Biomimetic Olfactory and Gustatory Biosensors

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The biomimetic olfactory and gustatory biosensing devices have broad applications in many fields, such as industry, security, and biomedicine. The development of these biosensors was inspired by the organization of biological olfactory and gustatory systems.

detection strategies olfactory and gustatory biomimetic biosensors

1. Biomimetic Olfactory and Gustatory Biosensors Based on Field-Effect Devices

Field-effect devices have been broadly used in the development of biomimetic olfactory and gustatory biosensors. In the field-effect devices, the conductivity of an underlying semiconductor layer can be modulated by the electric field of the gate electrode surface. It is an important physical mechanism of semiconductor devices.

1.1. Field-Effect Transistor

Field-Effect Transistor (FET) is an important field-effect device with excellent characteristics including high input resistance, low noise, low power consumption, large dynamic range, and ease of integration. It consists of the source electrode, drain electrode, and gate electrode. When no voltage is applied to the gate, the current channel between the source and the drain is not turned on, and the FET is at the cut-off state. Once the applied voltage of the gate exceeds the threshold voltage, the current channel between the source and the drain will be turned on to form a leakage current. The leakage current can be tuned by the voltage of the gate with an internal amplification effect.

Recently, CNT as sensitive material carriers have been widely used to combine with FETs for the development of biomimetic biosensors, which can be attributed to: (1) the tubular structure providing abundant binding sites and a wider detection range, (2) the high electrical conductivity and large carrier mobility providing effective direction for improving the interfacial properties of transducer devices, and (3) the excellent chemical stability and elastic mechanical properties being beneficial to improving the specificity, selectivity, and stability of the sensor. In addition, many studies usually utilized equilibrium constant K to reflect the dissociation rate of the combination between proteins and small molecules so as to achieve quantitative evaluation of protein affinity level with small molecules. FET-based biosensors were developed by coupling olfactory receptors as sensitive materials to

recognize different specific ligands, which can be widely applied in food quality analysis, disease diagnosis and environmental assessment. A single-walled carbon nanotubes-field effect transistor (swCNT-FET) was coated with polyethylene glycol (PEG) for the prevention of nonspecific absorption and functionalized with the human olfactory receptor 2AG1 (hOR2AG1), which was used to detect amyl butyrate (AB) selectively with ultrahigh sensitivity down to concentrations as low as 1 fM [1]. Carbon nanotube-based field-effect transistor (CNT-FET) was equipped with rectangular floating electrode functionalized with T1R2 venus flytrap (VFT) through N-acetyl-l-cysteine solution coating and amide bond formation, which achieved more ultrahigh detection on sweet tastants with a limit down to concentrations as low as 0.1 fM and highly stability with more than 28 days [2]. The dissociation constant of 10^{-12} M indicated the high affinity between VFT and sweet tastants and the similar constant of sucrose and sucralose contributed to their similar structure.

Graphene has a large specific surface area, good stability, electrical conductivity and excellent absorbability, which has been widely used to modify and perfect the transducer surface. A bioelectronic tongue was established based on graphene-FET platform with VFT domain of human taste receptor type 1 member 1 (T1R1) as sensing elements and the disodium 5′-inosinate (IMP) as the enhancing element for the specific detection of umami taste in real time [3]. This platform not only shows high sensibility for capturing ligands but also has an effective reusable property and a great storage stability to 5 weeks.

The developed biosensors have high resolution in distinguishing corresponding target molecules. Notably, the production of receptor proteins has the characteristics of simple, rapid and high-yield extraction, which provides a strong and beneficial basis for simulating human olfactory/gustation system.

Although olfactory and gustatory receptors can simultaneously detect and recognize different ligands in real time, the processing and effective analysis of large amounts of data is time-consuming and error-prone. The combination of biosensor and machine learning has become the mainstream of current researches. Among these methods, principal component analysis (PCA) was especially suitable for dimensionality reduction of large quantities of data, effective and quick extraction of data features and rapid realization of visual research, which have been widely used in data analysis for distinguishing various smells and taste substances. An artificial multiplexed super-bioelectronic nose (MSB-nose) was developed based on a liquid-ion gated FET coated with single layer graphene and functionalized with the assembly coding form of two different human olfactory receptors for the high-quality and high sensitive detection of AB and helional (HE) [4]. The PCA simulation results showed ultra-high accuracy with no less than 99%, indicating the MSB-nose can encode olfactory receptor combinations for distinct odor identification. Moreover, this biosensor successfully achieved super-highly sensitive AB and HE detection with a detection limit down to 0.1 fM and a good stability to 10 weeks. The equilibrium constant K of more than 10^{14} confirmed the perfect affinity between receptors and ligands.

