Ping Wang · Qingjun Liu
Chunsheng Wu · K. Jimmy Hsia *Editors*

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Preface

The integration of cells/molecules with micro/nano devices for the development of novel sensors with unique functions has attracted intensive interest and substantial research efforts in recent decades. Exciting progress has been achieved due to the combination of micro/nano fabrication technologies with biotechnologies, which introduced new concepts and scientific paradigms to this area. Fast advancements in micro/nano structured devices are providing unprecedented opportunities for them to couple with functional cells/molecules for the development of next generation of cell and molecular sensors. Micro/nano cell and molecular sensors have become increasingly important and found wide applications in a range of areas.

Recently, an impressive number of inventive designs and technological advances for micro/nano cell and molecular-based sensing have been made that greatly contribute to their potential practical applications. These types of micro/nano cell- and molecular-based sensors combine cells/molecules with micro/nano transducers to produce a biosensor and promise to provide a simple, accurate and inexpensive platform for various applications. However, the underlying mechanisms of micro/nano cell- and molecular-based sensing are still not fully clarified, which has become one of the main challenges for their further development and improvement. Considerable basic research on the cellular and molecular sensing mechanisms as well as the coupling of cells and molecules with micro/nano devices is still highly essential and favorable for the sake of practical applications and possible commercialization.

This book summarizes the development and implementation of micro/nano cell and molecular-based sensors. The organization of this book is based on the basic concepts and potential applications of micro/nano cell- and molecular-based sensors. In addition, this book attempts to introduce the key aspects and the future perspectives of micro/nano cell and molecular sensors, especially, the cell and molecular-based biosensors with electric cell–substrate impedance sensing (ECIS), microelectrode arrays (MEAs), light-addressable potentiometric sensor (LAPS), and field-effect transistors (FETs). Throughout the book, in every chapter, the most important and recent developments relevant to the subject matter are introduced.

This book focuses on cell- and molecular-based sensors using micro/nano devices as transducers. The definition, characteristics, type, and application of micro/nano cell and molecular sensors are introduced in this book. For living cell analysis, common micro/nano cell sensors are discussed to monitor the intra- and extracellular physiological signals, including the principle, design and fabrication, application. The two main cell sensors, ECIS- and MEA-based sensors are detailed on their cell impedance and potential study, respectively. For neurons study, neural network-based sensors are focused, including the formation of neural networks on solid surface and their chemical sensing. The book is also devoted to micro/nano molecular sensors and their applications. For molecular analysis, a label-free DNA field-effect device is presented for for DNA sensing and application. Micro/nano electrochemical sensors are described for ion sensing and measurement, which is applied in field environment and food analysis. Moreover, the basic structures and properties of micro/nano material are also introduced for recent development and application in biomedicine and food analysis. At the end of this book, the future trends of micro/nano cell and molecular sensors is prospected to establish the micro/nano electromechanical cell/molecular system and Intelligent biosystem for biomimetic devices, health care, and rehabilitation.

The topics covered by this book provide a comprehensive summary of the current state of micro/nano cell and molecular sensors as well as their future development trends, which would be of great interest to the interdisciplinary community active in this area. This book is also suitable to be a comprehensive perspective for the scientists and engineers in a wide range of areas. In addition, this book could inspire and attract more and more researchers and scientists, especially the young scientists, to this area to further advance the development of micro/nano cell and molecular sensors and broaden their application fields.

Hangzhou, Ping Wang
June 2015

Contents

Chapter 1
Introduction

Ping Wang, Qingjun Liu, Chunsheng Wu and K. Jimmy Hsia

1.1 What Are Smell and Taste Sensors

Biological olfactory and taste systems are natural chemical sensing systems that are crucial for almost all the creatures to sensing the chemical signals for various purposes such as survival, feeding, and breeding [1–7]. After long-term evolution, biological chemical sensing systems are able to detect and discriminate a large amount of chemical substances in complex environments with extreme high performances that currently cannot be matched by artificial devices. Functional components of olfactory and taste systems include olfactory and taste receptors/cells/tissues, which can be considered as natural olfactory and taste sensors because they can detect and transduce chemical signals presented by various odorants and tastants into biological signals such as neuronal action potential changes and releasing of neurotransmitters [8–10]. Inspired by the biological mechanisms of natural chemical sensing systems, different kinds of bioinspired smell and taste sensors have been developed by the combination of biological functional components for chemical sensing with various transducers [11–15]. Due to the unconventional utilization of biological mechanisms and functional components, bioinspired smell and taste sensors are characterized with high performances for chemical sensing and biochemical analysis, which exhibit high sensitivity, rapid response, and excellent selectivity.

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With the advances in biological mechanisms of olfactory and taste sensation, bioinspired smell and taste sensors have recently achieved significant progress, which have shown promising prospects and potential applications in many fields from basic research to industry. For example, the discovery of the gene family encoding vertebrate olfactory receptors (ORs) not only advances the basic research on olfactory mechanisms, but also promotes the development of bioinspired smell sensors [2]. As a result, various bioinspired smell sensors have been reported, in which biological functional components, such as olfactory tissues, olfactory sensory neurons, and olfactory receptors, have been utilized as sensitive elements for the detection of various chemical compounds [11, 15]. In addition, a non-linear neuronal network model, that is K serial models, was proposed based on the firing properties of neurons and neuronal networks at different anatomical levels of olfactory systems [16]. This model is based on the theory of nerve cell groups, which assumed that nerve cell groups formed by a similar set of cells with similar functions and features can be used as building blocks of the nervous system. On the other hand, the progress in the taste sensing mechanisms also promoted the development of bioinspired taste sensors. The discovery of important role of taste cell membrane in taste sensing [17] inspired the researchers to employ lipid-polymeric membranes that usually consist of differently charged lipids for capturing various tastant molecules [18–20].

The conventional standard methods for gas or liquid-phase detection and analysis usually rely on the utilization of precision laboratory artificial instrument like gas chromatography–mass spectrometry (GC-MS) [21]. These methods provide an accurate approach for the analysis of type and concentration of the single component in a mixture of substances. However, these methods are time-consuming, labor intensive, and expensive. The bioinspired smell and taste sensors provide novel solutions to overcome these drawbacks. In 1982, a novel gas sensing system, electronic nose (e-nose), was proposed by imitating the sensing mechanisms of biological olfactory systems [22]. In 1990, the first liquid analytical instruments based on non-specific taste sensor array—electronic tongue (e-tongue)—was reported [23]. An e-tongue using ion selective electrode array and pattern recognition algorithm was developed, which consists of a cross-sensitive chemical sensor array and pattern recognition algorithm and can be used to detect, analyze, and identify the complex chemical compositions [24]. After nearly 30 years of development, the e-noses and e-tongues have been applied in a large number of fields including food processing, environmental monitoring, public safety, and medical diagnostics.

However, limited by the development of sensor technology, the sensitivity, selectivity, and response speed of current e-noses and e-tongues are still far from that of biological olfaction and taste systems. To overcome the development bottleneck of traditional e-noses and e-tongues, in the in recent years, the concept of bioinspired smell and taste sensors have been increasing recognized as a novel approach. Figure 1.1 shows the differences in chemical sensing process by biological noses and tongues or electronic noses and tongues, which shows how the chemical signals of Brasil coffee can be detected and recognized. Unlike the traditional e-nose and e-tongues, bioinspired smell and taste sensors mimic the biological chemical sensing mechanisms and could achieve a similar performance to

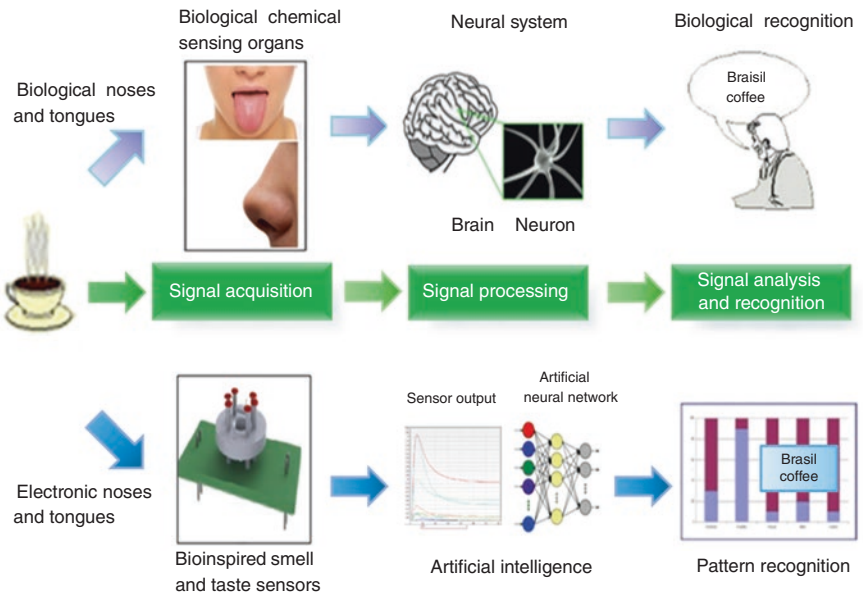


Fig. 1.1 Schematic diagram shows the comparison of biological noses and tongues with electronic noses and tongues for chemical sensing

biological olfactory and taste systems by the using of biological functional components for chemical sensing, which show prominent advantages such as high sensitivity, low detection limit, and excellent selectivity. Hence, a more broad application prospects could be expected.

1.2 Characteristics of Smell and Taste Sensors

Bioinspired smell and taste sensors consist of two main components: one is the biological functional components utilized as sensitive elements that can interact with target molecules and ions and generate the specific responses [11–15]. The other one is the physicochemical detector served as the transducer which can convert the responsive signals generated by the sensitive elements into physical signals that are easier to handle and analyze, such as electrical signals. As shown in Fig. 1.2, the composition of a complete detection system include the bioinspired sensors and the electronics and instrumentation for signal processing and display, which enable the display of detected signals in a more friendly manner. Due to the incorporation of biological functional components for chemical sensing, the biomimetic systems partly inherit the unique advantages of biological chemical sensing system such as rapid response, high sensitivity, and excellent selectivity.

At present, biological functional components used for the development of bioinspired smell and taste sensors include olfactory/taste tissues and cells,

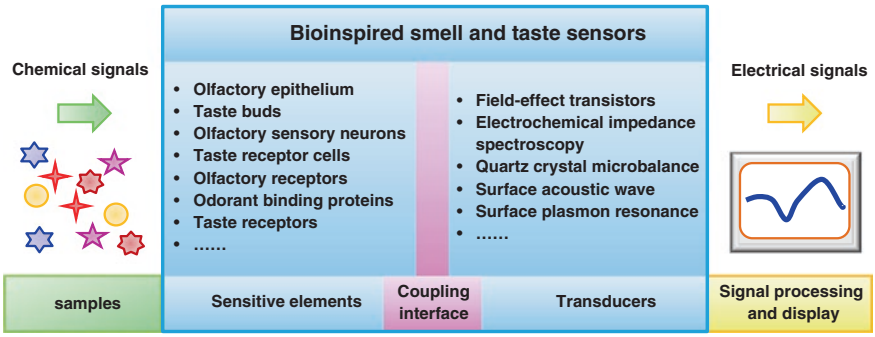


Fig. 1.2 Schematic diagram of composition and mechanisms of bioinspired smell and taste sensors for chemical sensing

olfactory/taste receptors and enzymes that can bind to the specific odorant or tastant molecules. Olfactory/taste-related proteins, such as the olfactory/taste receptor protein, and olfactory binding protein (olfactory binding proteins, OBPs), have been widely applied for the development of bioinspired smell and taste sensors due to their unique capability of binding to the specific target molecules [11]. Compared with tissues and cells, olfactory/taste receptor proteins have advantages of more easily storage for a long term, better stability, and activity, which contribute to the miniaturization and portability of bioinspired smell and taste sensors. On the other hand, although bioinspired taste sensors that utilize taste receptors as sensitive elements have achieved great progress, the current research is still at the cellular level, in which primary taste receptor cells or cell lines transfected with taste receptor genes are employed as sensitive elements for chemical sensing. Purification technology still cannot isolate purified taste receptors, which has greatly restricted the application of taste receptor proteins in bioinspired taste sensors. Bioinspired smell and taste sensors can be divided into two categories based on the detection mechanisms of different secondary sensors used transducers, which are electrical/optical detection and mass detection, respectively [11–15]. For electrical/optical detection class, conformational changes originated from the specific interactions between receptors and ligands cause the changes in the electrical/optical parameters, which can be detected by the various electrical/optical detection transducers such as field-effect transistors (FETs), microelectrode array (MEA), electrochemical impedance spectroscopy (EIS), and surface plasmon resonance (SPR). For mass detection class, the specific binding of receptors and ligands generate the mass changes that can be detected by the mass-sensitive devices such as quartz crystal microbalance (QCM) devices and surface acoustic wave (SAW) devices. The key issues for the development of bioinspired smell and taste sensors include (1) the preparation of functional biological components that can be used as sensitive elements for chemical sensing and (2) the effective coupling of sensitive elements with transducers. Based on olfactory receptors in the development of bionic type smell/taste sensor, key issues include the effective coupling preparation and olfactory receptor protein functional olfactory receptor

proteins and secondary sensors fixed. These issues have direct influence on the sensitivity, specificity, and stability of bioinspired smell and taste sensors.

1.3 Types of Smell and Taste Sensors

1.3.1 Electronic Nose and Electronic Tongue

Odorant commonly contains a series of functional chemical groups and some of them serve as ligands to combine with the specific receptors. The interactions between the odorant molecules and olfactory receptors exert combinatorial effects which are specific in the epithelium. Therefore, biological olfactory system has high sensitivity and specificity to perceive and discriminate a large number of odors in the environment. For great application potential in wide fields ranging from environmental monitoring to medical diagnosis, researchers have implemented relevant investigations and designed some electronic noses, depending on absorbability or catalysis properties of sensitive materials for specific odors. However, the sensitive materials seldom performed so perfectly as the biological olfaction which owns intact structure and function of cell population in the olfactory system with the native information coding.

1.3.2 Olfactory and Taste Cell-Based Biosensors

Cell-based biosensors, which treat living cells as sensing elements, can detect the functional information of biologically active analytes. Therefore, utilizing olfactory neurons and taste cells as sensitive materials to develop bioelectronic nose and bioelectronic tongue is one of independent trends concerning the research and development of electronic nose and electronic tongue, which makes use of biomolecular function units to develop highly sensitive sensors. Some experiments, such as insect antenna and human embryonic kidney-293 cells-based biosensors, have been tried and obtained high specificity and sensitivity to drugs or odors. However, the tissue or cells were not olfactory neurons or taste cells, and the parameters detected by those sensors were also not the action potentials of the cells. Therefore, a satisfactory bioelectronic nose or bioelectronic tongue should be a hybrid system of olfactory or taste cells and extracellular potential detection transducers.

1.3.3 Olfactory and Taste Epithelium-Based Biosensors

In intact epithelium, it is possible to estimate the electrochemical potential by keeping the neuronal membrane and environment intact after the epithelium surgically removed. Whereas cells were maintained in their native environment, the acute

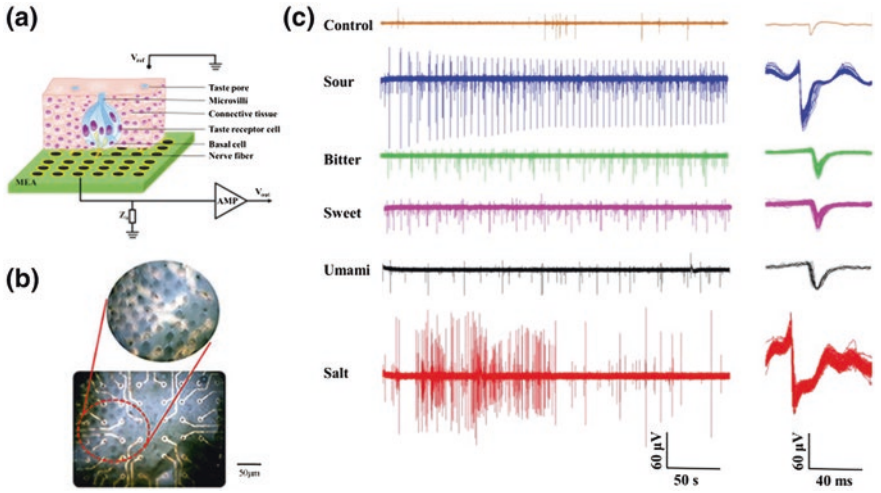


Fig. 1.3 A novel bioinspired taste sensor based on MEA chips. **a** Schematic plot of the bioinspired taste sensor composited by MEA and tongue epithelium accompanied with taste buds; **b** Microscope photograph of a MEA chip attached by tongue epithelial cells; **c** Extracellular potential of tongue epithelial cells stimulated by distinct tastants. (Reproduced with permission from Ref. [25]. Copyright 2013 Elsevier)

prepared intact epithelium had several advantages to the organotypic function over isolated olfactory receptor neurons or taste cells culture for bioelectronic nose and bioelectronic tongue: (a) the natural states of the neuronal populations of olfactory or taste receptor cells were well preserved. (b) The functional receptor unit of cilia on each 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 in the olfactory system. (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). Figure 1.3 shows the schematics of a novel bioinspired gustation sensor based on MEA chips and the detected signals in responses to different taste stimuli [25].

1.3.4 Insect Antenna and Binding Proteins Smell Sensors

In insects, the first event in odors detection is the capture of the molecules by some extracellular proteins and membrane-bound olfactory receptors. One kind of the major peripheral olfactory proteins in the reception of odorants is OBPs. The proteins involved as ligand selectors and transporters for triggering the olfactory signal transduction by binding the small hydrophobic molecules to enhance their aqueous solubility and transport them to specific olfactory receptors. As selective peripheral

signal filters to the actual receptor proteins, OBPs could be used as promising sense materials for a new generation of olfactory sensors with their increasing clear sensing properties. OBPs are robust enough to stand up to wide ranges of pH and temperatures for substantial mistreatments, without denaturing and losing their binding properties. In addition, OBPs are easier to be isolated and purified in the process of production compared with membrane protein of olfactory receptors. All of these will greatly enhance the practicability of those materials using in sensors.

