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A NOVEL BITTER DETECTION BIOSENSOR BASED ON LIGHT ADDRESSABLE POTENTIOMETRIC SENSOR

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This paper presents a novel biosensor for bitter substance detection on the basis of light addressable potentiometric sensor (LAPS). Taste receptor cells (TRCs) were used as sensitive elements, which can respond to different bitter stimuli with extreme high sensitivity and specificity. TRCs were isolated from the taste buds of rats and cultured on the surface of LAPS chip. Due to the unique advantages such as single-cell recording, light addressable capability, and noninvasiveness, LAPS chip was used as secondary transducer to monitor the responses of TRCs by recording extracelluar potential changes. The results indicate LAPS chip can effectively record the responses of TRCs to different bitter substances used in this study in a real-time manner for a long-term. In addition, by performing principal component analysis on the LAPS recording data, different bitter substances tested can be successfully discriminated. It is suggested this TRCs−LAPS hybrid biosensor could be a valuable tool for bitter substance detection. With further improvement and novel design, it has great potentials to be applied in both basic research and practical applications related to bitter taste detection.

Keywords: Taste receptor cells; bitter detection; bitter signal transduction; light addressable potentiometric sensor; biosensor.

1. Introduction

Taste sensation plays crucial roles in the detection of chemical substances, which can provide valuable information about the nature and quality of food. Taste receptor cells (TRCs) that form taste buds are the taste sensation elements for five basic taste qualities including sweet, bitter, sour, salty, and umami.[1,2] TRCs are specialized epithelial cells, bearing the properties of neurons, which can relay taste information via intracellular signal transduction pathway, resulting in cell depolarization and neurotransmitter releasing.[3] There are four morphological types of TRCs in each taste bud including type I (dark), type II (light), type III

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(intermediate), and type IV taste cells.[4] Type II taste cells mediate the signal transduction of sweet, bitter, and umami, which transmit the taste signals by ATP release due to the lack of classic synaptic connection with afferent nerves.[5–7] In contrast, type III cells can respond to sour stimuli and transmit the sour signals via synaptic connection with the taste afferent nerve fibres.[8] Type I and type IV taste cells are supporting cells and basal cells, respectively.[9] TRCs have intrinsic advantages for taste substance detection such as extreme high sensitivity, specificity, fast response, and powerful information processing ability. For bitter detection, thousands of bitter substances with great chemical diversity can be detected by a limited number of cellular sensors which consist of a subset of TRCs expressing only ∼30 bitter taste receptors.[10–13] The unique powerful capability of bitter sensation makes TRCs ideal candidates to be used as sensitive elements in biosensors for bitter detection.

On the other hand, with the development of micro-electro-mechanical systems (MEMS) technology, reliable extracellular recording chips provide us new approaches for the design and fabricate novel cell-based biosensors, which opened up an exciting realm for basic sciences as well as practical applications.[14] Various cell-based biosensors have been developed by combining cells with different extracellular recording chips, such as microelectrode array (MEA)[15,16] and field effect transistor (FET).[17–19] Both MEA and FET can monitor the extracellular potential changes in a noninvasive manner for a long term. However, both of them suffered greatly from the limited and discrete recording spots because it is hard to culture target cells on the desired spots such as the gate-electrode of individual FET and the tip of individual microelectrode. Light addressable potentiometric sensor (LAPS) is another commonly used extracellular recording chip, which can overcome the intrinsic geometry limitations of MEA and FET by scanning the light-pointer along the LAPS surface to select any desired spot for recording.[20] In addition, compared with MEA and FET, LAPS holds the advantages of simple structure, low cost, and easy integration. LAPS chip has been widely used to develop cell-LAPS hybrid for monitoring extracellular potential changes.[21–23]

The object of this work is to develop a novel TRCs–LAPS hybrid biosensor that can mimic the power of nature's gustatory system for bitter detection, which may provide a novel tool for bitter substance detection and have great potentials to be applied in many fields such as food safety, environmental protection, and drug discovery. For this purpose, TRCs isolated from rat taste buds were cultured on the surface of LAPS chip to serve as sensitive elements. LAPS chip was utilized as secondary transducer to monitor the responses of TRCs to bitter stimuli by extracellular potential recording. In this study, three bitter substances including $MgSO_4$, denatonium, and D-(-)-salicin, were used as bitter stimuli. Principal component analysis (PCA) was employed to analyze the recording data for bitter substance discrimination.