In addition to receptors, OBPs are another effective alternative sensitive material with hydrophobic β-barrel cavity, successfully providing a useful model system to study binding affinity with odor compounds. The sensing system opened a new pathway for accurate derivation of conformational events related to OBP interactions with an ultra-weak molecule [5]. In another study, odorant-binding protein 14 (OBP14) from the honey bee was used as the

sensitive material for quantitative recognition and detection of a variety of ligands containing a hydroxy group, such as homovanillic acid, eugenol, and methyl vanillate [6]. The results confirmed that OBP14 with a hydrophobic β-barrel cavity has a higher affinity with the ligands with the hydroxy group.

Although the solution to extract receptor or binding proteins may be very simple and fast, the conformation and biological activity of membrane proteins are still easily damaged during extraction due to various problems. In addition, it was still difficult to keep the activity, conformation maintenance and stability of extracted proteins in vitro. To challenge this problem, nanodiscs were reported and successfully extracted with a phospholipid bilayer structure composed of membrane scaffold proteins (MSPs) and phospholipids, providing powerful technical support for membrane protein research and olfactory and gustatory detection. Yang et al. developed a biomimetic olfactory biosensor based on oriented-immobilization nanodiscs embedded with trace-amine-associated receptor 13c (TAAR13c) as a sensitive element and CNT-FET with floating gold electrodes as a transducer element for highly-sensitive detection of death-associated odor, cadaverine (CV). The developed system successfully achieved detection on CV with a concentration range over five orders of magnitude and showed good selectivity toward other interference including diaminodecane, trimethylamine, ethanolamine, and glutamine. The equilibrium constant K between T13NDs and CV was estimated as 3.63×10^{11} M^{-1}, showing the high affinity between ligands and receptors and different periods of spoilage experiments, indicating the excellent practical application value for evaluations in food safety environments [7]. To improve the signal responses with similar strategy, the team firstly proposed an auxiliary method with 1 nM benzyl salicylate as enhancer materials. The K_d between hOR1A2 and geraniol was changing from 8.37×10^{11} M^{-1} to 1.64×10^{15} M^{-1} after the addition of enhancer materials, which indicated that the benzyl salicylate as the enhancer could obviously improve the binding affinity between ligands and receptors [8]. Recently, a portable biomimetic olfactory biosensor was successfully developed based on nanodiscs embedded with TAAR13c and TAAR13d for high detection of CV and putrescine (PT) with a limit down to 1 fM, respectively [9]. They also evaluated the binding degree of TAAR13 to CV and PT based on a SWISS-MODEL simulation [10], and the results showed that TAAR13 specifically combined with CV and PT was associated with Asp 112 and Asp 202 (both the amino groups of CV and PT formed salt bridges with Asp 112 and Asp 202 in TAAR13 at specific distances, respectively). Notably, the developed portable system can successfully achieve detection of diverse real samples on-site and monitor the time of freshness, providing a potential platform for the in situ and on-site monitoring of meat spoilage. However, utilization of nanodiscs embedded with receptors cannot completely analyze the ligand-induced intracellular signaling cascades. To challenge this problem, the emergence of nanovesicles containing both transmembrane proteins and contain cytoplasmic proteins, were successfully explored and widely applied. A biomimetic gustatory biosensor was proposed based on nanovesicles containing honeybee umami taste receptor, gustatory receptor 10 of Apis mellifera as sensitive materials CNT-FET with floating gold electrodes for high selectivity on l-monosodium glutamate (MSG) detection [11]. Notably, IMP has been widely used as the enhancing element for synergistic effects with MSG. Related reports suggested that the binding site of IMP close to human umami taste receptors expressing venus flytrap domain (VFTD) may strongly potentiate the umami taste intensity in insect taste systems, which was further confirmed with the equilibrium constant between AmGr10 and MSG changing from 1.77×10^{8} M^{-1} to 2.30×10^{9} M^{-1} in this developed biomimetic gustatory biosensor. Based on the similar detection strategies, another research proposed a new specific human olfactory

