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Dual functional extracellular recording using a light-addressable potentiometric sensor for bitter signal transduction

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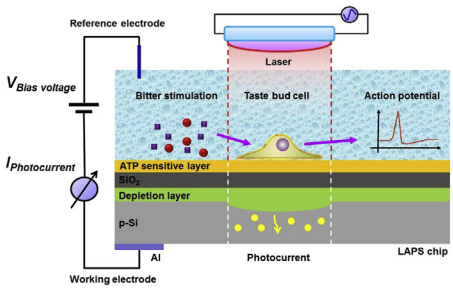
HIGHLIGHTS

- A biosensor was developed for extracellular recording of membrane potential changes and ATP release from single taste cell.
- Enhancive and inhibitory effects on the extracellular membrane potential changes and ATP release were efficiently recorded.
- Inhibitory effect of CBX also validates that LAPS extracellular recordings are originated from bitter signal transduction.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper presents a dual functional extracellular recording biosensor based on a light-addressable potentiometric sensor (LAPS). The design and fabrication of this biosensor make it possible to record both extracellular membrane potential changes and ATP release from a single taste bud cell for the first time. For detecting ATP release, LAPS chip was functionalized with ATP-sensitive DNA aptamer by covalent immobilization. Taste bud cells isolated from rat were cultured on LAPS surface. When the desired single taste bud cell was illuminated by modulated light, ATP release from single taste bud cells can be measured by recording the shifts of bias voltage-photocurrent curves (*I-V* curves) when the LAPS chip is working in discrete mode. On the other hand, extracellular membrane potential changes can be monitored by recording the fluctuation of LAPS photocurrent when the LAPS chip is working in continuous mode. The results show this biosensor can effectively record the enhancive effect of the bitter substance and inhibitory effect of the carbenoxolone (CBX) on the extracellular membrane potential changes and ATP release of single taste bud cells. In addition, the inhibitory effect of CBX also confirms LAPS extracellular recordings are originated from bitter signal transduction. It is proved this biosensor is suitable for extracellular recording of ATP release and membrane potential changes of single taste bud cells. It is suggested this biosensor could be applied to investigating taste signal transduction at the single-cell level as well as applied to other types of cells which have similar functions to taste bud cells.

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1. Introduction

Mammalian taste can detect and distinguish various taste signals presented by tastants using different kinds of taste bud cells, which play crucial roles in providing valuable information about living such as the nature and quality of food [1–3]. Taste bud is the taste sensation organ, which consists of four morphological types of taste bud cells [4]. Among them, type II cells are in charge of detecting sweet, bitter, and umami, which express the specific taste receptors and transduce the taste signals by a cascade of intracellular biochemical reactions [5–7]. It is proved that type II cells relay the taste signals to surrounding taste bud cells by ATP release in a cell membrane voltage-dependent manner, which plays a significant role in taste signal transduction [5,8]. The process of taste signal transduction is a complex and multistage process [9,10]. It is still far from complete understanding of mechanisms underlying taste sensation, especially the mechanisms of taste signal transduction. The main reason is attributable to the complications of taste signal transduction and the lack of powerful tools for the research of taste sensation. Specifically, the multiparameter measurement is highly desirable for the successful monitoring of taste signal transduction process. It is important to develop novel approaches for extracellular recording to investigate the signal transduction mechanisms of taste bud cells.

Light-addressable potentiometric sensor (LAPS) is a photoelectric semiconductor that is very sensitive to surface potential changes [11]. LAPS can measure any response that results in the changes of surface potential, such as cell membrane potential changes [12,13], ion concentrations [14,15], and charged molecules [16,17]. LAPS have two significant advantages compared to the field-effect transistor (FET) and microelectrode array (MEA). One is the large continuous surface, which makes it easy for the surface modification. LAPS chip can be functionalized with ultrathin sensitive membranes for detecting specific ions and charged molecules such as K^+, Na^+, and Ca^{2+} [18], 5-HT [19], and DNA [16,17]. The other one is the light-address ability, which can realize the single-spot measurement by illuminating the desired spot with a movable light. Versatile measurement principles of LAPS are capable of design and invent novel tools for multifunctional extracellular recording.

