in the pillars allows control of

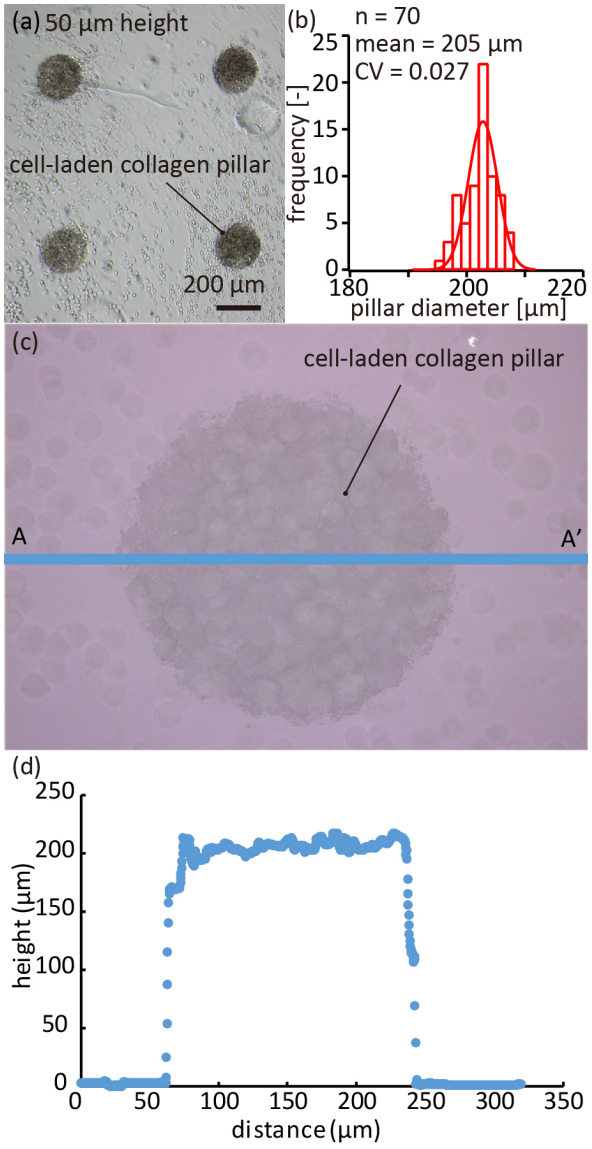

Figure 3. (a) Image of the cell-laden collagen pillars. (b) Diameter distribution of the pillars. (c) 3D laser micro scanning microscope image and height profile of the cell-laden collagen pillars. (d) Plots represent height of A-A' in figure c.

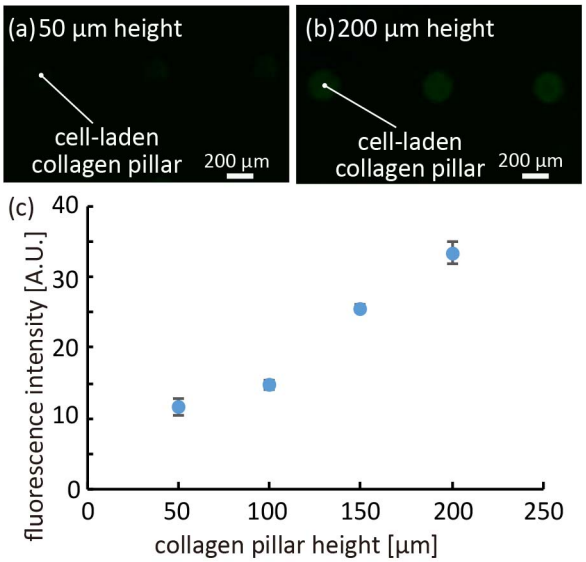

Figure 4. (a,b) Fluorescence images of cell-laden collagen pillars stained using calcein-AM detected by a CMOS imaging sensor. The collagen pillars heights are (a) 50 μm and (b) 200 μm. (c) Plots of fluorescence intensity detected by the portable odorant sensor according to the height of the cell-laden pillars: 50, 100, 150 and 200 μm. The number of samples are 6 each. The data is shown as mean ± standard error (SE).