receptor (OR) found using a cyclic adenosine monophosphate (cAMP) response element (CRE)-reporter gene assay and successfully achieved high sensitivity real-time detection of fungal contamination in grain down to 1 fM and high selectivity toward other interference including 1-octanol, 1-octanal, 3-octanol, and 1-octene [12]. Notably, some mammalian receptors composed of two different proteins, such as heterodimeric class sweet receptor based on hTAS1R2 and hTAS1R3 can also integrate with nanovesicles and achieve the detection and discrimination of sweeteners based on similar detection strategies. Its estimated K values in real drink examples were all ~2.0×10^{-3} M, showing that the developed sweet biosensor have great potential application in detection and monitoring sweeteners in real samples [13]. However, the complex cellular signaling processes induced by olfactory and taste receptors in response to specific ligands have always been difficult to analyze. To accurately analyze the GPCR reaction process, one research work proposed an effective method based on coupling ion channels with receptors to avoid complex cellular signaling processes and a successful reduction of the measurement errors caused by instability of various cellular components [14]. This created an olfactory biosensor system in which nanovesicles expressing olfactory receptors were covalently fused to Kir6.2 channels, when specific ligands that altered the conformational structure of receptors would directly result in the influx of potassium ions to activate Kir6.2 channels. This nanovesicle-based bioelectronic tongue showed ultrahigh sensitivity on AB down to concentrations as low as 1 fM and a good stability to 8 days, which successfully offers an effective platform for GPCR reaction process analysis and an alternative to labor-intensive and time-consuming cell-based analysis and to widely sensory assessment in the food and beverage industry, while the similar detection strategies that achieved a different detection limit may be attributed to the affinity between receptors and ligands and immobilization strategies, such as π-stacking and charge-charge interaction through PDL function. According to the collected research, it can be found that the physical immobilization method may provide a more suitable and stable micro-environment for nanovesicles in response to specific ligands. Detergent micelles belong to amphiphilic phospholipid structures, which have been widely used for stable environment construction of recombinant GPCR, a bioelectronic nose based on detergent micelles as a recombinant environment for the nematode olfactory receptor, ODR-10 expressed in Escherichia coli (*E. coli*) [15]. This successfully achieved highly sensitive detection of diacetyl with a limit down to 10 fM and distinguished it from other structurally similar substances in different environments, providing a useful tool for a variety of industrial applications.

Since an increasing number of micro-biomolecules was discovered and used as the sensing element, artificial synthetic peptide derived from receptors or the binding proteins' specific binding sites can be synthesized as another substitution of protein due to its high yield and stability. Detection of salmonella infection is relatively difficult and meaningful. A bioelectronic nose was proposed based on CNT-FET functionalized with odorant binding protein (OBP)-derived peptide expressed in Drosophila for the rapid detection of salmonella contamination in ham [16]. The system successfully achieved the sensitive detection of 3-methyl-1-butanol with a detection limit down to 1 fM. The corresponding equilibrium constant was more than 10^{13}, which suggested high affinity between peptides and targets. Unlike previous studies that used chromatographic instruments and strain counts to evaluate seafood freshness, a bioelectronic nose was developed based on CNT-FET functionalized with a synthetic peptide horp61m for rapid and non-invasive detection of gas trimethylamine (TMA). In order to evaluate the effectiveness of the biological electronic nose more accurately, the study adopted the sensory evaluation and gas chromatography-

mass spectrometry (GC-MS) as a standard for comparison. The results showed that peptide-based olfactory biomimetic sensor can effectively simulate the human olfactory system, which was very significant for the validation of practical application value of constructing biomimetic biosensor system [17]. A bioelectronic nose was developed based on swCNT-FET combined with microfluidic system (μBN) and functionalized with synthetic peptide horp61m for highly sensitive and good selectivity on gaseous TMA detection. μBN as a device for precise control and manipulation of micro-scale fluids was applied and widely developed to simulate the transport process of odorant and tastant substances in the human body [18]. In particularly, the developed integrated system successfully achieved a high sensitivity of 10 ppt for gaseous TMA detection and distinguished gaseous TMA produced by real spoiled seafood (oysters) without any treatments from other types of spoiled food, showing great potential in real-time rapid field analysis and providing an effective direction for simulation of human olfactory and gustatory functions.

1.2. Other Types of Biosensors Based on Field Effect Properties

Electrolyte-insulator-semiconductor (EIS) sensors are another type of field effect device, which has been widely used in chemical and biological sensing applications due to its small size and simple fabrication method. Utilization of EIS sensors as a transducer element can prosperously achieve detection on the function expression of sensitive materials through monitoring changes in transducer capacitance in respond to specific ligands. The biomimetic biosensors was fabricated based on EIS functionalized with ODR-10 [19], and 0.5% Brij58 was used as the detergent to promote the expression of the nematode olfactory receptor in *E. coli*, providing an effective method for batch production of sensitive materials. The system has characteristics of miniaturization, stability, and easy access to sensitive materials, and it provides a potential development direction for portable olfactory or gustatory detection device construction.

LAPS are another type of field effect device with an EIS structure. As the transducer element, LAPS has excellent light-addressable ability, making it suitable for the signal capture of single cell and tissue in response to specific ligands, obviously except patch-clamp technology. In the development of biomimetic sensors, olfactory and gustatory epithelium were used as sensitive materials and LAPS as the detection device for the determination of specific ligand combinations through change of photocurrent acquisition [20][21]. A dual functional extracellular recording biosensor was developed based on taste bud cells cultured on the surface of LAPS functionalized with ATP-sensitive DNA aptamer to realize the simultaneous detection of the membrane potential and ATP release of single taste bud cells responding to specific bitter ligands [20]. Another interesting concept has been proposed based on electrolyte-semiconductor (ES) structures, especially the introduction of indium tin oxide (ITO) with good conductivity and transparency as a semiconductor, facilitating the sensing system more simply, at a lower-cost, and with sensitive detection in chemical and biosensing applications. The report developed a novel bioelectronic gustatory biosensor based on an ITO-ES structure equipped with *E. coli* expressing hT2R4 as a sensing element without any additional steps of protein extraction and cell/protein immobilization for successful bitter substances detection through monitoring of the variation of space charge capacitance [22].