2. Materials and Methods

2.1. *LAPS chip fabrication*

LAPS chip was fabricated on the basis of n-type silicon wafer ($\langle 100 \rangle$, 10–15 Ω cm). For the first, a layer of SiO_2 with thickness of 30 nm was thermally grown at 1000°C on the surface of silicon wafer. Then the silicon wafer was ground to 100 μm thick from the back side to enhance the sensitivity. Afterwards, a 1 μm aluminum layer was evaporated on the back side of silicon wafer to create an ohmic contact, which will be used as the working electrode. LAPS chip was attached to the bottom of a detection chamber with a 5 mm-diameter hole in the bottom center. Platinum wire was used as the reference electrode which was immersed into the solution in detection chamber.

2.2. *TRCs isolation and culture*

TRCs were prepared from Sprague-Dawley rats according to the previous reported method.[24,25] Briefly, the rat was injected with 5-HTP (5-hydroxy-l-tryptophan, Sigma) (80 mg/kg) 1 h before sacrifice. The entire tongue was dissected. Then 1–1.5 ml tyrode's solution containing collagenase (2 mg/ml) and elastase (0.25 mg/ml) was injected under the epithelium of the tongue. Tyrode's solution consisted of 126 mM NaCl, 5 mM KCl, 5 mM NaHEPES, 1.25 mM $NaH_2PO_4 \cdot H_2O$, 10 mM glucose, 2 mM $CaCl_2$, and 2 mM $MgCl_2$ (pH = 7.4, adapted with NaOH). Afterwards, the lingual epithelium was incubated with divalent-free Tyrode's solution for 30 min and gently peeled from the underlying

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connective tissue. A fire polished glass pipette was used to collect circumvallate taste cells from epithelium by a gentle suction, which contain TRCs that can respond to bitter stimuli. Then the collected cells were cultured on the surface of LAPS chip for 2 h at 37°C under standard conditions of humidified air with 5% CO_2 in DMEM (Dulbecco's Modified Eagle's Medium) (Gibco, UK) to allow the attachment of cells on the surface of LAPS chip. Before cell culture, the surface of LAPS chip was coated with a thin layer of poly-L-ornithine and laminin (PLOL) mixture to enhance cell attachment.[21]

2.3. *LAPS test setup and measurement*

LAPS test setup used in this study was adopted from our previous reported setup.[23,25] Schematic diagram of the setup is shown in Fig. 1. LAPS chip with cell culture chamber was fixed under a microscope objective in the setup when the TRCs cultured on the surface of LAPS were ready for measurements. A He−Ne semiconductor laser (Coherent Co., USA) was used to generate the modulated light and focused to less than 10 μm. The focused light was moved on the surface of LAPS chip to select the desired TRCs for measurement by illuminating the cells to generate photocurrent. The wavelength of the light was 543.5 nm and the power was 5 mW. The responses of TRCs to bitter stimuli were monitored by recording the photocurrent fluctuations resulting from the changes of cell membrane potentials. The photocurrent fluctuations were subsequently transmitted into peripheral equipments and recorded by the electrodes of potentiostat (Model 273A, EG&G Princeton Applied Research) and the lock-in amplifier (model SR830 DSP, Stanford Research Systems). We used a 16-bit data collection card and the software of LABVIEW to collect, analyze, and store the data. Cell culture media or bitter stimuli were pumped alternatively into detection chamber by a peristaltic pump, which was controlled by a personal computer. Three bitter compounds dissolved in the cell culture media were used as bitter stimuli which included 30 mM $MgSO_4$, 1 mM denatonium, and 1 mM D-(-)-salicin. In order to exclude ambient light and to minimize background noise, the whole setup was shielded with a copper box and all measurements were performed at (37 ± 0.2)°C.