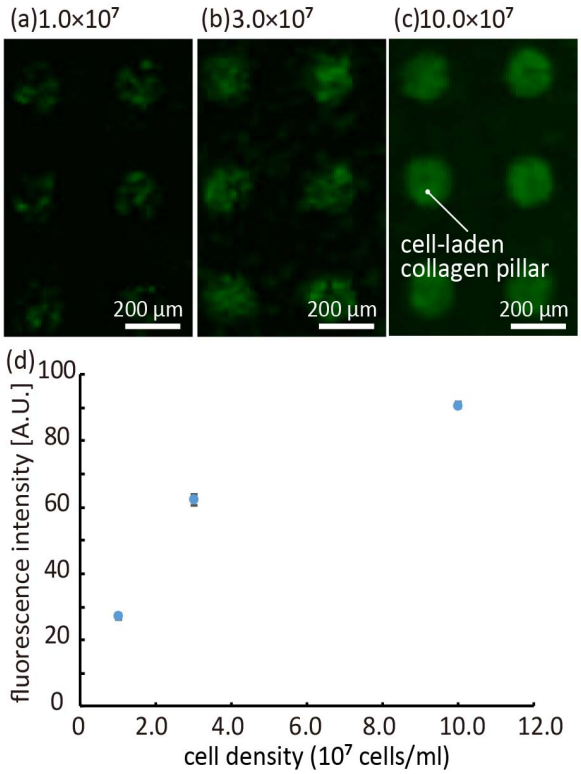

Figure 5. (a-c) Images of cell-laden collagen pillars containing cells with 1.0×10^7, 3.0×10^7 and 10.0×10^7 cells/ml cellular densities. (d) Plots of fluorescence intensity detected using the portable odorant sensor according to cellular densities of the cell-laden collagen pillars. The data is shown as mean ± SE.

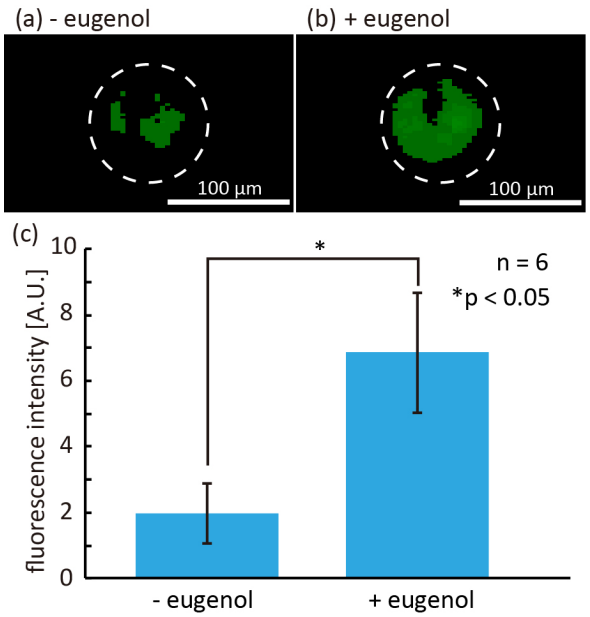

*Figure 6. (a, b) Images of the same collagen pillar consisting of HEK293T cells with olfactory receptors (a) before and (b) after applying eugenol to culture medium. White dashed lines show the edges of cell-laden collagen pillars. (c) Fluorescence intensities before and after the addition of eugenol. There are significant difference between them (t-test, *p < 0.05, n = 6). The data is shown as mean ± SE.*

fluorescence intensity of the pillars. Therefore, the cell-laden collagen pillars were suitable for detection of fluorescence using CMOS imaging sensor.

To investigate the relationship between cell density in the cell-laden collagen pillars and fluorescence intensity, we prepared the pillars with different cell densities: 1.0×10^7, 3.0×10^7 and 10.0×10^7 cells/ml. After staining these cell-laden collagen pillars with calcein, we measured their fluorescence intensity. As a result, fluorescence intensity increased corresponding to increase of cellular density (Fig. 5). These results indicate that high cell density promotes accumulation of fluorescence intensity in the pillars with the same height. Therefore, we regarded that 200 µm height cell-laden collagen pillars made with 10.0×10^7 cells/ml cell-mixed collagen solution was appropriate for the odorant detection.

Demonstration of odorant sensing

To verify detection of cellular odorant response using our portable odorant sensor, we measured fluorescence intensity of cell-laden collagen pillars consisting of cells transfected with olfactory receptor responding to eugenol (odorant molecule) before and after addition of eugenol. The fluorescence intensity after addition of 1mM eugenol was significantly higher than that of before the addition (Fig. 6). This result demonstrated that collagen pillars responded eugenol. Moreover, the CMOS imaging sensor detected the response of pillars to odorant molecule.

CONCLUSION

We fabricated the portable odorant sensor with the array of the cell-laden collagen pillars to detect cellular responses to odorant molecules by the CMOS imaging sensor. We believe that the portable odorant sensor will be applicable to quantitatively odorant detection in various fields as a substitution for working animals.

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