2. Biomimetic Olfactory and Gustatory Biosensor Based on Electrode Array

2.1. Micro-Electrode Array

A microelectrode array (MEAs) system can record the electrophysiological signals of sensitive materials in vitro and in vivo [23]. Isolated cells or tissues can be cultured directly on the MEAs surface and the membrane potential will be changed due to membrane surface receptor combined with target ligands causing redistribution of charge, which can be detected by this system. The MEAs system possesses unique long-term monitoring electrophysiological characteristics, making it useful in many applications, such as high-throughput screening for medicine, detection of a combination of cell electrophysiological functions and morphology and so on. This part would introduce olfactory or gustatory biosensors based on MEAs recording system and different types of sensing elements, such as cells and issue, from two aspects of in vivo and in vitro culture.

When the olfactory and gustatory systems are stimulated by external odorants and tastants, a cascade of reactions will occur in the corresponding cell and transport to respective intermediate stations for integration and procession. Direct utilization of in vivo mammalian system is a very effective strategy for olfactory and gustatory detection. An olfactory biosensor in vivo was proposed based on an array of mitral/tufted cells (M/Ts) as sensing elements coupled with MEAs as transducer elements for odorant discrimination [24]. The results showed the in vivo bioelectronic nose can successfully achieve natural odor detection and discrimination. Although the work indicated that a small percentage of M/Ts can carry enough information to distinguish odors, the position of the microelectrode in the olfactory bulb was not clear and the specificity and repeatability of the sensor could not be fully guaranteed. A genetically modified method was used as an effective solution to solve it. They developed murine M72 olfactory sensory neurons expressed with the green fluorescent protein through genetic modification for the relatively high precision location of multiple microelectrodes, which achieved high sensitivity and good stability and selectivity in long-term trinitrotoluene (TNT) detection [25]. This demonstrated that specific responses to odorants can be regulated by adjusting the expression of specific ORs. In the latest study, researchers genetically engineered olfactory epithelium to overexpress specific OR genes based on serotype 9 recombinant adenovirus-associated virus (AAV) for the detection of specific odorants [26].

In addition, an in vivo gustatory system can also achieve effective detection for the high-quality detection of bitter compounds in food and medicine. A microelectrode array was coupled with a rat's gustatory cortex for the selective and specific detection of a bitter compound down to 0.1 μM, exceeding both the in vitro conventional bioelectronic tongue and nose [27]. However, motion artifacts would generate when local field potentials were detected in the wake state of before and after bitter stimulation. Their work detected local field potentials in the rat's anesthesia states and achieved high sensitivity detection and high specificity screening of bitter compounds [28].

Utilization of high sensitivity of the mammalian olfactory system directly can achieve supernal value in multi-type odor discrimination and can avoid the complex means of extracting cell tissues and proteins. However, the development of biomimetic biosensing systems based on in vivo animals requires researchers with excellent