1.3.5 Smell and Taste Receptor-Based Biosensors

The most fundamental elements are smell and taste receptors in the cilia of smell and taste sensory neurons, which contribute greatly to the high-performance smell and taste system. The excellent properties of smell and taste receptors are generally recognized in the development of biomimetic smell and taste receptors-based biosensors. Over the past two decades, much work has been done in developing smell and taste receptors based biosensors due to their promising potential in many applications. The production of functional receptors is one of the most crucial factors in the development of smell and taste receptors-based biosensors, which should maintain their natural structures and native functions to recognize their natural ligands, low production costs, and ease of storage and long-shelf life.

1.3.6 Biomimetic Membrane-Based Taste Biosensors

This kind of taste biosensors is equipped with multichannel electrodes using a lipid/polymer membrane for the transducer. The sensor is considered to be an electronic tongue with global selectivity, which is defined as the decomposition of the characteristics of a chemical substance into those of each type of taste and their quantification, rather than the discrimination of individual chemical substances, by mimicking the human tongue, on which the taste of foods is decomposed into each type of taste by each taste receptor. The taste sensor is commercialized taste sensing systems SA 402B and TS-5000Z, which are the world's first commercialized electronic tongue system and are currently well known to be able to discriminate and quantify tastes. A lipid/polymer membrane comprising a lipid, polyvinyl chloride, and a plasticizer is used for the stage of receiving taste substances, the key technology of the taste sensor.

1.3.7 In Vivo Smell and Taste Biosensing System

The in vitro cultured environment leads to shortened cell/tissue survival, so the working life of biosensor is short. In vitro culture would also damage the intact

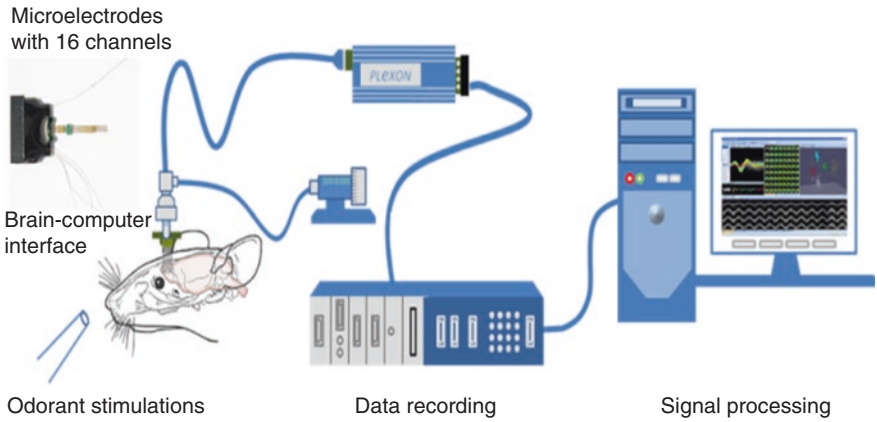


Fig. 1.4 Sketch map of bioinspired smell sensor based on implanted electrodes

nerve structure of olfactory and taste system and the natural pattern of neuronal activity may be changed. Multiple microelectrode implant technology could overcome these limitations by investigating electrophysiological properties of neuronal populations in vivo. Although this technology causes local tissue damage, it ensures integrity of biological nerve system. The propensity for multi-electrode electrophysiological investigation of neuronal populations is well established in both sensory and motor systems. For the in vivo biosensing system has characteristic of high sensitivity, continuous recording, and specificity, it presents a promising platform for specific trace odorant and tastants detection in real application (Fig. 1.4).

1.4 Application of Smell and Taste Sensors

The biological olfactory and taste systems can recognize and discriminate a large number of environmental chemical compounds presented by odorants or tastants with amazing performances. Inspired by the biological mechanisms of olfaction and taste systems for chemical sensing, many kinds of olfaction and taste sensors have been developed by the combination of biological functional components related to olfaction and taste with various secondary sensors mainly including QCM devices, SAW devices, FETs, microelectrode, SPR devices, and light-addressable potentiometric sensor (LAPS) [11–13, 15, 26, 27]. The biological functional components are usually utilized as the sensitive materials for the development of olfaction and taste sensors, which are mainly originated from the biological olfactory and taste systems at the tissue level, cellular level, or molecular level. Similar to biological chemical sensing systems, the olfaction and taste sensors are characterized with high sensitivity, rapid response, and excellent

specificity, which have shown great potential commercial prospects and promising applications in many fields such as biomedicine, environmental protection, pharmaceutical screening, and the quality control of food and water. In addition, the olfaction and taste sensors also provide novel approaches for the research of olfactory and taste signal transduction, which contribute greatly to the understanding of biological chemical sensing mechanisms. On the other hand, conventional electronic noses and electronic tongues also attracted more and more interests and have achieved significant progress in the recent decades due to their powerful chemical sensing capabilities and promising potential applications. Electronic noses and electronic tongues are usually equipped with olfaction and taste sensor arrays that can respond to diverse chemical compounds. It is thus possible to apply electronic noses and electronic tongues in the discrimination of special chemical combinations or patterns in a complex chemical environment, which can greatly broaden the application fields of olfaction and taste sensors.

1.4.1 Detection of Chemical Compounds

The most common applications of olfaction and taste sensors are connected with their capability of chemical compound detection with extreme high sensitivity and specific, which are highly essential and favorable in a wide range of fields related to the monitoring of specific chemical markers. The typical application fields include the agriculture, food safety, public safety, and anti-terrorism. In the agriculture field, an antenna-based olfaction sensor has been developed for the detection of (Z)-3-hexen-1-ol, which is a volatile chemical marker for plant damage [28, 29]. This olfaction sensor have been used to detect the plant damage in a glasshouse under real-world conditions, which can successfully distinguish single mechanically or beetle-damaged plants in background emissions of 1000 undamaged plants. In the field of food safety, an olfaction sensor have been developed based on the QCM device and odorant binding protein for the detection of volatile organic compounds indicative to *Salmonella* contamination in packaged beef, which can respond to 3-methyl-1-butanol and 1-hexanol with the detection limit as low as 5 ppm [30]. In the field of public safety and anti-terrorism, different kinds of olfaction sensors have been developed based on various chemicals sensing mechanisms for the detection of volatile chemical indicators of explosive materials as well as illicit substances, which have shown extreme high sensitivity and practical application potentials [31–34]. The taste sensors have also shown potential applications in these fields, especially in the fields of food safety. Various taste sensors have been developed, which can be used for the detection of specific tastants such as sour, sweet, and bitter substances [35–38]. These taste sensors can be used not only for the food safety monitoring, but also for the quality control of the food tasty that could have great influences on the consumption of foods. The olfaction and taste sensors have shown promising application prospects in these fields, but the research and development are still in the early experimental stage

and no commercialized olfaction and taste sensors can be found in the market. This also provides opportunities for the further development of olfaction and taste sensors to meet the special requirement of practical applications.

1.4.2 Research of Signal Transduction Mechanisms

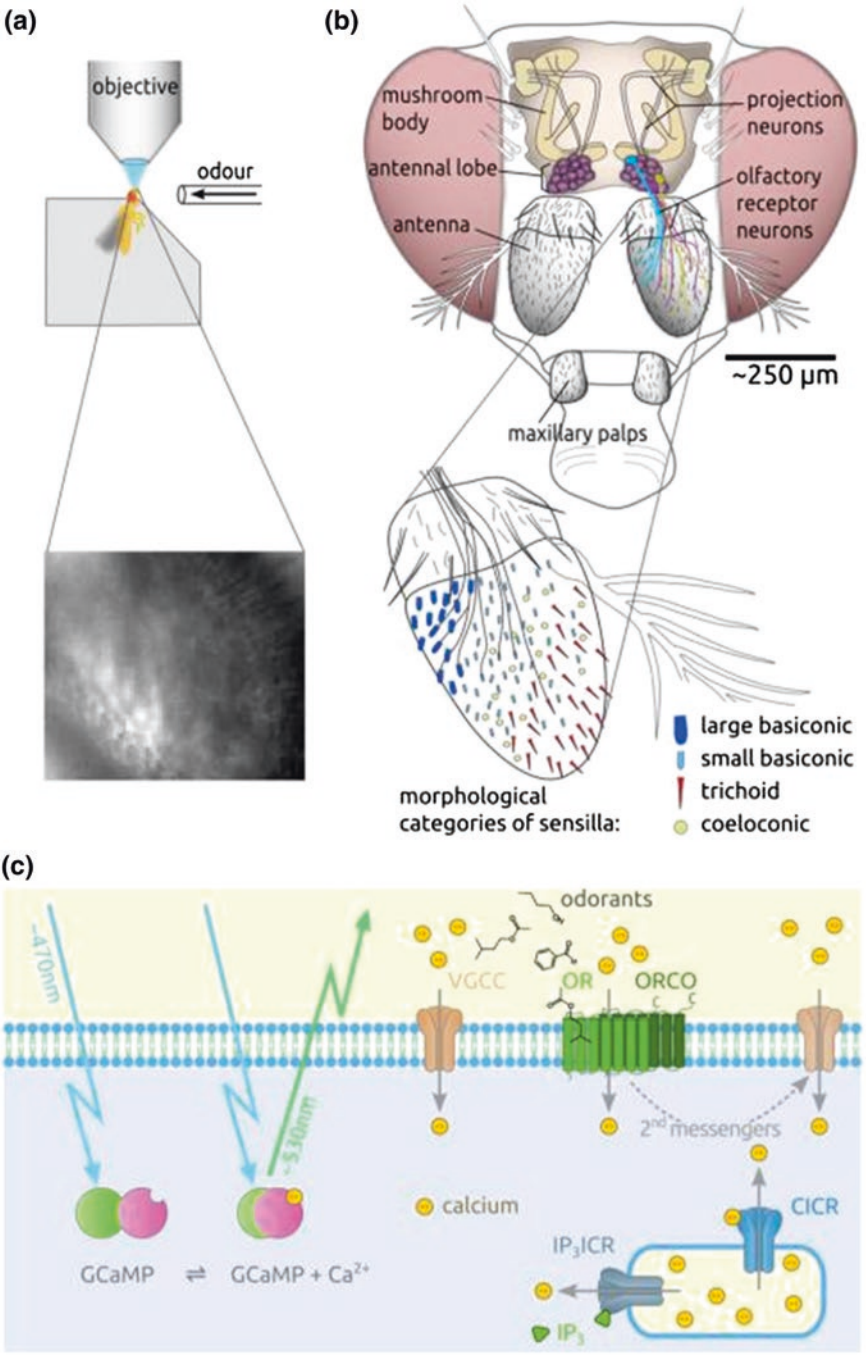
Another important application field of olfaction and taste sensors is related to the basic research of biological mechanisms of olfactory and taste systems for the detection of environmental chemical signals. In this field, olfaction and taste sensors are usually employed as promising alternative and useful tools or platforms for the further investigation of olfactory and taste signal transduction mechanisms, such as the identification of specific ligand-receptor pairs, the characterization of cellular physiology, and the electrophysiological recording of cells or tissues, which can the thus promote the research on biological olfactory and taste systems. For example, olfaction and taste sensors based on LAPS chip have been developed for the research of olfactory and taste signal transduction mechanisms at the tissue and cellular level, which can realize non-invasive, long term, and convenient measurements. An olfaction sensor developed on the basis of LAPS measurement setup has been utilized to record the membrane potential changes of rat olfactory sensory neurons (OSNs) to investigate the inhibitory effect of MDL12330A as well as the enhancive effect of LY294002 on the olfactory signals of OSNs [39]. In addition, based on the enhancive effect of LY294002 on the olfactory signals of OSNs, a novel strategy for the response enhancement of olfaction sensors was proposed, which indicate the using of LY294002 can enhance the sensitivity of olfaction sensors by 1.5 fold [40]. LAPS chip has been used as a novel platform to record the electro physiology property of living taste receptor cells in response to the five basic taste substances including NaCl, HCl, $MgSO_4$, sucrose, and glumate [41, 42]. Based on the similar LAPS measurement setup, cell-to-cell communications between different types of cells have also been investigated to explore taste sensation and analyze taste-firing responses [43, 44]. LAPS chip has also been applied in the development of taste sensors for the detection of different bitter compounds based on extracellular recordings on taste receptor cells, which can respond to different bitter stimuli [37]. In addition, LAPS chip can realize the non-invasive measurement in real-time for a long term. The extracellular recordings of taste receptor cells can successfully discriminate different bitter stimuli such as $MgSO_4$, denatonium, and D-(-)-salicin. More recently, a new method for the label-free functional assays of olfactory and taste receptors have been developed based on localized extracellular acidification measurement with a LAPS chip, which have been successfully applied in the functional assays of a human taste receptor, hT2R4, and an olfactory receptor of *Caenorhabditis elegans* (*C. elegans*), ODR-10 [45]. This biosensor provides a valuable and promising approach for label-free functional assays of chemical receptors as well as for the research of other G protein-coupled receptors (GPCRs). In addition to LAPS, olfaction and taste sensors

based on other types of transducers have also been widely used in the research of olfactory and taste signal transduction mechanisms which will be introduced in detail in the corresponding chapter sections of this book.

1.4.3 Breath Diagnosis for Cancer Diseases

One of the most attractive and promising applications of olfaction and taste sensors is in the fields of biomedicine, which are usually developed into electronic noses and electronic tongues by the incorporation of olfaction and taste sensor arrays. These applications are mainly based on the detection of volatile chemical compounds that are related to the clinical diagnosis of human body diseases such as cancer, infection, intoxication, and metabolic diseases. Human exhaled breath contains several hundreds of gaseous volatile organic compounds (VOCs) [46], which can be used as non-invasive chemical markers of some human body diseases such as lung cancer and diabetes [47]. As a non-invasive and convenient approach, electronic noses and electronic tongues have decisive advantages compared with conventional diagnosis methods, which is especially suitable for the early diagnosis of breath system diseases or diseases that have specific volatile markers in the breath air. The most typical application is using electronic noses to detect the exhaled breath air of patients for the early diagnosis of lung cancer. Lung cancer is one of the most deadly diseases both in men and women. Only 14 % of all lung cancer patients survived after 5 years diagnosis [48]. Its high-mortality results, in part, from the lack of effective tools to diagnose the disease at an early stage before it has spread to regional nodes or has metastasized beyond the lung. If the lung cancer can be discovered at its early stage and treated promptly, the 5-year survival rate would increase to 48 % [48]. The conventional methods for lung cancer detection, such as X-ray, computed tomography (CT), and bronchoscopy, are not suitable for early detection of lung cancer due to their inherent shortcomings like radiation exposure, invasion, or expensiveness. It has been demonstrated that there are 22 volatile chemical compounds, which are predominantly alkanes and methylated alkanes, can be regarded as markers of lung cancer [49, 50]. An electronic nose has been developed based on the virtual array of SAW gas sensors for the non-invasive and early diagnosis of lung cancer, which has been used to diagnose lung cancer patients in Run Run Shaw hospital [51]. This electronic nose provides an effective and promising approach for the early detection of lung cancer, especially suitable for the applications of large population screening.

In the recent decade, much effort has been devoted into the development of electronic noses for breath diagnosis. Another important example is the combination of single-stranded DNA (ssDNA) molecules with nanomaterial for the development of electronic noses for breath analysis [52]. ssDNA molecules with specific base sequences have been used to decorate the single-wall carbon nanotubes (swCNs) to build a nanostructure that can respond to the specific odorants.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ORCO
2nd messengers
calcium
CICR
IP3ICR
IP3
GCaMP ⇌ GCaMP + Ca2+

Fig. 1.5 Schematic diagram showing the basic detection mechanism of an in vivo electronic nose for the detection of cancer cells. **a** *Top* The antenna of a fixed fruit fly is exposed to fluorescent light. *Bottom* Fluorescent image of the *left* antenna of an Orco-GCaMP3 fly. **b** Structure of fly's olfactory organs. **c** Signal transduction mechanisms of volatile chemical compound detection with calcium imaging. (Reproduced with permission from Ref. [53]. Copyright 2001 Nature Publishing Group)

This kind of odorant sensitive DNA nanostructures have been applied in the development of electronic nose with a large set of diverse and sensitive odorant sensors to provide information-rich odorant-elicited signals for analysis by pattern recognition algorithms. It is demonstrated that this electronic noses can discriminate odorant homologs consisting of aldehydes and organic acids, which are usually found in human breath and other body emanations. It is suggested that ssDNA decorated swCNs have great advantages for the development of large, diverse, and sensitive sensor array due to the sufficient chemical diversity provided by the base sequences of ssDNA molecules and the high sensitivity provided by the nanomaterials.

A novel and exciting development and application of electronic noses is trying to use in vivo biological olfactory system for the detection of volatile chemical compounds that are breath from cancer cells [53]. Fruit fly's olfactory system was employed to detect cancer cells using in vivo calcium imaging to record the responses of olfactory receptor neurons on the fruit fly's antenna to the cell volatiles from different cell types. The basic mechanisms of the cancer cell detection are shown in Fig. 1.5. As shown in Fig. 1.6, the response patterns of this in vivo electronic nose to the volatile compounds from different cancer cell lines are variable. By the multidimensional analysis of antenna responses, this approach can successfully discriminate healthy mammary epithelial cells from different types of breast cancer cells. This shown the promising prospects of this kind of in vivo electronic nose to be applied in the clinical diagnostics, which is much more convenient than that of in vitro electronic noses with regard to the fabrication of olfactory sensor arrays.

Olfaction and taste sensor have achieved significant development and have been applied in a wide range of fields, which have demonstrated their promising prospects. With the multidisciplinary development and cooperation, olfactory and taste sensors will undoubtedly emerge and find promising prospects in many other application fields. Here we briefly introduce some typical and important application of olfaction and taste sensors as well as the electronic noses and electronic tongues in a general manner. For the details of each specific olfaction and taste senor, it will be introduced in the following corresponding chapters and sections.