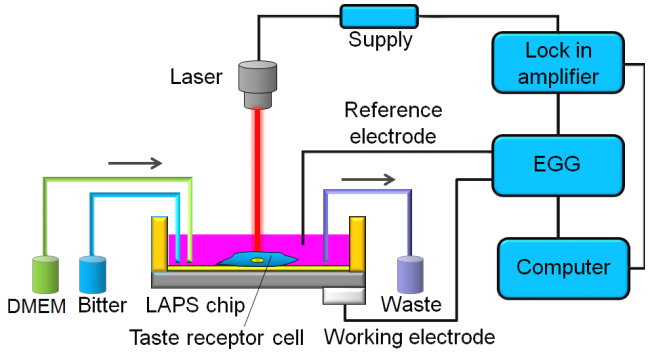

Fig. 1. Schematic diagram of LAPS test setup.

2.4. *Data analysis*

PCA was employed to process the recording data of LAPS chip. Six principle components were extracted from the recorded spikes, which were amplitude of spike, trigger cycle of spike, envelop area of spike, envelop area of spike with limited width, time of spike falling, and time of spike rising. Recordings from three types of bitter stimuli that is $MgSO_4$ ($n = 11$), denatonium ($n = 9$), and D-(-)-salicin ($n = 9$) were analyzed. Recordings of spontaneous spiking ($n = 29$) when there was no bitter stimuli applied, were also analyzed to serve as negative control.

3. Results and Discussion

3.1. *TRCs coupled with LAPS chip for bitter signal detection*

A subset of TRCs in taste buds can respond to a broad array of bitter substances and convert bitter signals into electrical signals by intracellular signal transduction pathway.[26,27] Figure 2 is the schematic diagram of bitter signal transduction pathway of TRCs in vertibate. TRCs for bitter sensation express bitter taste receptors, T2Rs, which is a family of approximately 30 highly divergent G protein-coupled receptors (GPCRs). Bitter sensation is initiated by the specific binding of bitter compounds to bitter taste receptors (R), which can activate specific G proteins (G). Then G protein activates PLC-β2, which can hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3). IP_3 can bind to IP_3R3 and result in the release of Ca^{2+} from intracellular Ca^{2+} stores. The increase of intracellular Ca^{2+}concentration can activate TRPM5. Finally, the activation of TRPM5 will lead to cell depolarization and ATP release. This cascade

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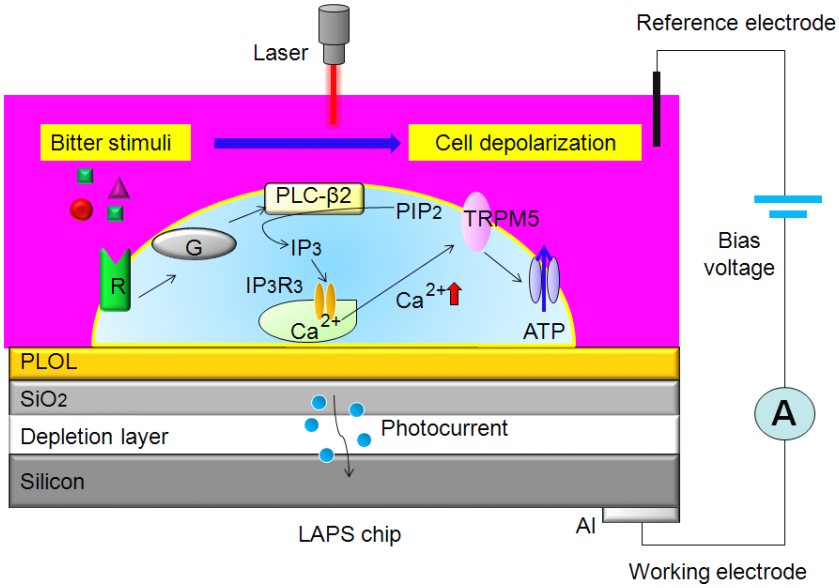

Fig. 2. Schematic diagram of bitter signal transduction pathway coupled with LAPS chip for bitter detection. R: bitter taste receptor. G: G protein. Note some additional intracellular regulatory pathways are omitted from this diagram for simplicity.

of intracellular enzymatic reactions ultimately leads to the generation of cell membrane potential changes, which can then be detected by LAPS chip. Figure 2 also shows the basic detection mechanism of LAPS with the structure of electrolyte insulator [SiO_2]semiconductor [Si] (EIS). When light illuminates LAPS chip, the light energy can be absorbed by the semiconductor to generate electron−hole pairs. With the bias voltage applied in depletion layer, the electron−hole pairs will lead to the generation of photocurrent that can be detected by peripheral circuit. When TRCs are cultured on the surface of LAPS chip, the cell membrane potential changes are coupled to the bias voltage, which can lead to the generation of corresponding fluctuation in photocurrent. Consequently, extracellular potential changes can be monitored by recording the photocurrent fluctuation. Therefore, bitter signals can be transformed into current information via this TRCs−LAPS hybrid biosensor, which could be used not only for the bitter detection but also for the research of bitter signal transduction.