experimental skills and experience, laboratories with sophisticated equipment, as well as the difficult to solve electrode stability, which seriously hinders its extensive development and application. An effective alternative on balance to resolve this problem and simultaneously implement spatiotemporal analysis is to isolate the primary olfactory and gustatory epithelium and plate it on the surface of planar integrated MEA system. The extracted primary tissues or cells contain their biological characteristics without completely changing and simulating the process of odorant and tastant sensing in vivo to reflect the state of the body in a certain extent. What's more, spatiotemporal methods involving time-domain and frequency-domain analysis were proposed and developed as the auxiliary analysis solution in such a sensing system to analyze the correlation between different channels and furtherly verify detection performance of the MEA system as the transducer element. In earlier work, [21] successfully obtained the olfactory code information of the intact olfactory epithelium induced by stimulating different ligands. Another bioelectronic taste sensor for natural and artificial sweeteners detection was developed based on the intact taste epithelium extracted and cultivated on the MEA surface [29]. In order to further visualize the response patterns of taste tissues to different sweet tastes, this utilized another machine learning, k-means algorithm as an auxiliary analysis tool to differentiate the expression of different sweet taste responses. The k-means algorithm can realize the classification of clusters with different characteristics, which is very suitable for the classification of continuous data sets with small dimensions and values due to its advantages of easy implementation, simple principle, and fast clustering speed. The peak ordering diagram of all sweetener induced signals was analyzed through the k-means algorithm and the corresponding typical waveform was clustered to each stimulus, successfully realizing the differential response of taste tissues to different sweeteners. In addition, the algorithm analysis informed that different sweeteners had an obvious and different response on the detection system and the values of artificial sweeteners showed a low concentration that was bigger than that of natural sugars with a higher concentration. What's more, the high specificity and detection effectiveness of this detection system was verified by its long-term signal recording results, dose-dependent increase curves obtained by the treatment with different concentrations, and mixture of the experimental results of two sweeteners with a similar chemical functional structure. The olfactory receptors contain metal binding sites and their conformational changes can be caused by binding to metals, such as colloidal nano-zinc isolated from blood, which might greatly improve the sensitivity of bioelectronics noses. One work confirmed the enhancer role of zinc nanoparticles and obtained the higher quality detection of electrophysiological signals produced by the olfactory epitheliums in response to different odors [30]. However, the limitation in stability and functional cellular localization of primary cell tissue extraction from the host animal always impedes such a biosensor's development. To challenge these problems, primary cells as sensitive materials have widely developed and applied. One work developed the bioelectronic tongue based on the MEA system and the primary taste cells obtained from fungiform papillae in the frontal tongue of female adult Sprague Dawley rats for effective noninvasive electrophysiological recording of taste receptor cells after and before acid stimulation. For exactly confirming the function of ASICs and PKDL channels on the sour taste coding mechanism, they still utilized the patch clamp system for the self-construction of Hodgkin–Huxley type mathematical model of taste receptor cells with acid-sensing in the whole cell [31]. However, the coupling degree of cells to the electrode surface greatly affects the efficiency of signal transduction in the biosensor system. Reducing the distance between the cells and the electrode surface to reduce the sealing resistance is an alternative effective solution. Specific hybridization between short complementary ssDNA strands was utilized to achieve controllable

orientation of olfactory cell immobilization sites on the sensor surface, which significantly improved cell signal transduction efficiency [32]. In addition to olfactory and gustatory related cells, olfactory and gustatory receptors can also be expressed in other systems, such as the gastrointestinal and respiratory tracts of mammals, the male reproductive system, and the brain and heart, providing a variety of possibilities olfactory and gustatory biomimetic biosensor construction. In recent research, primary cardiomyocytes endogenously expressing bitter taste receptors (Tas2r) and umami taste receptors (Tas1r1 and Tas1r3) were utilized as sensitive materials combined with the MEA system for the detection of bitter and umami substances [33].

2.2. Electric Cell-Substrate Impedance Sensing

Electric Cell-substrate Impedance Sensing (ECIS) can simultaneously measure the resistance changes and membrane capacitance changes of multiple groups of cells, as well as cultured cells and basement membranes. Activation of specific receptors on the cell surface will cause changes in cell morphology. Through the measurement of impedance spectrum, the ECIS technique can obtain and analyze the relationship between cell morphological changes and stimulation, extracellular matrix and cell proliferation in real time, providing abundant data for olfactory and taste signal transduction and coding and decoding processing. Except for cells specialized in endogenous expression gustatory receptor, several special cells with other functions also express some specific taste receptor, such as male mouse germ cells [34][35].

3. Biomimetic Olfactory and Gustatory Biosensor Based on Electrochemical Detection Method

The electrochemical analysis method has been widely used in application of biomimetic olfactory and gustatory biosensors due to its simple instrument, high sensitivity, high detection efficiency, and accuracy. The charge changes between the electronic conductor and the ionic conductor were detected to realize the qualitative and quantitative analysis of the substances according to the electrochemical properties of the substances in the solution and their change law. The three electrode systems consist of the working electrode (WE), reference electrode (RE), and auxiliary electrode (AE). The working electrodes were modified with packaged sensitive materials, the response of specific substance simulation was detected by monitoring the changes in its morphology, conformation, and electrical conductivity.

Glassy carbon electrodes (GCE) are commonly used as working electrodes in biomimetic biosensor construction due to its excellent characteristics of good electrical conductivity, high chemical stability, and small thermal expansion coefficient [36][37][38]. The combination of receptors and ligand has gradually become a popular issue in the bio-sensing field. The activation constant (*Ka*) was usually used to evaluate the affinity of receptors with ligands. The smaller the *Ka* value is, the higher the signal output efficiency produced by the ligand-receptor interaction. In addition, molecular docking can search the best matching pattern between molecules and ligands through geometric matching and energy matching mutual recognition, providing an effective analysis platform for exploring the combination pattern and affinity of olfactory and gustatory receptors and ligands. The calculated *Ka* through the sensing kinetics method showed that GMP have the highest affinity combined with hT1R1. Additionally,