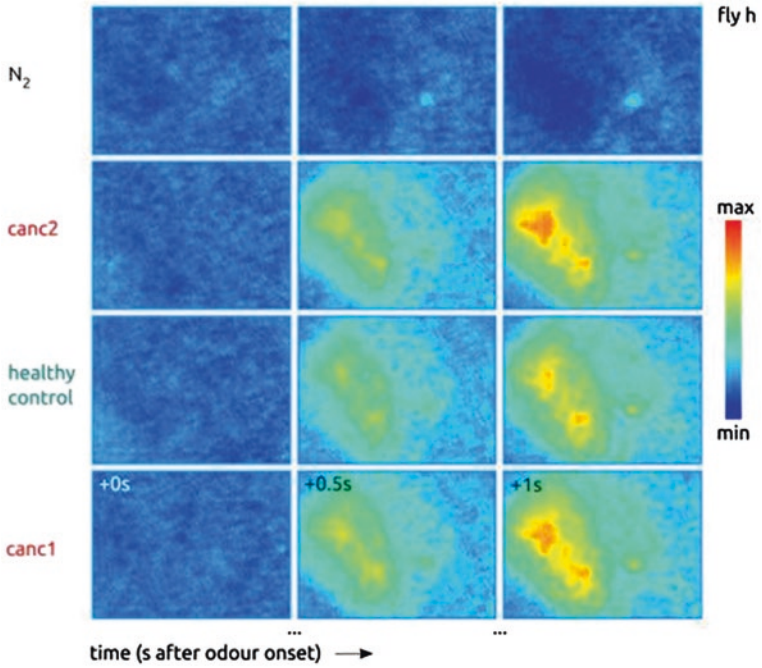

Fig. 1.6 Spatial response patterns of the fly's antenna to volatile compounds from different cancer cell lines. (Reproduced with permission from Ref. [53]. Copyright 2001 Nature Publishing Group)

1.5 Summary

This chapter briefly introduces the definition, types, and main application of bioinspired smell and taste sensor. Bioinspired smell and taste sensor include the design and manufacture of the sense of smell and taste sensitive biomimetic materials or biological materials; study of the recognition process of smell and taste; the design and manufacture of micro nano electronic and optical devices; and the research of different sensors and detection system. The research of bioinspired smell and taste sensors through the brain machine interface and micro nano electrodes is a new development direction. For different types of functional olfactory and gustatory receptors using genetic engineering technology, it will realize the novel bioinspired smell and taste sensors. Sensor miniaturization, integration, and intelligence can realize large-scale production to reduce the cost and improve the consistency of the device. These have been developed and developing bionic smell and taste sensors will be used in later chapters and introduced one by one.

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Chapter 2
Electronic Nose and Electronic Tongue

Yingchang Zou, Hao Wan, Xi Zhang, Da Ha and Ping Wang

2.1 Introduction

The sensations of smell and taste resulting from a series of specific and nonspecific molecular recognition can be used as an analytical tool in many industries to measure the quality of food, drinks, and chemical products. In a few cases, there are olfactory receptors or gustatory receptors which are specific for individual chemical molecules. However, most tastes and odorants are identified through a synthesis of the global chemical information from nonspecific interactions. Taking mammalian gustation as an example, the combination of "gustatory buds" which respond to five taste categories: sour, sweet, bitter, salty and umami creates a distinct pattern for each taste.

To mimics the nonspecific recognition, traditional electronic nose and electronic tongues that are composed of solid-state sensors array were developed. The sense of smell and taste are linked to a variety of different transduction schemes.

Herein, we review the research effort that has been carried out over the past years or so to create an electronic nose or electronic tongue, and then discuss some of the technologies that have been explored in what is essentially an intelligent chemical array sensor system. Finally, we summarize the applications of electronic noses to date and suggest where future applications may lie.

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2.2 Traditional Electronic Nose

An accepted definition of an electronic nose is "an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odor" [1] and tries to characterize different gas mixtures. Comparing with any other analysis techniques, such as gas chromatography and electronic nose systems are easy to build and could provide sensitive and selective analysis in real time. The present section focuses mostly on sensing techniques used in traditional electronic noses.

2.2.1 Principle and Structure

One cannot discuss electronic nose without comparisons to the biological nose. Upper panel of Fig. 2.1 shows a biological nose and illustrates the important features of this "instrument," while lower panel of Fig. 2.1 shows the artificial electronic nose. It is instructive for us to make comparisons between biological nose and electronic nose. For biological nose, mucous and vibrissae in nasal cavity implement filtering and concentration of odorant molecules. Odorant molecules are brought to the olfactory epithelium due to the passive pressure provided by the lung. Olfactory epithelium contains millions of sensing cells and olfactory receptors are located on the membranes of these cells. Receptors convert chemical signals into electroneurographic signals. The unique pattern of these Electroneurographic signals is interpreted by olfactory cortex neural network. Considering the general design of electronic nose, pump acts as the lung; inlet sampling system acts as the mucous and vibrissae in nasal; array of sensors acts as the olfactory receptors; and the signal processing system, e.g., computers, acts as the olfactory cortex neural network.

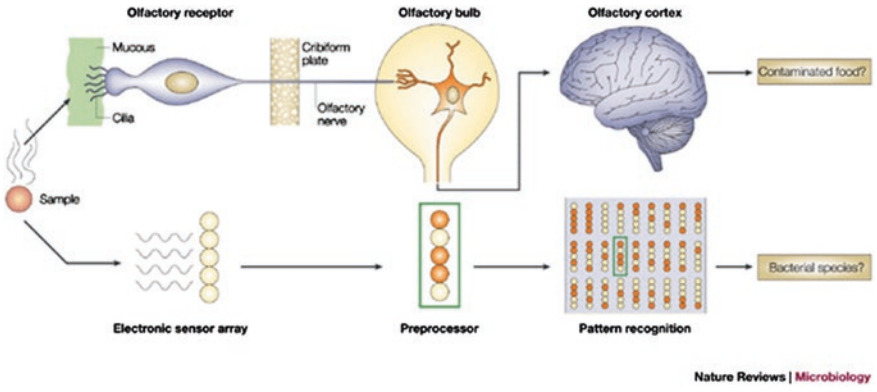

Fig. 2.1 Electronic nose devices mimic the human olfactory system [2]. (Reproduced with permission from Ref. [2]. Copyright 2004 Nature Publishing Group)

Table 2.1 Comparing electronic nose with human nose

Bio-nose	Electronic nose
It uses the lungs to bring the odor to epithelium layer	It employs a pump to smell the odor
It has mucus, membrane, and hair to act as filter	It has an inlet sampling system that provides filtration
The human nose contains the olfactory epithelium, which contains millions of sensing cells that interact with odorants in unique	Electronic nose has a variety of sensors that interact differently with a group of odorous molecules
The human receptors convert the chemical response to electronic nerve impulses whose unique patterns are propagated by neurons through a complex network before reaching the higher brain for interpretation	Similarly, the chemical sensors in the electronic nose react with the sample and produce electrical signals. A computer reads the unique pattern of signals and interprets them with some form of intelligent pattern classification algorithms

Electronic nose are used to characterize different gas mixtures as well as biological nose. However, there still exist some fundamental differences in both hardware and software. Details of comparisons between these two "noses" are listed below (Table 2.1).

In summary, an electronic nose is composed of two main components: sensing system and signal processing system. They are discussed in the following sections, respectively.

2.2.2 Sensing System

The sensing system consists of a sensor array is the "reactive part" of the instrument. The non-selectivity of some solid-state sensors (e.g., metal oxide sensors) was considered as a severe drawback. However, the idea of assembling arrays of such sensors with different sensitivities and selectivities was performed in the early 1980s. Although both the qualitative and quantitative information obtained from each sensor was highly ambiguous, their combination resulted in some sorts of "fingerprint" of the sample. With the help of statistical programs, the classification of samples into groups could be achieved [3].

Sensor technology has developed rapidly over the past decade, and this has resulted in a range of different sensor formats and the development of complex microarray sensor devices. The most commonly used sensors include metal oxide semiconductor (MOS) sensors, conducting polymer (CP) sensors, optical sensors, and piezoelectric sensors.

2.2.2.1 MOS Sensors

MOS sensors are one of the most commonly utilized sensor systems for the development of electronic noses to detect gaseous molecules. It has been known that adsorption or desorption of gaseous molecules on the surface of a metal oxide changes the conductivity of this material since 1962. When oxides are exposed to volatile organic compounds (VOCs), they are involved in a redox reaction on the surface of the MOS or act as oxidizing agents and, thereby, cause a shift in the resistance of the MOS. In detail, oxygen adsorbed from the air trap-free electrons from the conduction band of the semiconductor and builds up the potential barrier on the surface. The chemical reaction that results from adsorbed O_2 reacting with VOCs decreases the density of O2 on the surface, thereby reducing the electron trapping effect. The change in resistance depends on the VOC interacting with the adsorbed O2 on the semiconductor as well as the metal oxide. This phenomenon was first demonstrated using zinc oxide thin-film layers. Based on the results from ZnO, further metal oxides were examined as regards their properties of varying their conductivities with the composition of the gas atmosphere surrounding them, including ZnO [4, 5], WO_3 [6, 7], SiO_2 [8, 9], and TiO_2 [10, 11]. A schematic diagram of a MOS sensor is shown in Fig. 2.2a.

2.2.2.2 CP Sensors

CPs are widely used as sensor elements in electronic noses thanks to their ability to adjust their conductivity in response to organic compounds. CP sensor arrays

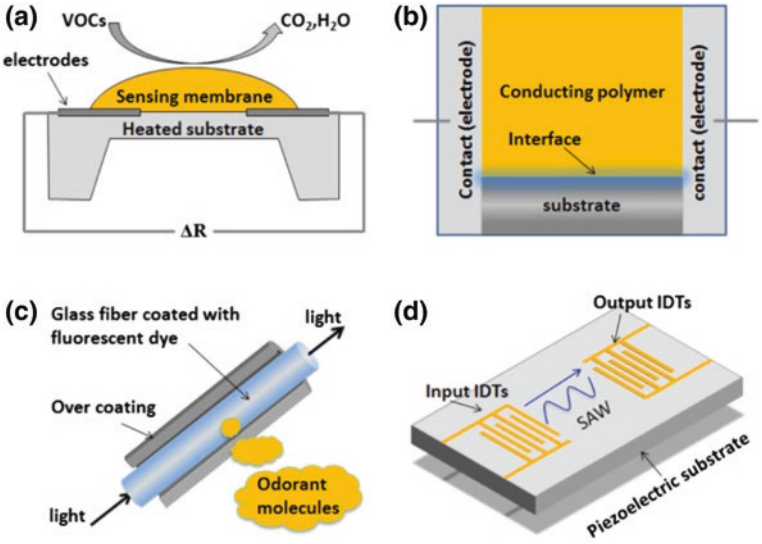

Fig. 2.2 Schematic diagrams of **a** MOS sensor, **b** CP sensor, **c** optical fiber and **d** SAW sensor

often consist of unique polymers with different reversible physicochemical properties and sensitivity to groups of volatile compounds to provide a broad specificity that overlaps with that of organic vapors. These organic vapors attach to and interact with the polymer surface, changing the resistance under ambient temperature conditions [12, 13]. Different polymers response to stimulated vapors with different physicochemical properties, and chemical modification of polymers also alters the properties of materials. Successful applications of conducting polymers to electronic noses as sensor elements have been conducted in several articles [14, 15].

Signals could be monitored for each sensor type, enabling an array to be constructed that has overlapping detection ranges for different groups of volatile compounds. However, sample presentation is crucial for CP sensor to avoid humidity and drift problems.

Figure 2.2b shows a schematic diagram of a chemiresistor which is the most common group of CP sensors. It consists of a pair of electrodes forming contacts to the CP, deposited on an insulating substrate. When a constant current is applied, the resulting potential difference at the electrode becomes the response signal.

2.2.2.3 Optical Sensors

Optical sensors have been widely used as gas sensors in many applications due to their response which could be measured precisely [16–18]. These sensors are based on a light source that excites the volatile molecules, and the signal can be measured in the resulting absorbance, reflectance, fluorescence, or chemiluminescence. Such output signals are detected using various detectors, including photodiodes, CCD, and CMOS cameras [19, 20].

The most classical optical sensors are optical fiber and surface plasmon resonance (SPR) sensor. Optical fiber sensors (Fig. 2.2c) utilize glass fibers coated with thin chemically active materials on their sides or ends that contain immobilized fluorescent dyes in an organic polymer matrix. The changes in dye polarity cause a shift in the emission spectrum due to interaction of VOCs with a light source. SPR technique is based on the fact that the field of surface plasma wave is extremely sensitive to changes in the refractive index of the dielectric when very close to the surface. The electrons in a piece of metal continuously and freely move like a charged cloud and are excited by light; this has been named surface plasmon resonance. When light is focused onto the surface, electrons become excited, and at a critical angle, called the resonance angle, reflectance falls to zero. Changes in the surface caused by chemical adsorption and molecular interactions alter the reflectance of the surface. This technique is a highly efficient method for detecting odorants [21, 22].

In summary, optical sensors are excessively sensitive and are able to identify individual compounds in mixtures. However, their connected electronics and software are complex and expensive. These sensors have a relatively short lifetime, which would also result in increasing the cost of detection.

2.2.2.4 Piezoelectric Sensors

Piezoelectric sensors have a radio frequency resonance under such electric potential and are highly sensitive to the mass change applied to the surfaces of piezoelectric sensors. Quartz crystal microbalance (QCM) and surface acoustic wave (SAW) sensors are two of the most useful piezoelectric sensors applied in electronic noses.

QCM is an advanced type of microbalance mass sensor. Its transducer based on the piezoelectric properties of quartz material has been implemented in sensors. QCM is made of a polymer-coated resonating quartz disk, vibrating at a characteristic frequency (10–30 MHz). Its oscillation frequency decreases, while the bounding-mass increases on the crystal surface. In detail, adsorption of gas molecules onto the sensing films deposited on the crystal surface would result in the shift in the quartz crystal (QC) resonant frequency. As the gas molecules adsorbed to the polymer surface, it reduces the resonance frequency and the decrease is proportional to mass of odorant adsorbed. QC which is equipped with metal electrodes (e.g., gold) is the basic material of the QCM sensor. The selectivity of QCM sensor is based on sensitive materials coated on sensor surface. The thickness of coatings affects the sensitivity of QCM sensors. In addition, temperature, humidity, and some other environmental conditions also have influences on sensitivity of QCM sensors. SAW and QCM are both mass-sensitive sensors. However, SAW uses a surface acoustic wave sensor, while QCM uses a BAW sensor. SAW sensors require waves to travel over the surface of the device. SAW sensors operate at higher frequencies (100–1,000 MHz) and thus generate a larger change in frequency. The structure of SAW is shown in Fig. 2.2d. The output transducer and input transducer are both interdigital transducers (IDTs) which are core components of SAW. When the environment of the transducer changes, e.g., gas molecules absorbed, the vibration frequency will change. Hence, the weight information of gas molecules can be obtained through comparing signals of output IDTs with input IDTs.

2.2.3 Pattern Recognition System

The use of multivariate analysis methods together with sensor arrays has shown to be very powerful. Two main issues are dealt with, to search for a structure and correlation in the data, or to make a model from a training set of data, which is then used to make predictions from test data.

2.2.3.1 Dimension Reduction

Principal component analysis (PCA) is a mathematical transform which is used to explain variance in experimental data [23]. The data matrix X consists of m

experiments, each consisting of n variables. In PCA, a transformation in the variable space is made. Let Y be another data matrix related by a linear transformation P [24].

$$\mathrm{PX} = \mathrm{Y} \tag{2.1}$$

X is the original recorded dataset and Y is a re-representation of that set. P is a rotation and a stretch which transforms X into Y. This transformation aims at having a minimum redundancy and a maximum signal. Considering the definition of covariance matrix, the goal of PCA is finding some orthonormal matrix P which results in a diagonalized matrix, where

$$C_Y = \frac{1}{n-1} YY^T. \tag{2.2}$$

Substituting (2.1) into (2.2), we rewrite in terms of linear transformation P.

$$C_Y = \frac{1}{n-1}(PX)(PX)^T = \frac{1}{n-1} PXX^T P^T = \frac{1}{n-1} P(XX^T)P^T \tag{2.3}$$

$$C_Y = \frac{1}{n-1} PAP^T \tag{2.4}$$

We defined a new matrix here. It is obvious that A is symmetric. A symmetric matrix can be diagonalized by an orthogonal matrix of its eigenvectors.

$$A = EDE^T, \tag{2.5}$$

where D is a diagonal matrix and E is a matrix of eigenvectors of A arranged as columns.

Now comes the trick. We select the matrix P to be a matrix where each row vector pi is an eigenvector of XXT. It means. Substituting into (2.5), we find. Note that E is a matrix of eigenvectors of A. That means:

$$C_Y = \frac{1}{n-1} PAP^T = \frac{1}{n-1} P(P^T DP)P^T = \frac{1}{n-1}\left(PP^T\right)D(PP^T) = \frac{1}{n-1}\left(PP^{-1}\right)D(PP^{-1}), \tag{2.6}$$

$$C_Y = \frac{1}{n-1} D. \tag{2.7}$$

That is to say is a diagonalized matrix. For PCA, the real goal is to obtain eigenvectors of XXT.

For PCA, there is no requirement to have any prior knowledge about classification of samples. It is a simple, effective, and stable multivariate analysis. But the most significant drawback of PCA is the uncertainty of the meaning of the principal components. On the contrast of PCA, other methods we introduce here are all supervising methods.

2.2.3.2 Classification and Prediction

Data often divided into two parts: training set and test set. Training sets of data are used to build classification models, while test sets of data are used to evaluate the classification model. The most useful methods for modeling are linear discriminant analysis (LDA), partial least squares (PLS) regression, and artificial neural nets (ANN).

LDA is method used in statistics, pattern recognition, and machine learning to find a linear combination of features which characterizes or separates two or more classes of objects or events. The resulting combination may be used as a linear classifier, or, more commonly, for dimensionality reduction before later classification. LDA is closely related to PCA for the reason that they both look for linear combinations of variables which best explain the data [25]. However, LDA explicitly attempts to model the difference among the classes of data. PCA on the other hand does not take into account any difference in class.