In order to utilize TRCs as sensitive elements for reliable extracellular recording, it is of great importance to couple TRCs with LAPS chip with high efficiency. For the purpose of enhancing the attachment of TRCs, we modified the surface of LAPS chip with a thin layer of PLOL mixture to improve the surface biocompatibility. The results of TRCs cultured on the surface of PLOL modified

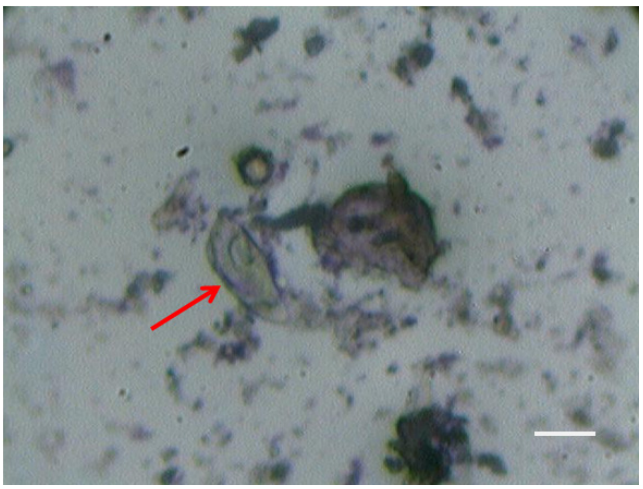

Fig. 3. Results of TRCs cells by Typan blue stain on the surface of LAPS chip with PLOL modification. Red arrow indicates single taste receptor cell which is elongated and spindle shaped (Scale bar: 20 μm).

LAPS chip was shown in Fig. 3. The results show that TRCs grow well on the LAPS chip. TRCs are elongated and spindle shaped which are distinguished from other round taste bud cells. This makes it possible for LAPS chip to perform reliable and high efficiency extracellular recording on TRCs.

3.2. *Detection of bitter substances*

To test the performances of this TRCs−LAPS hybrid biosensor for bitter detection, we utilize

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three different types of bitter substances disolved in cell culture media as stimuli which are 30 mM $MgSO_4$, 1 mM denatonium, and 1 mM D-(-)-salicin, respectively. Cell culture media without any bitter substance addition was used as the negative control solution. LAPS chip can perform real-time monitoring of single cell potential responses by illuminating the desired target cell with modulated light. For each cell we tested, the control solution as well as three bitter stimuli were applied sequentially. Between each stimulus application, the control solution was used to refresh the detection chamber and the interval for each recording was 5 min. By this protocol, the responses of 84 TRCs to bitter stimuli were monitored by recording the cell membrane potential changes. The results show that only 32 TRCs tested show responses to bitter stimuli. In addition, TRCs responded to only one out of three bitter stimuli tested. This may be due to the special pattern of bitter sensation, which does not use broadly tuned bitter-sensitive TRCs to recognize and discriminate bitter substances. Figure 4 shows the typical recordings of TRCs responding to different bitter stimuli. The results show the differences between control and bitter stimuli are obvious. As for negative cell control, no spike was recorded when bitter free or bitter containing solution was applied. A typical recording from negative cell control during denatonium stimulation was presented in Fig. 4. In cases of bitter responsive cells, when bitter free control solution was applied, only a few spikes were recorded, which is corresponding to the spontaneous spikes of cells. On the contrary, much more spikes were recorded when bitter stimuli were applied to the same cell, which are associated with bitter stimuli.

