VOLATILE ODORANT DETECTION BY CORNEAL EPITHELIAL CELLS USING A PERFUSABLE FLUIDIC CHAMBER

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ABSTRACT

This work presents a method to detect volatile odorant using corneal epithelial cells with olfactory receptors. Because the corneal epithelial cells can survive at constant exposure of air, these cells have ability to detect the volatile odorant at air-liquid phase in contrast to other parenchymal cells. Therefore, in the present study, we first fabricate a device that provides the air-liquid environment by (i) a dorm shaped chamber to confine the air and (ii) channels to maintain humidity of the cells on the bottom of the device. Using the device, we successfully detected the volatile odorant using the fluorescence imaging of the corneal epithelial cells that were transfected with the mammalian olfactory receptors.

INTRODUCTION

Since olfactory receptors could detect and recognize various odorant molecules, the receptors have been applied to olfactory biosensors using heterogeneous cells [1, 2]. Surface plasmon resonance, and electrochemical impedance spectrometry has been suggested to detect specific odorant with the biosensors using heterogeneous cells [3, 4]. However, these biosensors could only identify aqueous odorant molecules because olfactory receptors could be stable in aqueous buffer solution [5, 6]

Recently, it has been revealed that thin water layer covered with insect olfactory receptors helps to detect volatile odorant because the layer works as mucus that allows dissolving odors on the olfactory epithelium [7]. But monolayer of heterogeneous cells for transfection such as HEK cells could not survive at air-liquid phase.

EXPERIMENTAL RESULTS

To cultivate cells and detect the volatile odorant, an acryl device was fabricated. The device had two part; medium storage and odorant detection area. The detection part was designed as a dorm-shape to prevent diffusion of the gas to outside the device. A septum between inlet and outlet was formulated to control airflow. In addition, a septum, which was mimicked nasal septum in nasal cavity, between inlet and outlet was formulated to control airflow [8]. At the bottom of device, channels connected with the medium were fabricated to maintain air-liquid phase of cells and humidity of the collagen gel [9]. By lowering the height of medium in the storage, the medium in the detection area was removed [10, 11]. The cells and the collagen gel retain humidity using channels at the bottom of the device. The corneal epithelial cells were cultivated and transfected with mouse olfactory receptors on the gel within the detection area.

To clarify air control by the septum, we flowed ink into the device (Fig. 1). The ink gradually spread from inlet side chamber to outlet side chamber.

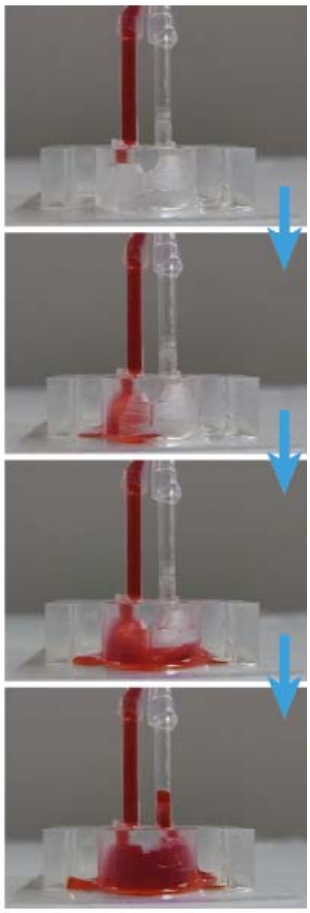

Figure 1: Flowing red ink into the device to clarify air control by the septum.

We evaluated whether the corneal epithelial cells had olfactory receptors and could recognize aqueous Eugenol that specifically reacts to the olfactory receptors in this study. The olfactory receptors were observed on the plasma membrane of the transfected cells by immunofluorescence staining (Fig. 2a,b). In addition, the calcium response sequentially increased with increasing the aqueous odorant concentration in calcium imaging using Fura-2/Am (Fig. 2c). These two results indicate that the olfactory receptors were successfully transfected into the corneal epithelial cells and preserve olfactory acuity.

Next, we did the calcium imaging to detect volatile odorant using the corneal epithelial cells with the olfactory receptors. Nitrogen gas used as carrier gas and was connected to a bottle of 1M eugenol solutions. The air pressure was 0.02 MPa. The fluorescence frequency was highly increased by stimulation of eugenol gas and decreased after flow of the nitrogen gas. Furthermore, the cells show no reaction to the ethanol gas that used as a negative control.

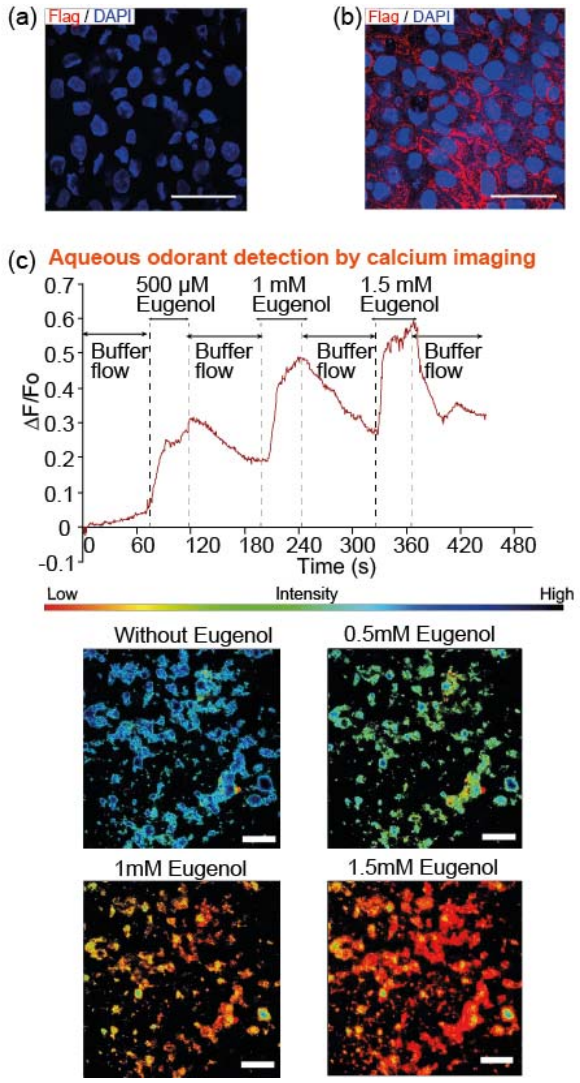

Figure 2: (a) No Flag that indicate olfactory receptor was observed at normal corneal epithelial cells. (b) Olfactory receptors were presented at plasma membrane of the transfected corneal cells. (Scale bars: 50 μm) (c) Calcium imaging with aqueous Eugenol stimulation. The flow rate was 1.5 min/ml. The calcium response increased with increasing the odorant concentration in liquid. (Scale bars: 100μm)

CONCLUSION

We supposed that the olfactory receptors successfully detected eugenol in the device because the collagen gel and channels around the gel preserved humidity to cover the olfactory receptors on the corneal epithelial cells Therefore, we believed that this device could be an effective biosensor to detect volatile odorant.

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