PLS regression finds a linear regression model by projecting the predicted variables and the observable variables to a new space. PLS is used to find the fundamental relations between two matrices (X and Y), i.e., a latent variable approach to modeling the covariance structures in these two spaces. A PLS model will try to find the multidimensional direction in the X space that explains the maximum multidimensional variance direction in the Y space. PLS regression is particularly suited when the matrix of predictors has more variables than observations, and when there is multicollinearity among X values. By contrast, standard regression will fail in these cases (unless it is regularized). In electronic nose system, PLS is often performed with each principal component obtained in PCA.

ANNs are computational models inspired by the way biological nervous systems. The key element of ANN is the novel structure of its information processing system. It is composed of a large number of highly interconnected processing elements (neurons in biological systems) working in unison to solve specific problems. ANNs can compute values from inputs and are capable of machine learning as well as pattern recognition thanks to their adaptive nature. ANN models are non-linear, thus being able to adapt to non-linear processes. An ANN can be divided in different layers, an input layer consisting of input signals, one or more hidden layers and an output layer. The hidden and the output layers consist of signal processing nodes, each connected to each other in a net, with the strength of the connections being set by a coupling weight. During learning, output values from the ANN are compared to true values and the coupling weights are adjusted to give a minimum sum of square errors.

2.3 Traditional Electronic Tongue

The human tongue mainly detects five tastes, salty, sour, sweet, bitter, and umami using gustatory receptor cells located in clusters called gustatory buds. After perception by gustatory cells, the taste information is transmitted via cranial nerves

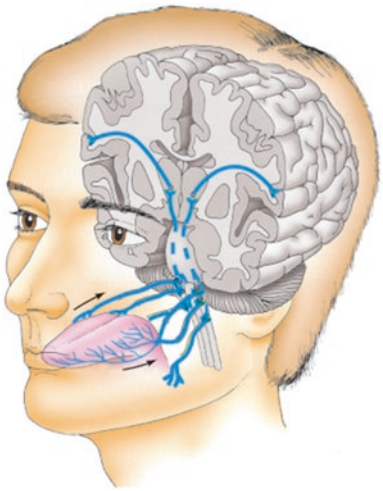

Fig. 2.3 The gustatory system of human (mindsmachine.com)

to brainstem nuclei. Eventually, all information is analyzed in cerebral cortex and different tastes are perceived. The whole gustatory system of human is shown in Fig. 2.3. The theory of electronic tongue originates from mechanisms of the gustatory system of human. Electronic tongues (e-tongues) can be considered as analytical instruments that artificially reproduce the taste sensation. These devices are typically array of sensors coupled to chemo-metric processing used to characterize complex liquid samples [26]. The schematic representation of the components of an electronic tongue is shown in Fig. 2.4. If properly configured and trained (calibrated), the e-tongue is capable of recognizing the qualitative and quantitative composition of multi-species solutions of different natures. The IUPAC technical report on the topic defines it as "a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on the pattern recognition(PARC) and/or multivariate data analysis" [27].

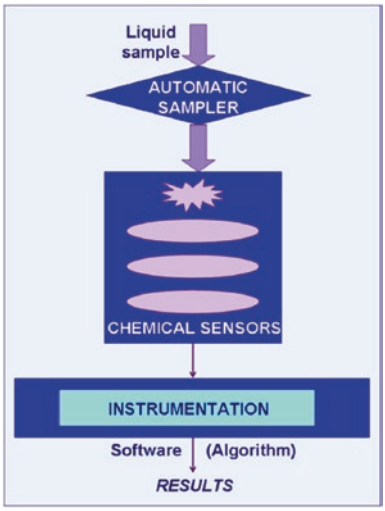

Fig. 2.4 Schematic representation of the components of an electronic tongue [26] (Reproduced with permission from Ref. [26]. Copyright 2010 Elsevier)

Regarding the sensor array used in the design of e-tongues, a wide variety of chemical sensors have been employed: electrochemical (potentiometric, voltammetric, amperometric, impedimetric, and conductimetric), optical, mass, and enzymatic sensors (biosensors) [28].

2.3.1 Potentiometric Sensors

In potentiometry, a potential is measured between two electrodes under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the solution. The potential that develops in the electrochemical cell is the result of the free energy change that would occur if the chemical phenomena were to proceed until the equilibrium condition has been satisfied.

The largest group among potentiometric sensors is represented by ion-selective electrodes (ISEs), the oldest and most widely used among them being a pH-sensitive glass electrode. Different approaches of potentiometric electronic tongues and taste sensors have been demonstrated. They have in common that they all measure the potential over a charged membrane. These membranes can be of different materials, which provide enough selectivity to different classes of chemical substances. Electronic tongues have, thus, been described based on an array of chalcogenide glass sensors, including conventional electrodes such as chloride-, sodium- and potassium-selective sensors, combined with a pattern recognition routine. The photograph of ISEs array and the measuring system in the group of Andrey Legin, St. Petersburg University (Russia), is shown in Fig. 2.5. The chalcogenide sensors show cross-sensitivity which has been preferably used for measurement of metal ions in river water and suggested or environmental and process-monitoring purposes [29–33]. This type of electronic tongues has also been combined with PVC membranes for testing of beverages [34].

Potentiometric ion and chemical sensors based on field-effect devices form another group of transducers that can be easily miniaturized and are fabricated by means of microelectronic technology.

Fig. 2.5 ISEs and the measuring system (Andrey Legin, St. Petersburg University, Russia)

Among them most studies are ion-sensitive field-effect transistors(ISFETs) [35] with different ion-selective membranes (often also called chemically sensitive field-effect transistors or Chem-FETs). ISFETs with bare gate insulator (silicon oxide, silicon nitride, aluminum oxide, etc.) show intrinsic pH-sensitivity due to electrochemical equilibrium between protonated oxide surface and protons in the solution. To obtain sensitivity to other ions, a polymeric membrane containing some ionophore may be deposited.

The LAPS [36, 37] is a semiconductor-based device with an electrolyte-insulator-semiconductor (EIS) structure, which is similar to ISFET in function. A dc bias voltage is applied to LAPS, so that a depletion layer appears at the insulator-semiconductor interface. When a modulated light irradiates LAPS from front or back side, an AC photocurrent inside the depletion layer could be induced as a measured signal. Amplitude of photocurrent is sensitive to the surface potential, and thus LAPS is able to detect the potential variation caused by an electrochemical even. Therefore, in principle, any electrochemical reaction that results in the change of surface potential can be detected by LAPS, including the ionic change [38] and redox effect [39]. By modifying the individual sensitive region with the polymer membrane or chalcogenide glass membrane which contains specific receptor molecules [40], relevant cations could be detected simultaneously. Most of the solid-state-based thin-film sensors suffer from an insufficient selectivity. Compared to the inorganic membrane, the organic one can overcome the problem with reasonable good selectivity [41] (Fig. 2.6).

2.3.2 Voltammetric Sensors

Voltammetry, in which a current is measured at a fixed potential, is a very powerful and often used technique in analytical chemistry. Depending on the potential applied and type of working electrode, redox active compounds are either oxidized or reduced at the working electrode, giving rise to a current. The sensitivity of voltammetric methods is often very high. The selectivity is, however, in many cases poor, since all compounds in a measured solution that is electrochemically active

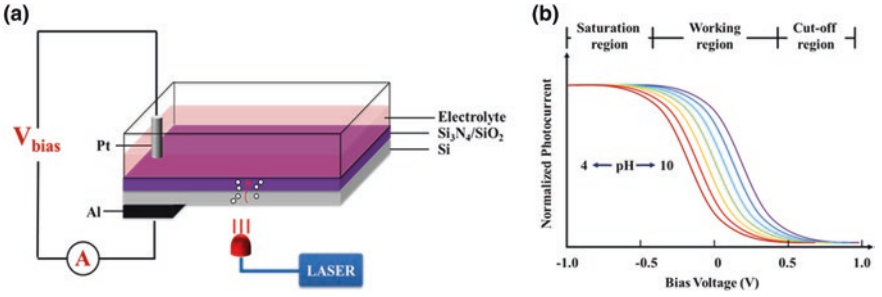

Fig. 2.6 **a** Working principle of the LAPS. **b** Characteristics I–V curve of n-type LAPS

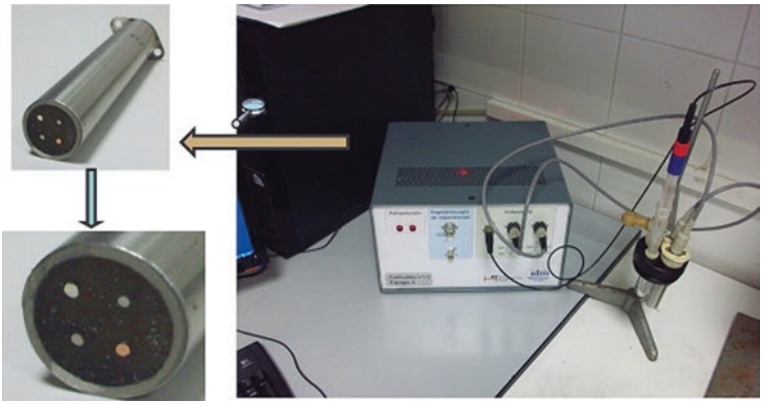

Fig. 2.7 A developed voltammetric electronic tongue for water quality monitoring (Reproduced with permission from Ref. [43]. Copyright 2012 Elsevier)

below the applied potential, will contribute to the measured current. Different ways to surmount this is, e.g., to cover the working electrode with a gas permeable membrane, only letting gases pass through, or to use pulse voltammetry. Voltammetry appears to have several advantages; the technique has been extensively used in analytical chemistry due to features such as its very high sensitivity, versatility, simplicity, and robustness. Besides, voltammetry offers a widespread number of different analytical possibilities, including cyclic, stripping, and pulse voltammetry. Depending on the technique, various kinds or aspects of information can be obtained from the measured solution. Normally, redox active species are being measured at a fixed potential, but using, e.g., pulse voltammetry, studies of transient responses when Helmholz layers are formed, also give information concerning diffusion coefficients of charged species. Further information is also obtainable by use of different type of metals for the working electrodes. Different metal electrodes can be used together with voltammetric measurements to classify different liquids [42, 43]. Still, the voltammograms contain a large amount of information and to extract this information, multivariate calibration methods have been shown to be rather efficient [44, 45] (Fig. 2.7).

2.4 Application of Electronic Nose

2.4.1 Food Evaluation

In the past, electronic noses have been developed for the classification and recognition of a large variety of foods, including meat [46, 47], fish [48, 49], grains [50, 51], fruits [52, 53], coffee [54, 55], beer [56, 57], beverage [58, 59], cheese [60], sugar [61], and vegetables [61], especially for the determination of the categories and freshness of food.

Yu and Wang [62] conducted an investigation about application of electronic nose in distinction of tea. Four tea groups (A120, A280, A380 and A600) with a different quality grade were employed. They sealed these teas (5 g) separately in vials of different volumes (50, 150, 250, and 500 ml), respectively. Then, they collected headspace compounds of these vials. The headspace generating time was 0.75, 1, and 2 h.

A portable electronic nose (PEN2) is used to detect these headspace samples. PEN2 is a commercial electronic nose system produced by WMA (Win Muster Airsense) Analytics Inc. (Germany). It consists of a sampling apparatus, a chamber containing an array of sensors, and pattern recognition software for data recording. The sensor array was composed of 10 MOSs, and its response was expressed as the ratio of conductance (G/G0). The headspace gas was pumped into the sensor chamber with a constant rate of 400 ml/min via a Teflon-tubing connected to a needle during the measurements process. When the gas accumulated in the headspace of vials was pumped into the sensor chamber, the ratio of conductance of each sensor changed. A computer recorded the response of the E-nose every second. When the measurement was completed, the acquired data was properly stored for later use. The temperature of the laboratory was kept 25 ± 1 °C.

In this research, response values of each sensor at 15, 30, 45, and 60 s were extracted and analyzed individually. PCA, LDA, and ANN were employed for data processing. One side, the optimum experiment conditions are decided in this research. On the other hand, four groups of tea were measured under the decided conditions, and authors evaluated the ability of PEN2 to distinct these four groups of tea. Only A120, A380, and A600 could be discriminated by PCA. However, the four tea groups were discriminated completely by LDA. The response value of the E-nose at 60 s was optimum to be used for discrimination. The method of ANN (network topology 20-12-4) was performed, and 90 % of the total tea samples were classified correctly using the back-propagation neural network.

PERES is the world's first portable "electronic nose"—a unique and innovative device and mobile application which enables users to determine the quality and freshness of pork, beef, chicken, and fish (http://www.getperes.com). It is designed to detect: whether a product is fresh, whether it is hazardous to health, whether there is a risk of food poisoning, and whether it has been left unrefrigerated for some time. The device has four types of sensors: temperature, humidity, ammonia, and volatile organic compounds sensors (Fig. 2.8a). To operate the device, the user simply directs it toward the food product and clicks a button (Fig. 2.8b). The device uses Bluetooth technology to transmit data to the user's smartphone or tablet, which displays detailed results with recommendations regarding the safety of the product (Fig. 2.8c). Users control PERES, start the sampling process, analyze the results of readings, and share their experiences with friends just by interfacing with a user-friendly environment on their phone or tablet (Fig. 2.8d).

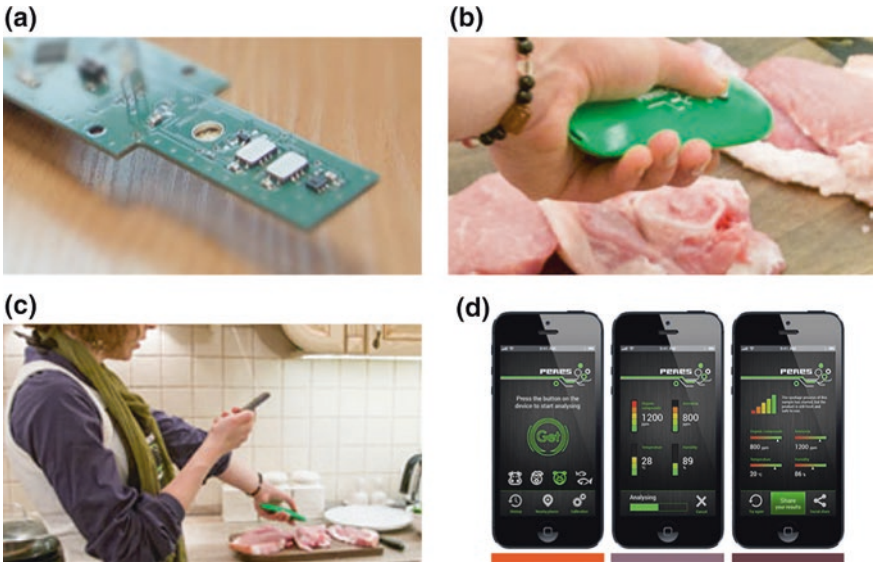

Fig. 2.8 A portable commercial electronic nose named PERES. **a** Structure. **b** User simply directs PERES toward the meat and clicks a button to complete detection. **c** A detailed result with recommendations regarding the safety of the detected meat would be provided on a mobile phone. **d** The user interface of PERES (http://www.getperes.com)

2.4.2 Public Security

One important application for the electronic nose is for use in detection of explosives. An electronic nose capable of detecting explosive substances may be used for the detection of landmines and for homeland security purposes [63]. Homeland security applications include screening people packages, luggage, and vehicles at key locations such as airports or government buildings, for the prevention of terrorist attacks.

Brudzewski et al. [64] designed a differential electronic nose which consists of two chemo-sensor arrays working in parallel to measure typical explosive materials including trinitrotoluene (TNT), pentaerythritolte-tranitrate (PETN), and cyclotrimethylenetrinitramine (RDX). The experimental system contains two sensor arrays instead of one. One of them forms the "measurement array" and the other forms the "reference array." The streams of air are delivered to both chambers of gas sensor arrays from the odor acquisition place through the socket outlet. The measurement channel contains inside the vapor of explosive material, while the reference one only the outside air, free of explosive odor. For either sensor array, 12 heated MOS sensors of Figaro series were used. The corresponding signals registered by the appropriate sensors of both arrays are subtracted from each other using the differential amplifier, then converted by A/D converter to the digital form and finally delivered to the computer interface for further processing,

Fig. 2.9 An example of a suitable effluence detection device—the Quantum Sniffer QS-H150 Portable Hand-held Explosives Trace Detector (anjian110.com)

which leads to the recognition of odor. PCA was employed to perform model recognition. 12 sensor signals corresponding to RDX, PETN, and TNT were mapped on two most significant principal components: PCA1 and PCA2. Results showed that these three kinds of typical explosive materials could be discriminated by the present electronic nose (Fig. 2.9).

2.4.3 Medical Applications

It has been reported that some volatile organic compounds (VOCs) in human exhaled breath can be potential biomarkers for lung cancer [65, 66]. Wang et al. designed an electronic nose based on surface acoustic wave (SAW) sensor combined with capillary column for early detection of lung cancer [67, 68].

The electronic nose consists of thermal desorption system, capillary column, SAW sensor, and data processing system on PC. VOCs in exhaled breath was enriched by an adsorption tube, desorption happened in the inlet of the capillary with high temperature, then VOCs were carried into the capillary to be separated by the carry gas. When VOCs come out from the capillary, there would be a frequency change because VOCs can attach to the surface of the SAW sensor independently owing to condensation. Comparing with general electronic nose, it contains one SAW sensor instead of a sensor array. Owing to capillary column, VOCs can be separated and would be detected one by one. That made the single sensor could work as a virtual gas sensor array. Authors extracted response of SAW sensor at 5 specific time points according to retention time of 5 kinds of potential biomarkers. PCA and ANN were applied for multivariate data processing.

Researchers at the University of Pennsylvania School of Medicine have demonstrated the effectiveness of an electronic nose device for diagnosing common respiratory infections, specifically pneumonia [69]. Doctors hope that the device—called the Cyranose 320, or e-nose—will provide a faster, more cost-effective, and easier-to-use method for accurately diagnosing pneumonia and, as a result, help

Fig. 2.10 Electronic Nose is used for Diagnosing Pneumonia (spectrum.ieee.org)

reduce over-prescription of antibiotics. Pneumonia is a serious bacterial infection that can cause serious injury or even death; indeed, it remains a leading cause of death in intensive care units (ICUs). All bacteria, as living organisms, produce unique arrays or mixtures of exhaled gases. The e-nose works by comparing "smellprints" from a patient's breath sample to standardized, or known, readings stored on a computer chip. These "smellprints" are created from both electrochemical and mathematical analysis of exhaled gases contained in a breath sample. Figure 2.4 shows the situation that diagnosis of infection by electronic nose (Fig. 2.10).