the analysis results of molecular docking model showed that the tight binding of TIR1 to the ligands depends on the formation of hydrogen bonds between its two rings (Asp147 and Ala170) and the amino/imino groups of the ligands. Notably, the hydrogen bond also formed between the phosphate group in GMP and IMP and the amino group at Asn69 to render the VFT active bag more tightly with ligands, which explained the reason why IMP and GMP can perform as enhancers for promoting umami perception [39]. What's more, the team also utilized the double-layer gold nanoparticle biosensor and combined with sensory evaluation as a control to realize the detection of five umami peptides produced from *T. flavidus*. The quantum chemistry and molecular docking results demonstrated that aspartic acid was the active site in the umami peptides. The meaningful work revealed the umami mechanism of peptides and provided a reasonable tool for rapid screening of umami peptides in food. Another interesting research, directly utilized VFT as the sensitive material element and designed a dual-signal amplifying sensor via CS-modified SWCNTs and PB-modified AuNPs-T1R1-VFT, which achieved highly sensitive detection on umami substances with a limit ranging from 10 fM to 100 fM and good selectivity toward other interference [40]. However, receptor purification steps are unavoidable in exploring the mechanism of ligand-receptor interaction of receptors in vitro. When the receptor was extracted from the cell membrane, the docking interaction with the membrane was lost. The conformational change caused by the ligand cannot fully achieve its conformational morphological change on the membrane. To challenge this problem, one research utilized a receptor protein embedding membrane as the sensitive material element to maintain its microenvironment and conformational morphology in respond to ligands. Its calculated Ka down to 10^{-15} showed capsaicine has the highest affinity with hTRPV1. Molecular docking simulation analysis demonstrated that Glu570 in the active pocket of hTRPV1 has a vital function in identifying spicy substances [41]. Furthermore, it also indicated that the combination of spicy substances and receptors can be promoted by spatial complementarity, hydrophobicity, hydrogen bonding and π-π interactions together. The extracted primary tissues can reflect the real state in vivo to some extent, which provides an effective tool for studying the interaction mechanism between receptors and ligands in vitro. However, it may be difficult for the cell/tissue with a certain weight to functionalize the glassy carbon electrode. To increase the amount of taste bud tissue attached to the biosensor surface and to prevent the tissue from flowing or falling off, a sandwich-type format was designed, which is composed of aldehyde starch gel solution and sodium alginate solution in proportion to form an upper and lower interlayer, and the taste tissue was successfully immobilized on the surface of the working electrode as a core.

Recently, taste buds were extracted from different regions of rabbit tongue tissue to construct a biomimetic biosensor, and its auxiliary analysis indicator *Ka* showed that the umami receptors in different tongue regions have different perceptions of MSG and IMP [42]. Another biosensor based on primary taste bud tissue and GCE can successfully measure capsaicin with a calculated activation constant reaching 2.0218×10^{-18} M and also analgesic compounds up to the attomole-level. Its related competition experiments and quantitatively the antagonistic strengths and inhibition constants investigation showed that capsaicin, AMG 517, lurelin B and tetrahydropalmatine are competitive allosteric regulatory ligands of capsaicin, while aconitine and anandamide are non-competitive and competitive mixed allosteric regulatory ligands. Results of these studies showed that such primary taste bud biosensors can precisely mimic the ligand-receptor interaction environment in biological taste systems to quantitatively illustrate the dynamic characteristics of cell or tissue receptor interaction with its ligand, the effect of

cell signal cascade, and the amounts of receptors and signal transmission pathways. However, GCE is easily polluted by some organic and metal compounds. Screen printing electrode also has been widely used in environmental security detection, auxiliary clinical diagnosis, and food composition detection [43][44]. It is a disposable electrode with advantages of being low-cost, maintenance-free, and multifunctional, with high repeatability between electrodes. Because of its high electrochemical performance, increasing numbers of studies have utilized it as the second transducer and made it functionalized with different types of receptors or cells to build up biomimetic biosensors for the detection and discrimination of different ligands. A novel taste biosensor was developed based on a screen printed carbon electrode (SPCE) functionalized with human recombinant OBP II a for detection of fat taste substances with a different length of alkyl chain [45]. For the detection and discrimination of complicated mixture components, two types of biological tongues were developed based on NCI-H716 from human enteroendocrine and STC-1 cells from mouse enteroendocrine, respectively [43]. The results demonstrated that STC-1 cell-based SPCE biosensor was highly correlated with quinine concentration and its taste perception ability was much better than that of the NCI-H716 cell sensor.