Various methods have been developed for ATP detection such as stand luciferin-luciferase assay [5], cell-based ATP-sensitive biosensor [20], cell surface attached firefly luciferase [21,22], myosin-functionalized cantilevers [23], and enzyme-functionalized Pt microelectrode [24]. However, these methods have some intrinsic limits such as tedious design and preparation of sensitive molecules, sophisticated responses of sensitive molecules to ATP, precise manipulation of measuring cells. Compared to methods mentioned above, aptamer-based methods have become more and more promising, which use ATP-sensitive aptamer as sensing elements [25,26]. Aptamers are nucleic acid ligands with high binding affinity to specific molecular targets [27,28]. It is thus tempting to combine the extracellular potential recording capability of LAPS with ATP-sensitive aptamer for the sake of providing novel solutions to the problems faced in developing multiparameter measurement biosensors for the research of taste signal transduction mechanisms.

This study presents a dual functional extracellular recording biosensor based on a light-addressable potentiometric sensor (LAPS) functionalized with ATP-sensitive aptamer for the first time, which can record both extracellular membrane potentials and ATP release from single taste bud cells. DNA competitor (with a full complementary sequence of ATP-sensitive aptamer) was covalently immobilized on the LAPS surface, which can capture ATP-sensitive aptamer by DNA hybridization. Taste bud cells prepared from rat were cultured on the LAPS surface. When the desired single taste bud cell was illuminated by a modulated light, ATP release can be measured by recording the shifts of bias voltage-photocurrent curves (*I-V* curves) when the LAPS chip is working in discrete mode, while extracellular potential can be monitored by recording the fluctuation of LAPS photocurrent when the LAPS chip is working in continuous mode. Further, a hemichannel blocker, carbenoxolone (CBX), which can dramatically inhibit the membrane current and ATP release of taste bud cells, was utilized to confirm the LAPS extracellular recordings are originated from bitter signal transduction. This dual functional extracellular recording biosensor cannot only be applied to taste bud cells for the taste signal transduction, but also be applied to other types of cells which have similar functions to taste bud cells, which can be accommodated by current devices or methods.

2. Methods and materials

2.1. LAPS chip fabrication and functionalized with ATP-sensitive aptamer

For LAPS chip fabrication, the silicon wafer (p-type, <100>, 3–8 Ωcm) was extensively cleaned and then was thermally grown a layer of SiO_2 on the surface under 1180 °C with a thickness of 50 nm. A thin film of titanium dioxide (TiO_2) was spin-coated onto the upper side of the silicon wafer. The silicon wafer was then dried in a furnace at 120 °C for 20 min and sintered at 500 °C for 60 min in the air with the temperature raised at 10 °C/min. Hydrofluoric acid was used to treat the back side of the silicon wafer to remove the SiO_2 layer. Afterward, 1 μm thickness aluminum layer was evaporated on the back side of the silicon wafer to create an ohmic contact and served as the working electrode. UV was employed to treat the upper side of the silicon wafer for the sake of improving the following silanization level. For silanization, the silicon wafer was dipped into the toluene solutions with 3-aminopropyltriethoxysilane (APTES) concentration of 0.1% (v/v) for 1 h and sealed at room temperature. The silicon wafer was then cleaned completely by rinsing in toluene and ethanol and cured at 120 °C for 1 h. Finally, the upper side of the silicon wafer was attached to a detection chamber with a 5 mm-diameter hole in the bottom center.

ATP-sensitive aptamer (5′-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′) and DNA competitor (5′-ACC TGG GAA TAC TCC CCC AGG T -3′, 5′-end with C6-NH_2 modified) were purchased from Takara Biotechnology Co. Ltd., Dalian, China. For LAPS chip functionalization, DNA competitor was covalently linked to LAPS surface by a crosslinking method. Amine residues were introduced to LAPS surface by silanization. Glutaraldehyde (Sigma-Aldrich, USA) was used to treat LAPS surface for cross-link reaction. DNA competitor was covalently immobilized by reaction between amine residues of DNA competitor with aldehyde residues of TiO_2 film. Bovine serum albumin (BSA) (1% (v/v), Sino-American Biotechnology Co. Ltd., China) was used to block unreacted aldehyde residues of LAPS surface to avoid nonspecific binding. The ATP-sensitive aptamer was then added and hybridized with DNA competitor. PBS was used to wash the LAPS chip to get rid of the excessive aptamer and avoid nonspecific adsorption. LAPS chips functionalized with ATP-sensitive aptamers were stored at 4 °C for further experiments.

2.2. Preparation of taste bud cells and fluorescent imaging

Preparation of taste bud cells was according to previously described method [29]. Briefly, Sprague-Dawley rat in a 5–7 days old was sacrificed. The entire tongue was later dissected and used for harvesting taste bud cells. For this, the lingual epithelium was

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