2.5 Application of Electronic Tongue

Electronic tongue was first proposed by Toko et al. [70–72] in 1990s for the intension of mimicking the functions of human gustatory receptors. The objective of electronic tongue is to study five basic taste substances: salty (NaCl, KCl, and KBr), sour (HCl, citric and acetic acids), bitter (quinine), sweet (sucrose), and umami (monosodium glutamate). Afterward, other tastes like astringent and pungent substances were investigated, and many expansive applications of electronic tongue have been explored including food evaluation, drink discrimination, and even hazards detection, etc.

2.5.1 Food Evaluation and Discrimination

Traditional food analysis is carried out using a wide range of methodologies based on chemical, biochemical, physic-chemical, and microbial principles aiming to determine the concentration or the presence of different compounds that directly participate in the characteristics of food. These traditional approaches are always destructive, time-consuming, and require laboratories equipped with complex and

expensive equipments. Additionally, the analysis should be carried out by specialized personnel which is not suitable for in situ or at site monitoring. As a comparison, electronic tongue indicates a promising technique in food analysis due to its capability of rapid and simple procedures for qualitative and quantitative analysis. Many applications have been investigated in food evaluation and discrimination.

Beullens et al. [73] presented an electronic tongue consisting of 27 potentiometric sensors for determining the sugar and acid profile of four tomato cultivars. Based on all these information, different multivariate data analysis techniques such as principle components analysis (PCA) and canonical discriminant analysis (CDA) were applied to detect differences in sugar and acid profiles between the four tomato cultivars. The potential of both the electronic tongue and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was used to predict the chemical composition of the sample using partial least squares (PLS). The electronic tongue is proved to be able to classify different tomato cultivars based on CDA.

Kaneki et al. [74] introduced an electronic tongue with potentiometric solid-state electrodes for pork freshness evaluation. Pt, CuS, and Ag2S electrodes were selected as solid-state electrodes to detect the organic compounds such as putrescine and dimethyl sulfide which were produced during the initial stage of putrefaction in meat. PCA was performed in the study by datasets from electric potential on each electrode for qualitative evaluation of pork freshness. And measurements were proved to be useful for qualitatively showing the degree of the pork freshness by analysis of the potential on electrodes. On the other hand, MRA was performed for quantitative evaluation of pork freshness by potential on electrodes and viable bacteria counts. Relationship between experimental values and predicted values of viable bacterial counts was analyzed by MRA. Results showed that the coefficient of determination (R2) was 0.762, suggesting a good linear correlation relationship.

In another study by Gil et al. [75], an electronic tongue consisting of 16 potentiometric electrodes was applied for fish freshness analysis. The potentiometric electrodes were the type metal, metal oxide, insoluble metal salts, and graphite as well. All electrodes were screened on to a surface substrate with different inks to manufacture the electronic tongue electrode array. Figure 2.11a shows the fabricated electrode array with 16 electrodes. Fish freshness indicators such as texture, pH, color, microbial analysis, total volatile basic nitrogen, and biogenic amines were determined versus time. The effectivity of the electronic tongue in the assessment of the evolution with time of fish fillets was evaluated. The electronic tongue was used to classify samples according to different time with PCA and artificial neural network (ANN). Figure 2.11b shows the PCA results for different metallic electrode response. Satisfactory cluster results were obtained in which fish fillets were distinguished with different days. Besides, the electronic tongue was used to predict the results obtained from chemical and biochemical analyses by building quantitative partial least square (PLS) models. A remarkable correlation was found between the electronic tongue formed by the 16 simple electrodes and parameters such as total biogenic amines, pH, TVB-N, and microbial analysis with correlation coefficients larger than 0.98.

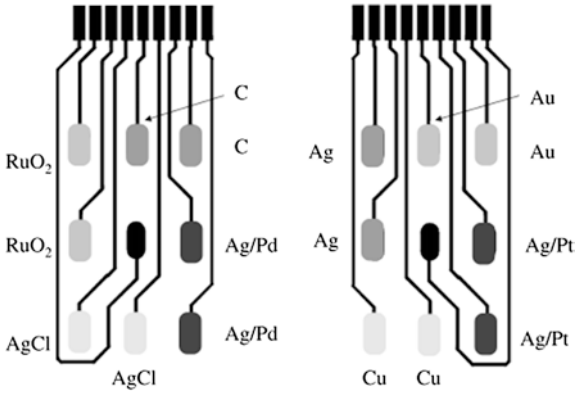

Fig. 2.11 **a** Electronic tongue design containing and array of 16 electrodes in thick-film technology; **b** PCA results for the metallic electrode response. Ellipses cluster together measurements carried out the same day (from Day1 to Day14) [75] (Reproduced with permission from Ref. [75]. Copyright 2008 Springer-Verlag)

Beverages recognition and discrimination are other fields which are widely applied with electronic tongue. Beverages industry, such as beer, wine, tea, mineral water, coffee, is in great demand for qualitative analysis [34]. Compared to conventional analytical tools such as various chromatographs, spectrometers, and electronic tongue show outstanding superiority in flexibility, simplicity, and costing for beverages analysis.

Vlasov et al. [76] introduced an electronic tongue based on the sensor array of nonspecific solution sensors to reliably discriminate various sorts of beverages. The electronic tongue sensor array includes two parts of potentiometric sensors: (1) conventional chloride-, sodium-, potassium-selective, and pH sensor, (2) specially designed nonspecific sensors with enhanced cross-sensitivities based on chalcogenide vitreous materials. Based on those electrodes, electronic tongue was used for qualitative and quantitative analysis of beverages. Figure 2.12 presents the discriminating abilities of the electronic tongue for beverages and different beverages can be distinguished apparently. Besides, quantitative performance of the electronic tongue for some ions (Cu, Fe, Mn, Zn, Ca, Mg, Na, Cl, and SO4) were also evaluated, in which satisfactory results were obtained in quantitative analysis with acceptable errors.

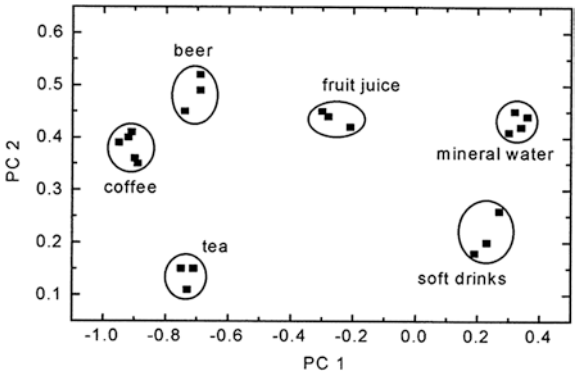

Fig. 2.12 Discriminating abilities of electronic tongue in different types of beverages by PCA [76] (Reproduced with permission from Ref. [76]. Copyright 2000 Elsevier)

Evgeny Polshin et al. introduced an electronic tongue comprising 18 potentiometric chemical sensors for quantitative analysis of beer. Fifty Belgian and Dutch beers were measured using electronic tongue and conventional analytical techniques with different physicochemical parameters including real extract, real fermentation degree, alcohol content, pH, bitterness, color, polyphenol, and CO2 content. Canonical correlation analysis (CCA) was used to study the correlations between electronic tongue data and physicochemical data. PLS calibration model was constructed based on electronic tongue data for physicochemical parameters prediction. The results showed that the electronic tongue was capable of predicting parameters including real extract, alcohol and polyphenol content and bitterness, which could be used for the evaluation of beer quality.

2.5.2 Water Environment Monitoring

Water environmental pollution has received extensive attentions worldwide due to its severe toxicity to humans. However, environmental monitoring requires onsite measurements simultaneously of a wide range of different chemical compounds and species [23]. The robustness, sensitivity, and broad selectivity are the main obstacles for on-sit monitoring. Electronic tongue with global selectivity, good stability, and high sensitivity turns to be a promising approach for environmental monitoring. Using electronic tongue with cross-selectivity, it is plausible to realize real-time multicomponent measurement in samples with a convenient and simple instrument. Various data processing methodologies such as PCA and ANN are used in electronic tongue to analyze the data of sensors, from which effective information are extracted for quantitative analysis. In fact, many studies have been investigated for water environment monitoring based on different electronic tongues.

Di Natale et al. [77] introduced a sensor array of ion-selective electrodes to simultaneously detect concentrations of a number of chemical species in solutions. In the electronic tongue system, 22 electrodes, which are mainly based on chalcogenide glasses variously doped and conventional electrodes, were used for cross-selective measurements of eight cations and anions (Cu, Cd, Fe, Cr, Zn, Cl, SO4, and H). Different data analysis approaches were utilized to ensure the best performance of the electronic tongue, including multiple linear regression (MLR), partial least squares (PLS), non-linear least squares (NLLS), and back-propagation neural network (BP-NN). About 150 chemical solutions were measured in order to construct robust calibration models of different analysis approaches. The results showed that modular models could significantly reduce the errors for concentration prediction of different ions with multiple approaches coupled. And very good concentration prediction results (mean relative absolute error <6 %) were obtained to validate the feasibility of the electronic tongue for environmental monitoring.

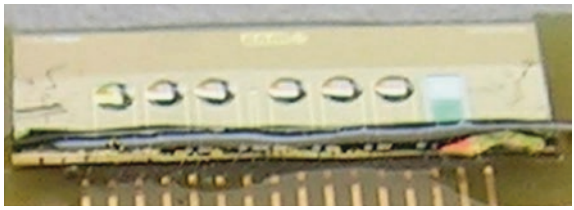

Fig. 2.13 **a** The photograph of the chemical multi-sensor array; **b** Error analysis table of the PLS prediction of the concentration of K^+, Na^+, Ca^{2+}, and Cl^- in water samples [78] (Reproduced with permission from Ref. [78]. Copyright 2006 Elsevier)

Moreno et al. [78] presented an electronic tongue based on a monolithically integrated array of chemical sensors. The electronic tongue was composed of six independent ion-selective field-effect transistors (ISFETs), an interdigitated platinum electrode (IDS), and a silicon diode used as a temperature sensor. The photograph of the chemical multi-sensor array is Fig. 2.13a. K^+, Na^+, Ca^{2+}, and Cl^- ISFET-based sensors were obtained by depositing different photocurable membranes onto their gates. IDS was used to measure conductivity and redox potential. Partial least square (PLS) model was calibrated and used for concentration prediction. The concentration prediction results are shown in Fig. 2.13b, in which SEP means the standard error of prediction and r is the regression coefficient. From the results, the slope of the regression for all ions is quite close to 1.0 and the intercept to zero, which indicates very good quality of the prediction. The electronic tongue demonstrated very good performance for quantitative determination of K^+, Na^+, Ca^{2+}, and Cl^- in samples, which could be further used for water quality assessment and discrimination.

In spite of the cross-selectivity of traditional electronic tongues, some studies have also been investigated for cross-talk rejection and increasing the selectivity of sensors. Ha et al. [79] introduced a multi-sensor array based on light addressable potentiometric sensor (LAPS) with polyvinyl chloride (PVC) membrane modification for determination of heavy metal cations. Cross-talk was decreased by heavily doping part of the silicon substrate with boron and fabrication thick oxide formation on its surface. The schematic diagram of the multi-sensor array measurement setup is shown in Fig. 2.14. Three electrodes system was used for electrochemical characterization, and a potentiostat was utilized for potential control. The results showed that different light addressable potentiometric sensors demonstrated very high sensitivity and rapid response time to zinc, cadmium, and lead. And long-term stability was also investigated that the standard deviation was less that 0.12 mV in continuous tests for 4 h.

Besides traditional analytical methodologies used for environmental analysis such as spectroscopy and analytical chemistry, electronic tongue also plays a very important role in water environment monitoring. Compared to other approaches, electronic tongue presents significant advantages in terms of detection time, instrumentation cost, on site measurement, and multielement analysis.

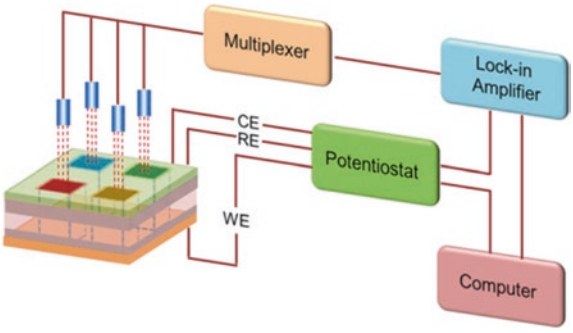

Fig. 2.14 Schematic diagram of the multi-sensor array measurement setup (Reproduced with permission from Ref. [79]. Copyright 2012 Elsevier)

2.5.3 Process Monitoring

The electronic tongue has developed rapidly in recent years due to its potential application in various fields. Besides the applications mentioned above, the electronic tongue is also used for process monitoring in industry. In some industries such as dairy industry, brewery industry, and fermentation industry, it is very important to monitor the quality parameters in the process. Normally, control of the process in these industries is achieved by controlling the time for different events, while no real-time information could be obtained in the process. Analytical tools for process monitoring should withstand complicated environments in the process, and no extra substance is allowed to be introduced in the background environment in case of pollution. Thus, some approaches such as electrochemistry are very hard to realize in the process monitoring due to the reference electrode. Electronic tongue with good ruggedness and simplicity is developed as a promising tool for industrial applications.

Winquist et al. [80] presented a specially designed voltammetric electronic tongue for application in the dairy industry. Two different electronic tongues were used, one consisted of three working electrodes of gold, platinum, and rhodium embedded in a dental material; the other consisted of four working electrodes of gold, platinum, rhodium, and stainless steel embedded in PEEKTM. In case of pollution from the reference electrode, two-electrode configuration was used to measure the current with large amplitude pulse voltammetry. All electrodes were directly immersed in the process line to monitor the conductivity, turbidity, and temperature in real time. The study showed that milk from different sources with different qualities could be identified. The information provided guidance for off-flavors monitoring, which is very concerned in dairy industry.

Parra et al. [81] introduced a novel hybrid sensor array based on voltammetric electrodes to monitor the aging of red wines and to discriminate wine samples aged in oak barrels of different characteristics (wood origin and the toasting level). The hybrid sensor array was formed by three families of chemically modified electrodes, including polypyrrole sensors doped with a range of counterious, carbon paste electrodes modified with metallophthalocyanine complexes, and carbon

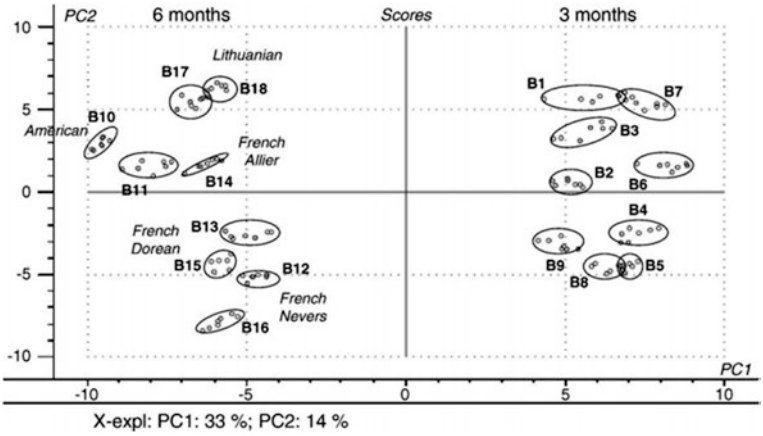

Fig. 2.15 PCA score plot obtained from the electronic tongue data of the wine aged during 3 and 6 months [81] (Reproduced with permission from Ref. [81]. Copyright 2005 Elsevier)

paste electrodes modified with perylene imide derivatives. The diversity of the sensing materials has allowed obtaining a high cross-selectivity in the responses of the sensors forming the array. All information acquired by the electronic tongue was used for principal component analysis and soft-independent modeling of class analogy to confirm the high capability of discrimination and classification of the electronic tongue. Figure 2.15 presented the PCA score plot for the wine aged in the nine oak barrels during 3 months (B1–B9) and during 6 months (B10–B18). Wine samples were discriminated into two groups: red wines aged during 3 months were situated on the left side and wines aged during 6 months were located on the right side. The results indicated that the electronic tongue was able to monitor the process of aging in oak barrels.

2.6 Summary

Various electronic noses and electronic tongues have been developed by the combination of nonspecific sensors array. A significant feature of traditional electronic nose and electronic tongue is the global selectivity which is important in recognition of odorants and tastes. It results from the nonspecific characteristics of solid-state sensor array. Comparing with the other artificial olfactory and gustatory techniques that discussed in the following chapters, traditional electronic nose and electronic tongue have absolute superiorities in some aspects, e.g., low cost, rapid detection, and convenient operation. Those features made them widely used in food industry, environment evaluation, and public security.

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Chapter 3
Olfactory Cell-Based Smell Sensors

Yanli Lu and Qingjun Liu

3.1 Introduction

Olfaction is initiated by the target chemical molecules binding to their corresponding receptors or ion channels. Subsequently, through cellular signaling pathways, chemical signals are translated to electrical signals. The electrical signals are propagated along the neurons axons to the upper organs, where the signals are processed and encoded, and the output signals are then transmitted to the brain. At last the brain can decode the signal and discriminate the corresponding olfaction. Taking advantages of the phenomena learned from nature, biometric engineers have already made many devices for practical applications for odor detections [1–4]. One of the most widely studied devices is smell biosensors based on olfactory cells.

Taking bioactive units as sensitive elements, the novel artificial olfaction systems in vitro were firstly developed and studied by Göpel and his colleagues [5]. It inspired us that those olfactory neurons and biomolecules were promising sensing materials to establish smell sensors. As the encapsulated arrays of molecular sensors, such as receptors and ion channels, cells can maintain physiological stable manners and be responsive to analytes via native cellular machinery. One of the most widely sensing signals that emitted by cells and detected by sensors or transducers are electrophysiological signals that related to cell functions [2, 3, 6–8]. Characterized by rapid response and excellent selectivity as well as high sensitivities, cell-based biosensors have been successfully applied in many biomedical and environmental studies. Along with analyte sensing and analysis, this kind of

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biosensors could also provide the advantages of *in situ* physiological monitoring using cells. Olfactory cell-based biosensors are among these biosensors that using olfactory cells as sensing elements, to detect the interactions between different analytes and the olfactory cells.

