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Olfactory mucosa tissue-based biosensor: A bioelectronic nose with receptor cells in intact olfactory epithelium

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ABSTRACT

The biological olfactory system can distinguish thousands of different odors. Bioelectronic nose based on olfactory cells can be developed to realize the biomimetic design of an electronic nose. In this study, olfactory mucosa tissue of rat was isolated and fixed onto the surface of a light-addressable potentiometric sensor (LAPS), with the natural state of the neuronal populations and functional receptor units of the cilia well preserved. The electrical properties of the tissue–semiconductor interface were analyzed by the volume conductor theory and the sheet conductor model. Then, the local field extracellular potentials of the receptor cells in the olfactory mucosa tissue were simulated and monitored. The results suggested that this tissue–semiconductor hybrid system was sensitive to odorants stimulus. We believe that the receptor cell-based biosensor technology is a valuable tool to record data with high information content with respect to odors stimulation in the intact cellular environment of the olfactory epithelium. Due to the advantages of intact epithelium, this novel technology will potentially bridge the gap between conventional in vitro methods and complex in vivo experiments for a bioelectronic nose.

1. Introduction

The biological olfactory system can perceive and discriminate a large number of odors with very high sensitivity and specificity. Due to the great potential commercial prospects, lots of engineering researches of the olfactory system have been carried out for a long time [1]. The electronic nose, which can mimic olfaction systems of humans and animals to detect odors by its sensitive materials, is just one of the newly developing technologies. Its detection ability mainly depends on the sensitive materials' capability of absorption or catalysis. Compared to the biological specific binding of the odorants to the olfactory receptor neurons, electronic nose still has limitations in sensitivity and specificity, although great progress in this technique has been achieved for the environmental and medical detection [2].

Cell- and tissue-based biosensors, which treat living cells as sensing elements, can detect the functional information of biologically active analytes [3–5]. The neural cell and tissue can be extracted from primary sources and cultured in vitro. Then, the electrical activities directly relating to cellular functions can be detected by microelectronic sensor chips. This novel biosensor technique, characterized with high sensitivity, excellent selectivity and rapid response, has been applied in many fields ranging from environmental sensing to biomedical diagnosing.

In vertebrates, the initial events in olfaction occur as odorant molecules bind to the receptors of the olfactory receptor neurons. At the end of the last century, Gopel et al. first proposed to utilize olfactory neurons as sensitive materials to develop a bioelectronic nose [6–8]. They suggested that all of the biomolecular function units could be used to develop highly sensitive sensors for electronic noses. In recent years, some experiments have been tried to express olfactory receptors in heterologous cells (i.e. *Escherichia coli* cells or human embryonic kidney-293 cells), and coupled these cells to transducers (i.e. quartz-crystal microbalance or surface plasmon resonance). The designs have obtained high specificity and sensitivity to drugs or odors [9–11].

Odorant molecules bind to receptors in the ciliary membrane of the olfactory receptor neurons, initiating the olfactory signal transduction cascade and contributing to depolarization. Action potentials evoked by odor-induced depolarization propagate through the axons of receptor cells to the olfactory bulb, where odorant information is further processed and transmitted to the brain [12–16]. Therefore, a satisfactory bioelectronic nose should be a hybrid system of olfactory neurons and cellular potential

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detecting transducers. Our group has reported an olfactory biosensor based on light-addressable potentiometric sensor (LAPS) to investigate the extracellular potentials of the primary cultured olfactory cells under stimulations of the odorants [17]. It has been proved that those receptor cells and olfactory bulb neurons cultured on the sensor surface are sensitive to environmental change.

The whole intact olfactory mucosa tissue is one of much better candidates for the sensitive elements of the bioelectronic nose, as it preserves natural state of the neuronal populations and can be obtained easily. In this study, we analyzed the basic recording theories of the olfactory tissue coupled to LAPS, and then fixed the isolated olfactory mucosa of rat on the surface of LAPS to detect its extracellular potentials. Both the simulation and experiment results suggest that the tissue–semiconductor hybrid system is sensitive to odorant stimulation.

2. Theories

2.1. Theories of the LAPS

LAPS is a kind of semiconductor sensor commonly used for surface potential detection [18]. With a light spot illuminating on LAPS, the semiconductor absorbs energy and leads to energy band transition and produces electron–hole pairs. If LAPS is biased in depletion, the width of the depletion layer is a function of the local surface potential value. When LAPS is biased in reverse voltage, the depletion layer is enlarged.

When a modulated light spot is illuminated at LAPS, the photocurrent generated (Fig. 1). It can reflect the local value of the bias voltage. Some experiments have been designed to investigate LAPS as a possible cell–semiconductor hybrid system to monitor the extracellular potentials [19,20].