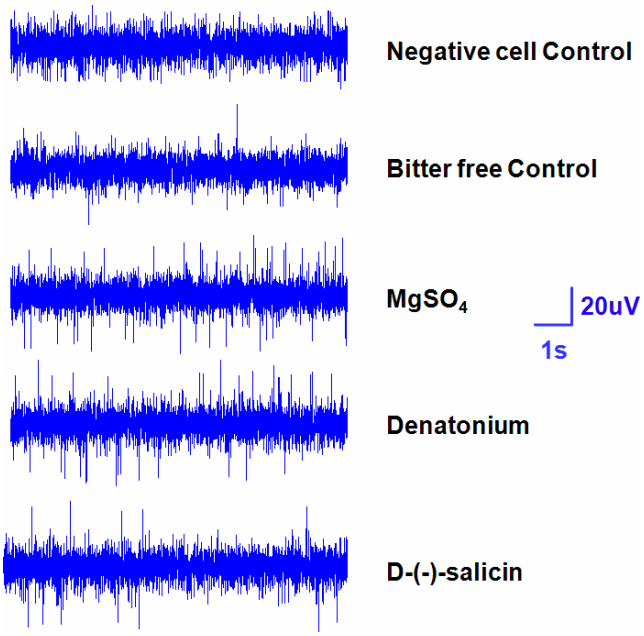

Fig. 4. Typical recordings of LAPS chip from taste bud cells in response to different bitter free or bitter containing solutions.

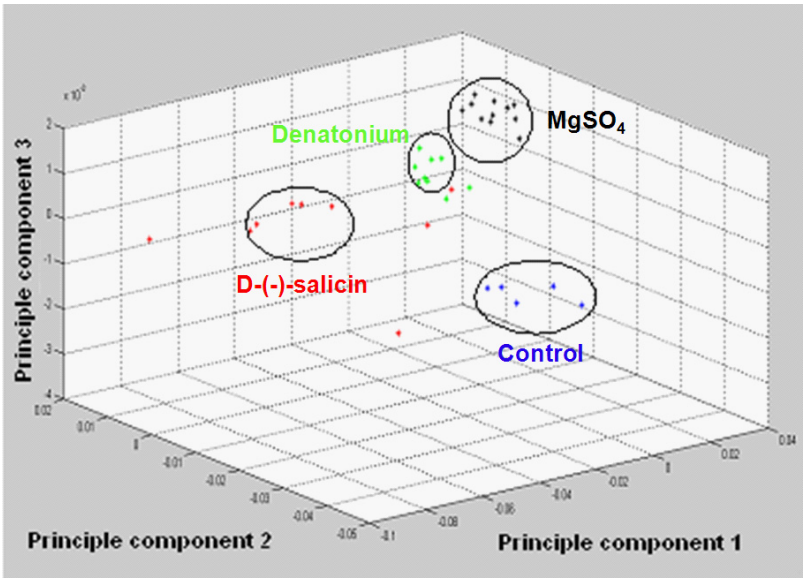

Fig. 5. PCA of LAPS recorded data upon control and three different bitter stimuli. Three principle components are amplitude of spike, trigger cycle of spike, and envelop area of spike. The contribution rates of three principle components are 63.9%, 16.5%, and 10.3%, respectively.

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However, the differences between each bitter stimulus were not apparent and need further data process. It is hard to be used directly for the discrimination of different bitter substances.

Therefore, we employed PCA to process the recording data of LAPS chip for the purpose of bitter substance discrimination. We performed PCA on the recording spikes of control ($n = 25$) and three bitter stimuli, which included $MgSO_4$ ($n = 11$), denatonium ($n = 9$), and D-(-)-salicin ($n = 9$). As shown in Fig. 5, the control firing and the responses of three different bitter stimuli can be classified into distinct clusters, which indicate that different bitter substances used in this study can be discriminated. All the results suggested this TRCs−LAPS hybrid biosensor could be useful for discriminating different bitter substances and could serve as a valuable tool for bitter detection.

4. Conclusion

In this work we developed a bitter taste biosensor by using TRCs as sensitive elements and LAPS as transducers. The results indicate LAPS chip can effectively record the responses of TRCs to bitter stimuli used in this study. Bitter substance detection results demonstrate that this TRCs−LAPS hybrid biosensor can successfully discriminate three different bitter substances tested in this study. It is suggested this biosensor could be a valuable tool for bitter substance detection. This is the first critical step of developing bitter receptor-based biosensors. With further improvement and novel design, this biosensor could be used to test a large number of structurally diverse compounds to establish the structure−function relationship between ligand and bitter taste receptors, which may eventually contribute to the bitter signal transduction as well as to the prediction of any compound's bitterness.

Acknowledgments

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