Compared to sensitive materials of traditional electronic noses, the bioactive cells that were extracted from primary sensing organs and cultured in vitro and were more promising. Sensors incorporated mammalian cells have the distinct advantages of responding in the manners that can offer insight into the physiological effects of an analyte [1, 4, 9–11]. Especially, in order to get much better sensitivities, selectivities, and faster responses, some approaches even tried to knocking in or knocking out some special receptors on cell lines to get measurements of the receptor-ligands interactions incorporated with cell-based biosensors [12–14].

In our previous studies, utilizing cultured olfactory cells as sensing elements, we have established the microchips of smell sensors [1, 2]. Moreover, novel bioelectronic noses that combining electrode arrays with intact olfactory epithelium, which were extracted from the primary olfactory system with structural and functional integrity of the receptor cells well preserved, was also developed. The olfactory cell and sensor hybrid systems, taking advantages of both elements, can detect real-time extracellular signals under odorant stimulations for prolonged periods. In this chapter, we will mainly give the detailed discussions about the olfactory cell-based smell systems and the promising applications, while the descriptions about receptors and epithelium tissues can be found in other chapters of this book.

3.2 Theories of Olfactory Cell-Based Smell Sensors

3.2.1 Biology of Olfactory Receptor Cells

There are two to three thousand distinct olfactory receptor neurons containing in the animal olfactory epithelium. Cultured rat olfactory neurons are excitable and can respond to odors [15]. Although there is a large family of receptors neuron types, numbering approximately 1000, each receptor cell class can respond to many different odorants. Thus, any particular odorant activates a substantial subset of these receptors, on the order of hundreds of receptor types. The great variety, exquisite specificity, high sensitivity and fast response of olfactory receptor neurons make them an ideal candidate for olfactory cell-based biosensors.

The mechanisms of signal detection and transduction in olfaction is an electrophysiological process, mainly taking place among olfactory epithelium receptor cells and their corresponding mitral cells in olfactory bulb, and then the signals transferred to the olfactory cortex [16]. In this electrophysiological way, odors are apperceived. The investigation has found out that if odor information were not encoded into action potentials, the information could not be transmitted exactly to the olfactory bulb [17]. Therefore, a satisfactory olfactory cell-based biosensor

should be a hybrid system of olfactory neurons and extracellular potential detection transducers. However, there are many difficulties for olfactory cell-based biosensors. Neuron cultures were more rigorous in their choice of sensor substrates. Therefore, the measurement of the electrical responses of neural cells or even neural networks presents a major challenge. Although special receptors on cell lines also can be used to determine and characterize the response profiles of the receptors and provide functional assays of receptors on cells, the electrical properties of cell lines are very difficult to be recorded in the biosensor design.

3.2.2 Electrophysiology of Olfactory Cells

In vertebrates, when olfactory mucosa epithelium is exposed to odorants, sensory transduction occurs in the cilia of olfactory receptor neurons [11]. The olfactory receptor neurons and the ion channels involved in the process of response to odorants are shown in Fig. 3.1. Initially, an odorant molecule binds to a seven-transmembrane-domain receptor protein on the olfactory cilia. Then, a G-protein-coupled cascade may be triggered and the enzyme adenylyl cyclase is activated, which results in an increase in intraciliary adenosine-3′,5′-cyclic monophosphate (cAMP). When cAMP increases, it forms directly gates ciliary channels. The opening of cyclic nucleotide-gated (CNG) channels allows calcium and other extracellular cations into the cilia, generating an inward current. When large amounts of Ca^{2+} accumulate in the cilium, Ca^{2+} -gates chloride [Cl(Ca)] channels were activated, creating an outward flow of Cl^{-} ions and amplifying the inward current that results in membrane depolarization and the generation of action potentials in the cell soma. The Cl(Ca) channels remain open until enough calcium is extruded from the cilia via the Na/Ca exchanger (NCX) [18, 19].

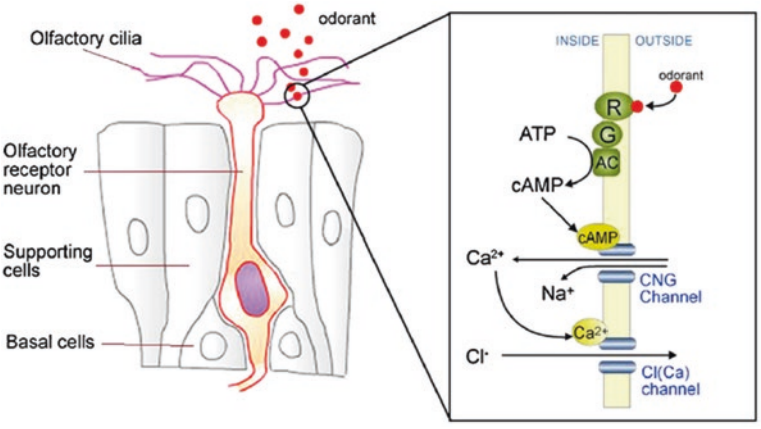

Fig. 3.1 Olfactory receptor neuron and the ion channels in the cilia. (Reproduced with permission from Ref. [11]. Copyright 2010 Elsevier)

After odor stimulation, ions flow in and out of the membrane causing ion channel currents which can be modeled as the following:

$$I_{\mathrm{CNG}} = \frac{\mathrm{cnmax} \cdot \mathrm{cAMP}^{n1}}{\mathrm{cAMP}^{n1} + (\mathrm{inhcng} \cdot \mathrm{hmc}_1)^{n1}} \cdot (\mathrm{vcng} - V). \tag{3.1}$$

With

$$\mathrm{inhcng} = 1 + \frac{(\mathrm{inhmax} - 1) \cdot \mathrm{CaCaM}^{\mathrm{ninhcng}}}{(\mathrm{CaCaM}^{\mathrm{ninhcng}} + \mathrm{kinhcng}^{\mathrm{ninhcng}})}, \tag{3.2}$$

where cnmax was the maximum conductance of CNG channels; n1 was the hill coefficient of the CNG channel activation function; hmc1 was the concentration of cAMP needed to achieve half-maximum activation of the CNG channel; vcng was the reversal potential of CNG channels; inhmax was the maximum CNG channel inhibitor factor; kinhcng was the concentration of CaCaM needed for half-maximum inhibition of the CNG channel; ninhcng was the steepness of the sigmoid; inhcng represented the fold-increase in K1/2 of the CNG channel as a function of CaCaM concentration.

$$I_{\mathrm{NCX}} = F\mathrm{vol} \cdot J_E, \tag{3.3}$$

where Fvol was the product of Faraday's constant and ciliary volume, and JNCX was the Ca^{2+} flux through NCX.

$$I_{\mathrm{Cl(Ca)}} = \frac{\mathrm{clmax} \cdot \mathrm{Ca}^{n2}}{\mathrm{Ca}^{n2} + \mathrm{hmc}_2^{n2}} \cdot (\mathrm{vcl} - V), \tag{3.4}$$

where clmax was the maximal conductance of Cl(Ca) channels; n^2 was the hill coefficient of Cl(Ca) channel activation function; hmc2 was the concentration of Ca^{2+} needed to achieve half-maximal activation of Cl(Ca) channel, and vcl was the reversal potential of Cl(Ca) channels.

The total currents were described as:

$$I_{\mathrm{total}} = -(I_{\mathrm{CNG}} + I_{\mathrm{ClCa}} + I_{\mathrm{NCX}}) \tag{3.5}$$

I_{total} was the total current produced by the olfactory tissue after odor stimulation. In the equation above, we only considered the CNG current, Cl(Ca) current and NCX current which contributed mainly to the total current while neglected other currents, such as leak current whose value was small.

3.2.3 Theories of Cell Electrophysiological Recording

The electrophysiological signals that related to cell functions were the most widely testing signals in olfactory cell-based biosensors. In this part, we will take

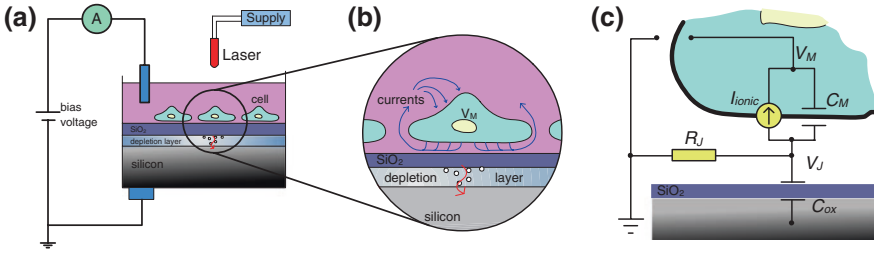

Fig. 3.2 Principle of the olfactory-LAPS system. **a** The schematic of cell-based biosensor using LAPS. **b** Simplified cell-semiconductor interface. **c** Schematic circuit of the cell-LAPS hybrid system. (Reproduced with permission from Ref. [1]. Copyright 2006 Elsevier)

light-addressable potentiometric sensor (LAPS) as an example to give a brief introduction about the theories of the cell electrophysiological recording.

LAPS is a commonly used semiconductor chip [1, 11, 20]. Lots of experiments have been done that investigate on LAPS as a possible cell-semiconductor hybrid system to monitor the extracellular potentials of the cells [21, 22]. Based on basic detection theories of the extracellular potential, olfactory neurons can be cultivated on the surface of LAPS to monitor their potentials (Fig. 3.2a). Moreover, LAPS can monitor the potential of exciting olfactory cell in a non-invasive way [2]. Since the surface of LAPS is laterally unstructured, cells can adhere without any spatial restrictions. When the cell produces potential changes by the ionic currents of the Na^+, K^+, and Ca^{2+} (Fig. 3.2b), which was equal to the change of bias voltage, and its photocurrent given corresponding fluctuation. The simplified schematic circuit of the cell-LAPS interface was shown in Fig. 3.2c. Based on the theories of cell-silicon junctions and circuits [23]:

$$\frac{V_J}{R_J} = C_M \frac{\mathrm{d}(V_M - V_J)}{\mathrm{d}t} + I_{\mathrm{ionic}}, \tag{3.6}$$

where V_J is the transductive extracellular potential, V_M the transmembrane potential, C_M the membrane capacitance per unit area, and I_{ionic} the total ionic currents through the cellular membrane, R_J the seal resistance. Because when the cell produces V_M changes, ionic and capacitive currents flow through the membrane. The concomitant currents along the cleft give rise to V_J between the cell and chip, which is equal to the change of bias voltage of the sensor.

3.2.4 Modeling of Cell Electrophysiological Recording

In order to interpret and analyze the properties of electrophysiological signals of olfactory cells, there should be a model to simplify them. Here, we will give an example. Through phase locking to a common underlying oscillatory potential,

olfactory processing can be achieved in the absence of synaptic interactions between neurons [24]. When olfactory cells were isolated from rat and cultured on the surface of sensors, they often exhibited spontaneous excitatory potentials. If cells were exposure to odorants, the cells can response in the form of oscillatory current. So in Eq. 3.7, $\times 0$ was set to 10 μm. g_{leak} and λ_{sheet} were 13.3 nS/μm^2 and 35 μm, respectively, obtained from [25]. Thus, field potentials can be written as [11]:

$$V_{\text{field}}(x,t) = \frac{I_{\text{total}}(t)}{13.3} \begin{cases} 1-\exp\left(-\frac{10}{35}\right)\cosh\left(\frac{|x|}{35}\right), & |x|<10 \\ \sinh\left(\frac{10}{35}\right)\exp\left(-\frac{|x|}{35}\right), & |x|>10 \end{cases} \tag{3.7}$$

Odorant molecules bound to the cilia with a G-protein-coupled cascade increased the concentration of cAMP, which was to directly activate a CNG channel and to make the intracellular Ca^{2+} change producing oscillatory current responses I_{CNG}. The outward flow of Cl^- ions amplified the inward current and resulted in the current $I_{\text{Cl(Ca)}}$. Extrusion of calcium from the cilia via the Na/Ca exchanger (NCX) caused the current I_{NCX}. $I_{\text{total}}(t)$ values in Eq. 3.7 were the sum of I_{CNG}, $I_{\text{Cl(Ca)}}$ and I_{NCX}, which were first calculated by MATLAB. Then, we used Eq. 3.7 to get the simulation results shown in Fig. 3.3.

Three axises were distance axis, time axis, and the normal axis representing the voltage changes, respectively. During simulation, the duration time was defined as long as 30 ms, and the distance was assumed to be as long as 200 μm. For the output results, the maximum peak value was calculated to be about 30 μV, whose position was the recording site. As time went on, signal amplitudes decreased, presenting an oscillatory phenomenon, whereas the oscillation period was 3.3 ms. The farther the distance was away from the recording site, the smaller the amplitude was, and even became a negative peak.

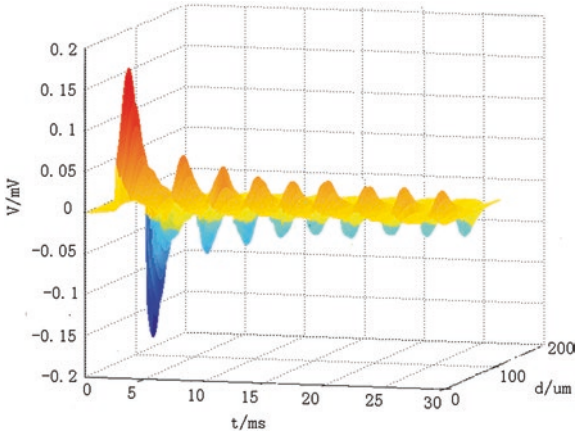

Fig. 3.3 Extracellular potentials simulation of olfactory receptor cells. (Reproduced with permission from Ref. [11]. Copyright 2010 Elsevier)

3.3 Design of Olfactory Cell-Based Smell Sensors

3.3.1 Culture Olfactory Receptor Cells on the Sensors

Olfactory receptor neurons are bipolar nerve cells. From their apical pole, the neurons extend dendrite to the epithelial surface, where they expand cilia, which are specialized for odorant detection. The coupling of olfactory cells with sensors is the key step to develop biosensors in order to meet the specific performance requirements for odorant detection.

Dissociated culture of olfactory receptor neurons can be prepared according to the established basic culture methods. And, to improve the biocompatibility of the silicon device [26], surface of the sensor, such as LAPS, can be coated with poly-L-ornithine and laminin mixture to promote the cells attaching to the surface of the sensors [21]. Cells were maintained for one week in LAPS device, containing 10 % fetal calf serum, at 37 °C under standard conditions of humidified air with 5 % CO_2. The cells were fed every 2 days with fresh DMEM [1].

At the same time, researchers also focused on the olfactory cells growth on biocompatible polymer films electrodeposited on silicon microsystems [27]. Monitored of the adhesion and proliferation rates of rat neuronal cell cultures indicated that polyethyleneimine (PEI) and polypropyleneimine (PPI) were the best substrates for cultivating olfactory cells. Recently, a study described the employment of a DNA-directed site-specific cell immobilization method, which can achieve controllable and high-efficient coupling between cells and the sensing microelectrodes [28]. In which, a pair of complementary thiol-modified single-stranded DNA (ssDNA) can be designed and synthesized. One of them can covalently attach to the plasma membrane of olfactory cells. The other one can be used as ssDNA probes and immobilized on the gold surface of the electrodes. Based on the mechanism of complementary hybridization, cells can be site-specifically immobilized for smell sensors.

3.3.2 Bioengineered Olfactory Cells with Specific Receptors

Recent studies showed that using cells to express specific olfactory receptors could be achieved by gene engineering [29, 30]. Through transient transfection, olfactory receptors can be expressed on the plasma membrane of different cells. Heterologous cell systems with expressed olfactory receptors, such as in yeast, Escherichia coli, human embryonic kidney (HEK)-293 cells, have been researched for biosensors [12–14, 31]. Generally speaking, the stages of cell-surface expression of olfactory receptors include olfactory gene screen and clone, transfer of recombinant vector to cells, transfection, immunocytochemistry or flow cytometry, odorant stimulation, and assay of functional activation.

The establishment of a robust heterologous expression system for mammalian olfactory receptors facilitated the high-throughput deorphanization of these

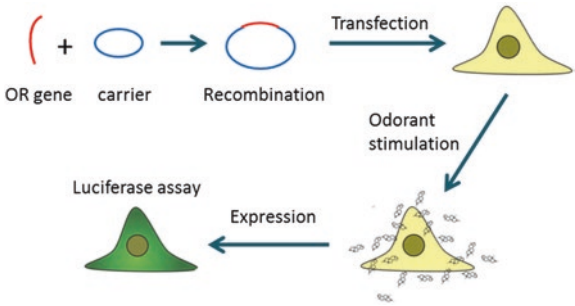

Fig. 3.4 Schematics of cell-based olfactory receptor synthesis and expression

receptors by matching them to their cognate ligands. The special protocols have detailed the methods used for evaluating the cell-surface expression and measuring the functional activation of receptors for transiently expressed receptors in cells [31]. As shown in Fig. 3.4, the mammalian olfactory receptors, contained in a mammalian expression vector such as pCI, can be transfected into HEK293T- or the HEK293T-derived Hana3A cells along with a luciferase reporter gene construct driven by a cyclic AMP-responsive element promoter and luciferase construct drive by a promoter. After transfection, cells could be stimulated with potential odorants. Activation of the receptor leads to accumulation of cAMP, which turns on the expression of the luciferase reporter gene. The expression of the specific olfactory receptors can be inferred though luciferase assay.

Through modifying receptors on the surfaces of cells, they can interact with odorants that are not their natural partners. It was one promising way to control signaling processes in different cells and in the cell lines. These hybrid biosensors, however, have an inevitably short functional period because the lifetime of the living cells. Furthermore, the reaction time was much longer than that in the original olfactory systems possibly because non-linear amplification by neural systems was not effective [32]. However, with the mechanisms of odorant discrimination in olfaction at a receptor level elucidated step by step, the functional evidence of these putative receptors recognizing and responding to specific odorant molecules could improve the development of the olfactory cell-based biosensors greatly.