The interdigitated electrode (IDE), another alternative type of electrode, is a finger-shaped or comb-shaped electrode with periodic patterns in the surface obtained by electrochemical processing. As the core component of electrical signal transmission, it has been widely utilized in various applications, such as biomedical, environmental monitoring, and food safety due to its high collection rate, high signal-to-noise ratio and feedback effect similar to amplification. When the receptor immobilized on the IDE electrode surface binds to the corresponding ligand, its conformational change might increase the contact area between the mobile ion and the electrode, thereby making the electron transfer process easier, which has a great influence on the dielectric and conductive properties of the staggered electrodes. An olfactory biosensor was developed using IDE as transducer element and OBPs from an oriental fruit fly as sensitive material for the detection of insect semiochemicals at low concentrations through surface impedance variation monitoring [46]. The molecular docking model showed that the three-dimensional structure of BdorOBP2 has a central hydrophobic pocket, exposing a variety of chemical groups connected with pheromone chemicals through hydrophobic, polar, electrostatic, and π-stacking interactions to form stable conformational changes. Olfactory receptor-derived peptides (ORPs) were chemically immobilized onto single-walled carbon nanotubes (SWCNTs) on interdigitated electrodes based on Steglich esterification reaction (SER) and native chemical ligation (NCL), which achieved high responsiveness and stability to TMA with a detection of limit down to 0.01 ppt [47]. This novel ORP sensor has potential applications for environmental safety, food quality control, and healthcare.

4. Olfactory and Gustatory Detection Strategies Based on Optical Measurement

4.1. Ca^{2+} Imaging

Intracellular calcium ions have temporal and spatial properties in neurocyte communication. In the resting state, the calcium ion concentration in most neuronal cells is approximately 50 to 100 nM. Once the cell is excited, intracellular calcium ion concentrations will instantaneously rise to 10 to 100 times. Therefore, detection of the

variation of intracellular calcium ion concentration could be an effective way to monitor the cell mobility through special fluorescent dye or a calcium ion indicator. The influx of extracellular calcium ions includes voltage-gated calcium ion channels, ionic glutamine receptors, and nicotinic cholinergic receptors. The olfactory or taste biosensors introduced here were mainly based on calcium influx of ligand-gated channels.

Calcium ion indicators include chemical calcium ion indicators and genetically encoded calcium ion indicators. Fluo-4 AM was used as a chemical calcium ion indicator in an olfactory biosensor in which HEK-293 cells expressing hORs was cultured on the porous membrane to construct the microfluidic device for odor detection [48]. As receptors on the membrane surface bind to specific odor molecules, the calcium concentration greatly increased from the opening of the calcium ion channel and the release of endoplasmic reticulum into the cytoplasm. Then, the intracellular Fluo 4 would combine with calcium ions and emit green fluorescence under a 488 nm excitation light, which can be captured for odor information analysis. A sperm-cell gustatory biosensor was developed based on this Ca^{2+} imaging principle and innovative combination with flow cytometry to capture the green fluorescence for successfully bitter substance detection [49].

A series of cellular cascades and signaling codes constitute the overall response to external stimuli. To simulate the signal transduction environment of cells in vivo, the idea of a co-culture system was proposed. It has been widely used in many studies, such as oncology [50] and tissue engineering [51] to clarify intrinsic information transmission and material transportation in biological tissue [51][52]. A co-culture in vitro system was constructed based on gustatory cells and neurons to monitor cellular signaling and substance exchange and to better understand the mechanisms of taste perception [51]. This system made gustatory cells and neurons incubating with synthesized DNA-lipid conjugates, which was composed of single-stranded oligonucleotides, polyethylene glycol, and phospholipids, in order to achieve the close connection between the two kinds of cells. When the specific bitter molecules stimulate a co-culture in vitro system, continuous calcium influx responses between gustatory cells and neurons can be monitored by Fluo 4-AM. However, all neurons are also stimulated by bitter substances, which may cause more calcium ion influx and increase the complexity of data analysis of this system. For improvement of selectivity of calcium influx [51], agarose gel was used to cover the surface of the co-culture in vitro system to reduce the influx of calcium ions in nerve cells stimulated by taste substances. Results showed the feasibility of this approach, which can be used to improve the signal-selective cell response of multiple types of cells in a co-culture in vitro system [52].

4.2. SPR Measurement

Surface plasmon resonance (SPR) is a plasma wave-based optical measurement method, which has the excellent characteristics of real-time, label-free, and high-throughput monitoring for target detection. The signal is proportional to the refractive index (RI) of the medium near the gold metal film, indicating that any changes in the medium environment at a distance of 300 nm from the surface can be captured, which can perform an effective highly-sensitive transducer element for biomimetic olfactory and gustatory biosensor construction. When sensitive materials combine with specific ligands on the SPR surface, the changes of properties of the surface medium or the amount of attachment would cause resonance angle variation. A biomimetic olfactory biosensor was

functionalized with liposome containing hOR3A1 as the sensitive material and based on SPR as the transducer element for the detection of helional [53]. Notably, in addition to functional peptides from olfactory binding proteins or receptor protein-binding domains, peptide sequences can also be obtained from antibody sequences of target receptors in virtue of molecular docking tools. Three TNT-binding peptide sequences were successfully obtained from the CDR1 heavy chain of anti-TNT antibody through antigen docking simulation. The function of the peptide was evaluated by SPR [54]. SWCNT was used as the transducer substrate to enlarge specific surface area through π stack functionalization for significant improvement of sensitivity and selectivity with the detection limit changing from 3.4 ppm to 772 ppb on 2,4,6-trinitrotoluene (TNT) recognition [55]. The system showed excellent performance on TNT explosives with enhanced sensitivity and excellent selectivity and long-term stability, providing an effective platform for environmental safety applications.