Our laboratory has used LAPS to monitor potentials of exciting cells, such as cardiomyocytes and neurons, cultured on LAPS in a long-term non-invasive way [21,22]. Due to the ionic currents of the Na^+, K^+ and Ca^{2+} in cell membrane, the excitable cells can produce extracellular potential change, which was equal to the change of bias voltage on LAPS. And, the change of bias voltage corresponded to photocurrent of LAPS. By focusing the light spot on a target cell, changes of the extracellular potentials were recorded by measuring the local surface potential at the illuminated region of LAPS.

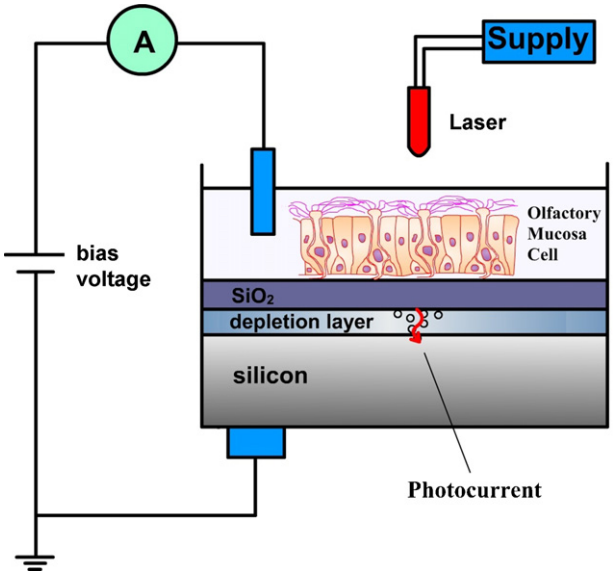

Fig. 1. LAPS system of the olfactory mucosa tissue cells on the sensor surface.

2.2. Theories of the in intact epithelium

Although isolated receptor cells are excitable and can respond to odors [17,23], the environment of the neurons *in vivo* is much more complex. The olfactory epithelium contains several cell types [24]. The olfactory tissue in Fig. 1 is the cartoon diagram of major cell types of the olfactory mucosa. The basal progenitor cells can differentiate into supporting (sustentacular) cells or into olfactory receptor neurons. The supporting cells and glandular cells secrete a mucus layer that coats the luminal surface of the epithelium. The olfactory receptor neurons are bipolar cells. One process of each cell is a single, unbranched axon that forms synapses in the olfactory bulb. On sufficient depolarization, the neuron can generate one or more action potentials. The other process of each neuron is a dendrite that projects into the mucus and terminates in an olfactory knob or vesicle. From each knob, fine olfactory cilia emanates into the mucus. Thus, odorants are detected on the surface of the olfactory epithelium by cilia.

In intact epithelium, it is possible to estimate the electrochemical potential by keeping the neuronal membrane and environment intact after the epithelium surgically removed [25–26]. Whereas cells were maintained in their native environment, the acute prepared intact epithelium had several advantages to organotypic function over isolated olfactory receptor neurons culture for bioelectronic nose:

(a) The natural states of the neuronal populations of olfactory receptor cells were well preserved.
(b) The functional receptor unit of cilia on each olfactory receptor cell would not be damaged.
(c) Extracellular compartments present *in vivo* (including supporting cells and basal cells) were preserved.
(d) The mucus layer with odor binding protein generated by Bowman's glands and supporting cells were preserved.
(e) The intact epithelium allowed simpler acute preparation and easier visualization, without strictly controlled cell culture conditions (i.e. nutrient media, pH, temperature, and listerize).

2.3. Field potentials of the epithelium tissue

For cells cultured on LAPS, theories of the area-contact model or the point-contact model of the cell-silicon junctions were used to illuminate current and voltage of the oxidized silicon surface [17,27]. In contrast to individual cultured cells, olfactory receptor cells are embedded in intact olfactory epithelium of about 100 μm thickness between the insulating silicon dioxide of LAPS and an electrolytic bath on reference potential (Fig. 2). In a tissue of densely packed cells, the electrical property of the tissue–semiconductor interface has been well analyzed by the volume conductor theory and the sheet conductor model [28].

The average field potential V_{field} of tissue on the semiconductor chips could be described by the volume conductor theory, as shown in Eq. (1):

$$-\frac{1}{\rho}\left(\frac{\partial^2 V_{\text{field}}}{\partial x^2}+\frac{\partial^2 V_{\text{field}}}{\partial y^2}+\frac{\partial^2 V_{\text{field}}}{\partial z^2}\right)=j_{\text{source}} \quad (1)$$

where the potential V_{field} arose from currents per unit volume j_{source} of cellular sources. ρ was the resistivity of the olfactory mucosa tissue, assuming the tissue was isotropic and homogeneous. From Eq. (1), the curvature of the potential was proportional to the current-source density with the Cartesian coordinates x, y and z.

Neglecting the details of the current flow from tissue to bath, we described the sheet conductor model for LAPS recording. In the model, we described the shunting effect of the bath by an ohmic conductance per unit area g_{leak}, the tissue itself by a sheet resistance