3.3.3 Fabrication of Sensors and the Detecting Systems

Except for sophisticated patch clamp, there are many extracellular potential detection methods, such as field effect transistor (FET) array and microelectrode array (MEA) cell-based biosensor. Such methods can be called neurochip, offering a non-invasive and long-term method to monitoring cells and tissue [3, 33]. However, these methods are confined to measuring the potential only at a limited number of sites, such as the tip of individual microelectrode and the gate-electrode of individual FET, which make it difficult to culture cells on the place where the very sites designed. But by scanning light-pointer along its surface, the surface

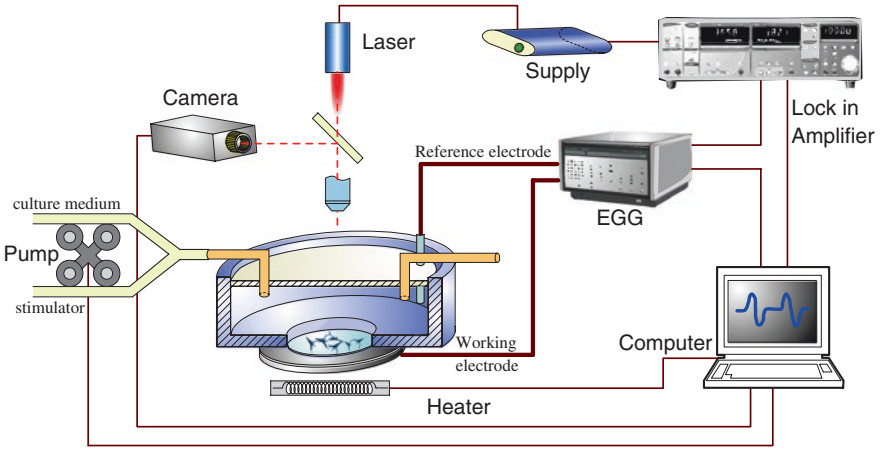

Fig. 3.5 Experimental system based on LAPS for electrophysiological sensing of olfactory cells. (Reproduced with permission from Ref. [1]. Copyright 2006 Elsevier)

potential at any desired position can be detected by LAPS [1, 34]. So we will introduce the fabrication of LAPS and the detecting systems in this section.

The LAPS chip and detecting setup were just similar to the system we have reported [34]. The experimental system is shown in Fig. 3.5. The LAPS consists of an electrolyte-insulator [SiO_2]-semiconductor [Si] (EIS) structure [21]. When the cell produces potential changes, photocurrent of the LAPS shows corresponding fluctuation, which is transmitted into peripheral equipments through working electrode of potentiostat.

At the same time, olfactory receptors expressed in heterologous cell systems, including ODR-10 that was expressed in *E. coli*, human receptor OR17-40 in *Saccharomyces cerevisiae* yeast cells, and rat receptor, I7, on the surface of human embryonic kidney (HEK)-293 cells and so on, also need design special sensor systems to recording the cellular responses [1, 12, 35]. Therefore, despite LAPS, there are several other sensors that utilizing these receptors for recording the cell electrophysiological signals, such as bioMEMS system, quartz-crystal microbalance (QCM), electrochemical impedance spectrometry (EIS), and surface plasmon resonance (SPR). All of these cell-based biosensor techniques are characterized with high sensitivity, excellent selectivity, and rapid response [4, 14, 35, 36].

3.4 Application of Olfactory Cell-Based Smell Sensors

3.4.1 Extracellular Sensing of Olfactory Cells to Odorants

Based on the previous studies about the response of olfactory cells to odorants, extracellular sensing of olfactory cells by LAPS will be illustrated in this part. In order to primarily testify the feasibility of odorants detection, different

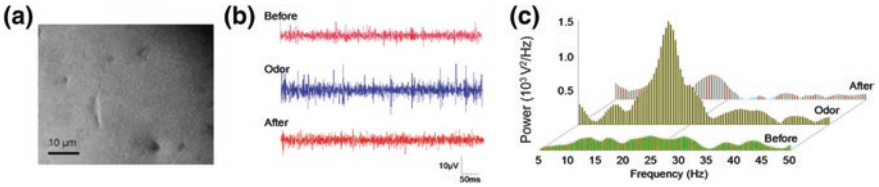

Fig. 3.6 Odorant-elicited extracellular potential of the olfactory receptor cells. **a** Olfactory cells cultivated on the LAPS. **b** The odorant presentation elicits regular, large-amplitude extracellular potential. **c** The odorant uniquely and consistently elicits strong 24 Hz frequency component extracellular potential. (Reproduced with permission from Ref. [1]. Copyright 2006 Elsevier)

concentrations (1, 25, and 50 μM/ml) of acetic acid (CH_3COOH, an organic acid, with a distinctive pungent odor) were taken as stimulant to olfactory receptor neurons in the study of the LAPS system (Fig. 3.6) [1]. We got the typical potential peaks of olfactory receptor cells cultured on the sensor. It was sustained in the whole course of the acetic acid's stimulation to the receptor cells. With FFT analysis, we also found that olfactory receptor neurons showed a specific appearance of 24 Hz, occurred repeatedly to the stimulant. The amplitude of the frequency was increased in a concentration-dependent manner and disappeared along with the stop of the odorant stimulation 10 min latter. The frequency signal represented the binding of the odorant to the receptor neurons, and only the receptor neurons gained odorant sensitivity.

Based on digital compensation of frequency domain, the surface potential at all illuminated regions can be measured simultaneously by analyzing the resulting photocurrent on the multi-light LAPS [37]. The olfactory neurons with sensor can be examined with multi-light systems to measure potential changes of neurons simultaneously. This work is necessary to develop bioelectronic nose, for each neuron in an odor coding assembly responds with an odor-specific temporal firing pattern consisting of periods of activity and silence [38]. These correlations can suggest whether olfactory neurons have influence on one another and respond in synchrony to temporal pattern or not. Observations of correlated firing also can provide more information of olfactory neurons connections and signal processing. This reveals a new potential application of this novel olfactory cell-based biosensor. Since the mechanism of olfactory sensory neurons is a complex pattern of neuronal networks, which makes the olfaction coding and decoding become very difficult to study by the current electrophysiological recording techniques, such as patch clamp [16].

To take advantages of extracellular potential detection, novel hybrid smell sensors were designed expressing ionotropic odorant receptors into dissociated neuronal cell cultures [32]. The combination of receptors and ion channels brings significant advantages such as easy functional expression, prolonged lifetime, and an ability to amplify the weak ionic currents of odorant receptors.

3.4.2 Cell-Based High-Throughput and Automatic Odorant Screening

In recent years, the development of cell-based high-throughput screening systems have attracted much attention of researchers who study drug screening mechanisms and characterization of G-protein coupled receptors (GPCRs) [29, 39]. Although olfactory receptors constitute the largest group of receptors that play a critical role recognizing and discriminating odors, only a few receptors have been characterized, and most remain orphan. The conventional cell-based assay system for characterizing G-protein coupled receptors, including olfactory receptors, was very laborious, time-consuming and often required an expensive assay system. Thus, researchers have developed simple, miniaturized odorant screening methods by combining micro-electro-mechanical system (MEMs) technique and visualization technique for detecting odorant responses (Fig. 3.7).

The new approaches of the high-throughput odorant detecting systems provided important potential advantages over the conventional odorant screening system [39]. First, cells can be cultured and transfected on a small-sized well providing an inexpensive and efficient culture environment for cellular assays. Second, because the receptor gene printed micro-well is highly stable based on polyethylene glycol (PEG), it can be fabricated in large quantity and can be stably stored for odorant screening. Thus, there was no need to conduct transfection of cells with olfactory receptor genes every time before seeding the cell on the micro-well. Finally, various receptor responses to the specific odorant can be visualized simultaneously by fluorescence imaging and patterned on miniaturized platform demonstrating its applicability to the medical, biological, and industrial fields.

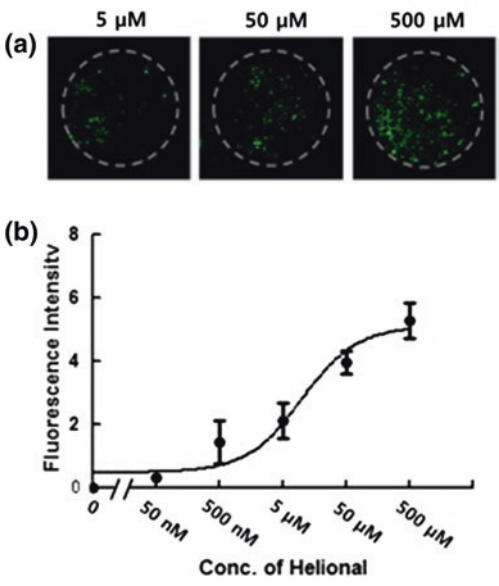

Fig. 3.7 Cell-based high-throughput responses of olfactory receptor to odorant. **a** Receptor of hOR3A1 expressing cells were stimulated with 50 nM to 500 μM of helional. The fluorescence increased with the concentration of helional. **b** The fluorescence intensity of each well was calculated and shown as line plot. (Reproduced with permission from Ref. [39]. Copyright 2010 Elsevier)

Especially, if combined with the sensing device and automatic control system, these smell sensors could achieve stable and reproducible odorant sensing [40]. Sensor systems for culture and measuring cell responses (e.g., electrophysiological recording and even fluorescence imaging), typically, employ a noise reduction system to cancel out background noise. However, with the development of micro- and nano-fluidic systems, semiautomatic or automatic devices can be used to install cells for stable and reproducible sensing [41]. Integrated with robotic systems, the cell-based biosensors can potentially be incorporated into portable systems for monitoring environmental and physical conditions.

3.4.3 Applications of Olfactory Cell-Based Smell Sensors

First, the potential of the olfactory systems, which can detect innumerable chemical agents with unparalleled sensitivity and selectivity, would be of immense value in the detection of environmental toxins and chemical warfare agents even at sublethal levels. Danny Dhanasekaran and colleagues genetically engineered a yeast strain with rat olfactory receptors and genetically linked it to the expression of green fluorescent protein [40]. When the olfactory receptor "smells" the odor of 2,4-dinitrotoluene, an explosive residue mimic, the biosensor turns fluorescent green.

Olfactory cell-based biosensors also have been developed for assessing food quality that mimics the way receptors in a canine nose. Seunghun Hong and colleagues have designed an olfactory cell sensor that detects hexanal, a volatile compound produced when food is past its best [41]. The sensor consisted of nanovesicles with cells expressing canine olfactory receptors specific for hexanal (Fig. 3.8). Then, the vesicles were immobilized onto carbon nanotube transistor (CNT). When the hexanal bounded to the vesicles, this caused an influx of Ca^{2+} into the vesicles and increased the nanovesicles' potential in the vicinity of the CNT. Finally, hexanal can be detected by the conductance changes of CNT the

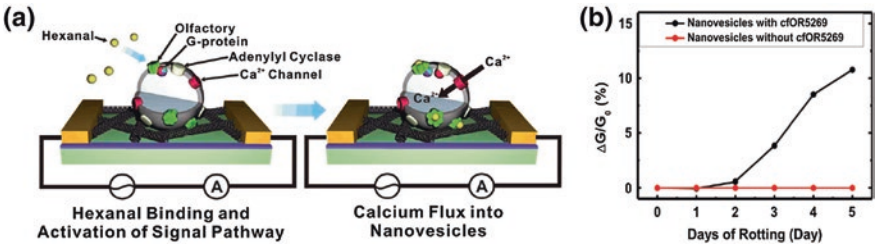

Fig. 3.8 The nanovesicles sit in the carbon nanotube channel. When hexanal is present, it binds to the olfactory receptors, such as cfOR5269, causing an influx of Ca^{2+} ions into the vesicles leads to a decrease in the conductance in the channel (**a**). Graph showing the conductance changes in OCBs after the introduction of spoiled milk (**b**). (Reproduced with permission from Ref. [41]. Copyright 2010 Elsevier)

channel. They tested the sensor with spoiled milk and found that the conductance changed measurably and the response increased as more days went by. The sensor could detect hexanal down to 1fM, even when it was mixed with its analogs pentanal, heptanal, and octanal.

For biomedical fields, studies suggested that a number of volatile substances from human body, such as the exhaled breath, are probably connected with the condition of the person [42]. Some compounds that through the human sense of smell have closed relationships with certain diseases, such as acetone is related to diabetes mellitus. Based on the dog's sensitivity olfaction, many previous researches have shown that trained dogs were able to detect bladder, lung, or breast cancer in urine [43–45]. However, it is expensive and time-consuming to get the trained "sniffer dogs." Therefore, olfactory cell-based smell sensors that maintained the selectivity and sensitivity of animals' olfaction showed great potentials in disease detection.

3.5 Summary

This chapter mainly demonstrated the olfactory cell-based smell sensors, which was developed from conventional cell-based biosensors and electronic nose researches, and investigated the responses of the olfactory cells, especially olfactory receptor neurons, under stimulations of odorants. Combined with sensors or transducers, the olfactory cell-based smell sensors employed immobilized olfactory cells with odorant receptors as sensing elements to detect intracellular and extracellular physiological parameters and produced responses through the interaction between odorants and cells. The biosensors will be gradually improved by better preservation of the cell function and the development of new micro- and nano- sensor technologies. As the biosensors' performance improved, we believe that olfactory cell-based smell sensor technology will be well applied in the fields of food safety, environmental monitoring, and health care.

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Chapter 4
Smell Sensors Based on Olfactory Epithelium

Qian Zhang and Qingjun Liu

4.1 Introduction

The appearance of bioelectronic noses plays a great role in promoting the development of olfaction studies and odor detections for that these smell sensors take advantage of biological sensitivity of their bioactive materials and their sensor sections [1–3]. Olfactory cell-based smell sensors are of course among typical kinds of bioelectronic noses that are still active on the stage, which have been introduced in the previous chapter. However, some studies have found that neuronal mechanism in intact epithelium differs substantially from those determined in isolated olfactory receptor cells, which may due to differences in structure and function of cell population compared with biological olfactory system and lead to deviation in biological olfaction mechanism studies [4, 5]. Besides, isolated olfactory receptor cells were difficult to obtain, which was inconvenience in production and operation of these biosensors.

Studies showed that, in intact epitheliums, it was possible to estimate the electrochemical potential by keeping the neuronal membranes and environment intact after the epitheliums surgically removed [6–8]. The acute prepared intact epitheliums had several advantages to organotypic function over isolated olfactory receptor cell cultures for bioelectronic noses: (a) the natural states of the neuronal populations of olfactory receptor cells can be well preserved. (b) The functional receptor units of cilia on each olfactory receptor cell will not be damaged. (c) Extracellular compartments present in vivo (including supporting cells and basal cells) can be preserved. (d) The mucus layers with odor binding proteins generated

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by Bowman's glands and supporting cells will be preserved. (e) The intact epitheliums allow simpler acute preparation and easier visualization, without strictly controlled cell culture conditions (i.e., nutrient media, pH, temperature, and listerize).

Inspired by these, Liu et al. managed to establish a new type of bioelectronic noses, which combined intact olfactory epithelium with effective detecting instruments, such as microelectrode arrays (MEAs) and light-addressable potentiometric sensor (LAPS), called olfactory epithelium-based biosensors [9–11]. Researches have verified that such bioactive units extracted from primary sources can show higher sensitivity, better selectivity, and faster response compared with sensitive materials of traditional electronic noses [12–16]. With advanced tissue culture and micro-fabrication technologies, this kind of biosensors takes full advantages of both elements and can extract signals conveniently from the primary olfactory system with structural and functional integrity, showing great potentials in detecting real-time extracellular signals in the presence of odor stimulations [17, 18].

This chapter attempts to give a full sight of olfactory epithelium-based smell sensors. Taking olfactory epithelium and microelectrodes bio-hybrid systems as typical examples, detecting theories, modeling, manufacturing technique, and signal analysis methods of these bio-hybrid systems are all introduced. With gradually in-depth understanding of these biosensors, more suitable and powerful applications in the fields, such as food safety, environmental protection, and health care, will be found with these effective approaches.

4.2 Theories of Olfactory Epithelium-Based Smell Sensors

4.2.1 Structure and Sensing Mechanism of Olfactory Epithelium

In most vertebrates, the mechanism of olfactory systems is an electrophysiological process in which chemical signals are transduced into perception [19]. The initial olfactory sensing takes place in the olfactory epithelium which is a specialized epithelial tissue connected to the olfactory bulbs (Fig. 4.1a). Three types of cells compose the epithelium (Fig. 4.1b), and among them olfactory receptor cells are main sensing cells with axons that penetrate into the central nervous system, with supporting cells producing mucus and a heterogeneous population of proliferative basal cells serving as sources of new receptor cells [20].

The olfactory receptor cells extend their dendrites into the nasal cavity, ending in knobs calling cilia, which project into the mucus of the olfactory epithelium. These cilia are covered a large number of olfactory receptors that can specially interact with odor molecules, with the olfactory signal transduction cascade initiated and depolarization induced, leading to action potentials propagating through axons of the neurons [21, 22]. As a result, the olfactory epithelium completes the process to transduce chemical stimuli into bio-electricity signals, which can be detected by chemical or physical sensors such as MEAs.