High-throughput analysis has always been an important work of mass component detection in life science research. Surface plasmon resonance imaging (SPRi) offered a very promising multi-functional high-throughput research platform for complex odorant and tastant recognition. SPRi can be developed based on microarray device, which has all the advantages of SPR and can successfully realize real-time monitoring of various molecular action processes. A biomimetic olfactory biosensor was successfully developed based on SPRi as the transducer element for β-ionone and hexanal detection [56]. The SPRi combined with OBP derived from OBP3 by modifying different amino acid residues, and the VOC-induced conformational changes of OBPs were detected based on the SPRi signals amplification. Different receptor materials were functionalized in the microarray, indicating that the obtained olfactory/taste encoding information is nonlinear, abundant, and complex compared with that composed of pure receptors. With the continuous maturation of the extraction technology of various olfactory/taste receptors, olfactory binding proteins and their derived peptides, and the development of the combined analysis ideas with various popular algorithms, development of such sensors will become mainstream in biomimetic olfactory and gustatory biosensing platform construction. The SPRi microarray detection greatly provides a potential platform for mixture of odorant and tastant detection and furtherly promote development of biomimetic olfactory and gustatory biosensors to replace mammals smell/taste function in real life.

4.3. Other Types of Optical Measurement Methods

The luciferase reporter gene system is another efficient fluorescence measurement method which uses luciferin as a substrate to detect firefly luciferase activity. A bioluminescence system consists of luciferin and luciferase, which is an effective detection method for detecting the interactions between transcription factors and DNA in the promoter region of the target gene. It was used to develop a dataset for other researchers to analyze olfactory coding information [57]. The in-vivo cultured cells could express the same specific OR and respond to specific odor, which can be monitored through SynaptopHluorin imaging [58].

In addition, interferometer imaging can realize the combination of one or more light waves and produce interference patterns. The interference patterns produced by different light wave combinations produced by different types of receptor-ligand binding on the working elements may be different. The contained olfactory and taste coding information may be very abundant and worth analyzing. Aryballe Corporation developed a novel

portable olfactory biosensor based on Michelson interferometer functionalized with different types of functional peptides and equipped with machine learning as the fast analysis method, which has been commercialized and widely used in the automotive industry [59].

5. Biomimetic Olfactory and Gustatory Biosensor Based on Mass Sensitive Device

Quartz crystal microbalances (QCM) is a typical quality sensitive sensor based on the piezoelectric effect of quartz crystal. With its high sensitivity, good chemical stability, and quantitative detection of high precision, it has been widely applied in many fields of biosensor technology, such as detection of the interactions between biological macromolecules, gaseous material analysis, etc. The interactions between the biomolecule and the target ligand can be monitored by detecting the output of the variation frequency. The excellent characteristic of easy synthesis, easy modification, high yield, and stability of peptides make it widely developed as a sensitive material for various biomimetic biosensors. Molecular docking technology can not only be used to explore the binding mode and affinity of receptor ligands but also to provide a suitable platform for the analysis and screening of new peptides through introducing predictive models to optimize trial-and-error analysis protocols and to minimize experimental issues such as non-specific identification. It was found that the amino acids in the composition of the peptide determine the affinity of the peptide to various olfactory and gustatory molecules, such as effective aspartic acid, is the active site of the umami peptide to capture ligands. Another research performed a virtual screening analysis to evaluate the affinity binding properties of 5 different peptides (cysteinylglycine, glutathione, Cys-Ile-His-Asn-Pro, Cys-Ile-Gln-Pro-Val, Cys-Arg-Gln-Val-Phe) with 14 volatile compounds simultaneously compared with the detection results of the biosensor constructed based on QCM. The experimental results showed that virtual screening can predict the sensing ability of pentapeptides with an accuracy rate of 93% [60]. In a follow-up work, the team used a semi-combinatorial virtual approach to extract a subset of 120 tripeptides from the complete tripeptide library (8000 elements) that were used as scaffolds to generate 7912 tetrapeptide combinatorial libraries. Then, according to the experimental procedure, five tetrapeptides (IHRI, KSDS, LGFD, TGKF and WHVS) were selected via virtual affinity and cross-reactivity. Together with PCA analysis, the results demonstrated that the final running virtual screening equipped with experimental conditions required to implement the biosensor closely matched with the real experimental data obtained from the biosensor array [6]. Therefore, the virtual screening method provides a new potential tool for peptide profile expansion and offers a fast, low-cost, and non-invasive procedure for peptide-based gas biosensors to distinguish different targets.

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