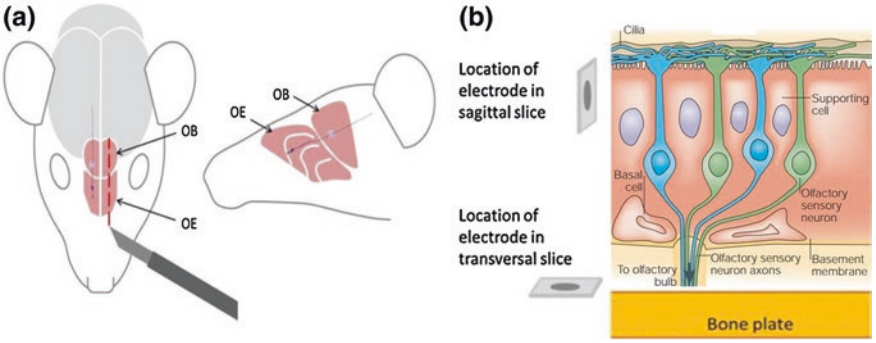

Fig. 4.1 **a** Illustration of the sagittal slice direction through the olfactory epithelium and bulb with a representative olfactory receptor cell and its connection to the bulb. (Reproduced with permission from Ref. [19]. Copyright 2013 Elsevier). **b** Illustration of the location of the MEA electrodes when using transversal of sagittal slices. (Reproduced with permission from Ref. [20]. Copyright 2004 Nature)

4.2.2 Epithelium Electrophysiological Recording on Microelectrodes

MEA chips have been extensively employed in tissue electrophysiological signal recording and can directly detect the extracellular activities related to cellular functions [15, 23–25]. With all transduction molecules located in cilia of olfactory receptors, olfactory receptor cells in olfactory epitheliums mainly use one molecular signaling system for transduction. Once an odor molecule is captured by an olfactory receptor, the receptor has conformational changes and stimulates a G protein. After that, adenylyl cyclase will be activated to form cyclic adenosine monophosphate (cAMP), which can bind to specific cation channels to induce opening of them and influx of Na^+ and Ca^{2+}. Consequently, Ca^{2+}-activated chloride channels are opened, generating ion current flow and membrane depolarization [16, 26–28].

With basal membrane contacted with microelectrodes of MEA, these electrophysiological activities can be recorded in the form of transmembrane potentials, including the rate of action potentials ("spikes") and groups of action potentials ("bursts") [29].

4.2.3 Modeling of Epithelium Electrophysiological Recording

When the olfactory epitheliums were attached on the multiple metallic film sites of microelectrodes, a conductive cleft would appear between the tissue and the electrode composing of extracellular solution [11]. Once action potentials of the

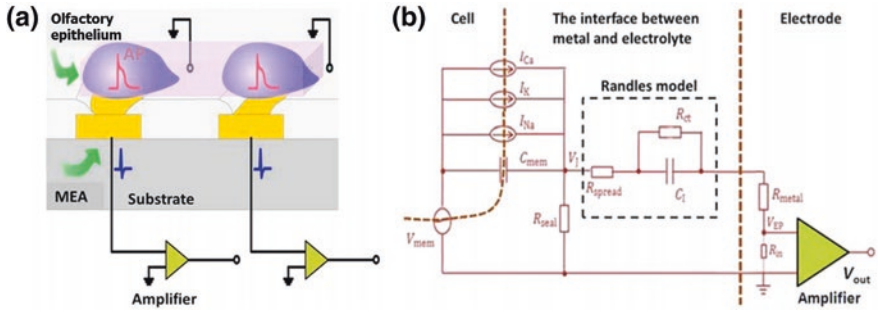

Fig. 4.2 **a** Schematic of the measurement setup of the olfactory epithelium and MEA bio-hybrid system. **b** Equivalent circuit of the signaling pathway in the system based on Randles model. (Reproduced with permission from Ref. [30]. Copyright 2014 American Chemical Society)

epitheliums were induced, ions flowing out of the cell membrane would move in the solution and could be detected at different sites of MEAs (Fig. 4.2a). Thus, the electrochemical properties of the epithelium-electrolyte interface are the basis for the tissue electrophysiological recording.

Based on the H-H theory and the solid-electrolyte interface model, the transmembrane current of the cell-electrode junction can be given by:

$$\boldsymbol{I_M = C_M \frac{dV_M}{dt} + I_{ionic}}, \tag{4.1}$$

where V_M is the transmembrane potential, C_M is the membrane capacitance per unit area, and I_{ionic} is the current induced by ions flowing through ion channels in the cellular membrane. This current of the olfactory receptor cells can be defined as:

$$\boldsymbol{I_{total} = -(I_{CNG} + I_{ClCa} + I_{NCX})} \tag{4.2}$$

where I_{CNG} represents the inflow of Ca^{2+} and other extracellular cations through the cyclic nucleotide-gated (CNG) channels; I_{ClCa} represents the outflow of Cl^- through Ca^{2+}-gated chloride (ClCa) channels; and I_{NCX} represents outflow of Ca^{2+} via the Na–Ca exchange protein.It has been known that extracellular potentials monitored by MEAs are due to ions flowing through the cell membrane. Then the total field potential of a receptor cell can be given by:

$$\boldsymbol{\frac{V_i}{R_{seal}} + \frac{V_J}{Z_{electrode} + Z_a} = C_M \frac{d(V_M - V_J)}{dt} + I_{ionic}}, \tag{4.3}$$

where V_i is the transmembrane voltage. V_J is the polarization voltage detected by electrode, which represents general extracellular potential detected by MEA. R_{seal} is the seal resistance, which can be defined as:

$$\boldsymbol{R_{seal} = \frac{\rho_{seal}}{d}\frac{l}{\omega}}, \tag{4.4}$$

where ρ_{seal} is the sealing resistivity, d is the thickness of average patch-to-insular distance, l and ω are the length and width of the effective portion of electrode coupled to the patch of the tissue, respectively [11, 30]. According to these, the equivalent circuit of the olfactory epithelium-MEA bio-hybrid measurement setup can be expressed as the Randles model (Fig. 4.2b).

4.3 Design of the Olfactory Epithelium-Based Smell Sensors

4.3.1 Preparation of Microelectrode Arrays

MEAs are utility multichannel sensing devices which integrate multiple microelectrodes to form arrays [31]. With olfactory epitheliums attached on MEAs, the integration of these electrodes allows simultaneous and long-term extracellular recording of extracellular potentials of the epitheliums at different sites (Fig. 4.3a), which can provide important information regarding network structure and function that is difficult or impossible to obtain using other electrophysiological techniques [29]. Therefore, MEAs have wide applications in detection of tissue

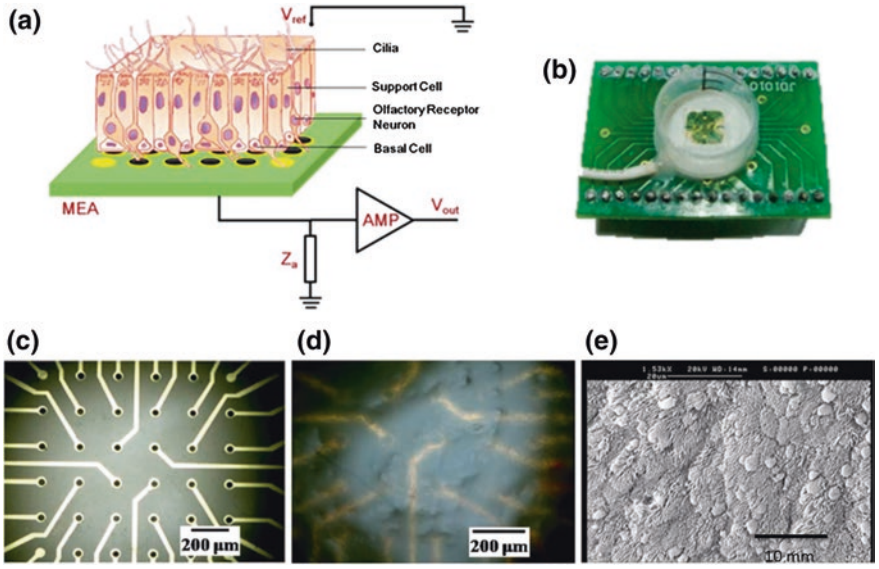

Fig. 4.3 **a** Illustration of olfactory epitheliums fixation on MEAs. **b** Photo of a 36-channel MEA chip. (Reproduced with permission from Ref. [11]. Copyright 2010 Elsevier) **c** Microphotograph of microelectrodes of a 36-channel MEA. **d** Microphotograph of the intact olfactory epithelium fixed on electrodes. **e** SEM photograph of the intact olfactory epithelium. (Reproduced with permission from Ref. [17]. Copyright 2011 Elsevier)

electrophysiological signals and studies on the signal firing mechanism, such as in vitro excitotoxicity testing and drug discovery [32, 33].

Figure 4.3b displays a photo of a 36-channel MEA chip. The fabrication and preparation procedures for such chips are described as follows [3, 11]. Microelectrodes of MEAs were fabricated by depositing Au, Ir, Pt, or other metals onto glass or silicon substrate. Then, the photoresist was spinning coated onto the metallic layer and exposed to ultraviolet light under the mask with defined electrode layout. As a result, the metal without protection by photoresist was removed, while the electrodes and interconnects are left. Subsequently, Si_3N_4 or SiO_2 layers for electric isolation of interconnects were deposited onto the chips by the process of plasma enhanced chemical vapor deposition (PECVD). Finally, the electrode pattern and external contact pads ware formed by wet etching in HF solution. Besides, the microelectrodes can be electrodeposited by platinum black (Fig. 4.3c) to improve the signal-to-noise ratio of the chip [34].

4.3.2 Isolation and Fixation of Olfactory Epitheliums

Isolated neurons require demanding extracellular culturing environment and odor delivery while the intact epithelium can easily be obtained and preserves native state, which brings great convenience for odorants detection [17]. Some studies even have introduced MEA recordings within sagittal slices of the olfactory tissues with attached bulbs instead of transversal slices (Fig. 4.1b), for that sagittal slices provide enhanced contact between olfactory cells and electrodes, resulting in higher signal-to-noise ratios [19].

Sprague Dawley rats are sources of olfactory epitheliums. In experiments, they were euthanized and their heads were hemisected in a midsagittal plane with the blade passing between the septum and the lateral wall. The olfactory epitheliums covering on the septum were removed from underlying cartilage and bone carefully [7]. Isolated epitheliums were then rinsed with Ringer's solution and placed with cilia receptors side up on the sensors' surface (Fig. 4.3a), by which cilia of the epitheliums could be exposed to stimuli with olfactory receptor cell axons attached on the electrodes (Fig. 4.3d). After rinsing, the solution would be removed from the MEA petri dishes and these tissues would be fixed by plastic ring-shaped frames covered with tightly stretched pieces of mesh.

Observed by the scanning electron microscope (SEM), the olfactory cilia formed a dense meshwork expanding naturally on the olfactory epitheliums, indicating well-preserved cilia with basic structures of receptor neuronal populations (Fig. 4.3e). The olfactory epitheliums on the MEAs should be kept in standard perfusate to maintain their biological activities, for preserving neuronal connections between epitheliums and bulbs and improving functionality and survival of olfactory receptor cells [3].

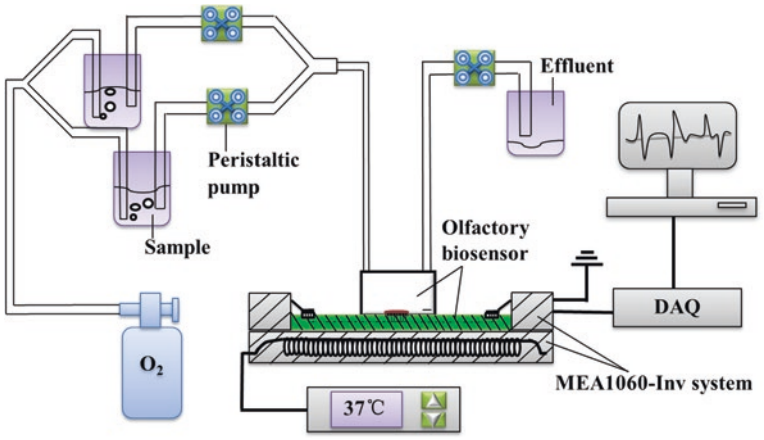

Fig. 4.4 A typical olfactory epithelium and MEA bio-hybrid system

4.3.3 Sensor System and Data Processing

Olfactory biosensors can achieve stable and reproducible odorant sensing if combined with the sensing device and automatic control system [35]. Thus, it is also important for construction of the olfactory epithelium-based systems. Figure 4.4 displays a typical olfactory epithelium biosensor detection system with sample sinks, peristaltic pumps, data collection and amplification section, temperature controller, a data acquisition card (DAQ), and software controlling section on the display screen.

As odorants were sensed in a liquid state in the nasal cavity, odor experiments with this hybrid system contain following steps: Odorants under test will be dispersed into standard perfusate and filled with sample sinks. With let-in and let-out rates controlled by peristaltic pumps, the data collection and amplification section can acquire the bio-electricity signals induced by odor stimuli and detected by MEAs. Temperature controller was necessary to maintain the bio-activities for the olfactory epithelium. Under the help of DAQ in recording rate, multi-channel detection results will be displayed on the screen, which can be similar to what accepted in biological bulbs [17].

4.4 Application of Olfactory Epithelium-Based Smell Sensors

4.4.1 Recording of Electrophysiological Signals

The olfactory epitheliums with native nervous tissues can be easily exposed, dissected, and manipulated without damaging its functional integrity [4]. The defined epithelial strata afford facile identification of extracellular electrophysiological

recording sites with microelectrodes [18]. After specific design of MEA chips for attachment of olfactory epitheliums and construction of the whole bio-hybrid systems, transmembrane potentials of the epitheliums could finally be recorded at different sites synchronously. The olfactory epitheliums were preserved to keep native state for in vitro environments with suitable temperature, humidity, nutrient solution, etc., and generally could be stimulated by odorants.

Based on theories of the tissue recordings on MEAs, the transmembrane potentials were identified as transductive extracellular potentials, which was due to the reduction of electrode seal impedance and the improvement of tissue adhesion [18]. Figure 4.5a shows a typical transductive extracellular potential recording detected by the 16-channel olfactory epithelium-based biosensing systems. The microelectrode recordings showed that many receptor cells would respond to the presentation of odorants, and these cells might be distributed across wide areas of the olfactory epitheliums, which were consistent with the widespread distribution of each receptor gene [20, 21].

Olfaction is initiated by molecular interactions of odorants with the olfactory receptors in the epitheliums. The molecular structure and their spatial molecular arrangement of interacting groups would be very important to the olfactory sensing [36, 37]. Olfactory epithelium-based smell biosensors using living cells expressing special olfactory receptors could even simultaneously distinguish different types of chemicals that had differences in double bond isomerism or functional groups [35]. The primary events recorded by MEAs were extracellular potentials, but a series of additional information could be extracted from these signals [29]. To achieve analysis and discrimination of odorants, various analysis methods could be applied in studies on the olfactory epithelium-based smell sensors [3, 8, 11, 17].

4.4.2 Time and Frequency Analysis of Multi-channel Signals

One of the most intuitionistic manners to analysis response signals recorded by olfactory epithelium-based biosensors was time-domain analysis, by which basic characteristics representing response intensity of different stimuli, such as average amplitudes, durations, frequency rates and signal shapes, were typically assessed. MEAs had the benefit of detecting signals of many cells synchronously, which was convenient to comparatively analyze recorded information in parallel. Experiments based on the in vitro neurons coupling to MEAs suggested that synchronism of activities in neuron networks was considered as one of the elementary features [38–40]. Generally, one of multiple channels on MEAs with the most response signals was chosen for long-term analysis (Fig. 4.5b), which probably was where the specific olfactory receptor cell for the odorant located. A segment of recording data could be extracted from the single channel and then be classified by signal

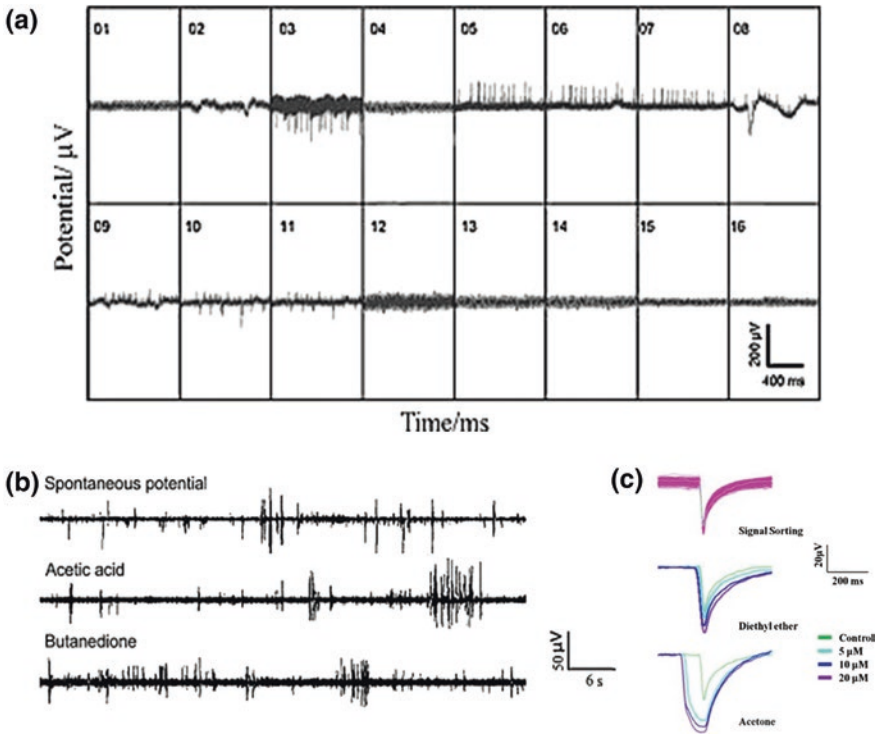

Fig. 4.5 **a** Multi-channel recording electrophysiological signals of olfactory epithelium. **b** Recording of electorphysiological signals of one channel after odorant stimulation. (Reproduced with permission from Ref. [11]. Copyright 2010 Elsevier). **c** Signal sorting of spontaneous signal clusters and their average signals in the clusters (the *green thick lines*). (Reproduced with permission from Ref. [17]. Copyright 2011 Elsevier)

sorting under the specific condition for available parameters, and different odorants could induce different signal shapes (Fig. 4.5c), which were the basis of odor discrimination by time-domain analysis [11, 17].

Frequency and power spectrum analysis of olfactory cells responses to odors was often used to calculate the distribution of frequency band during odor presentation [41, 42]. One typical frequency spectrum analysis method was sonogram by the color-coded method. Figure 4.6a is a typical time-frequency distribution sonogram, which indicates that response signal waveforms of olfactory epitheliums may have diverse evident time-frequency distribution features under different odor stimulations [18]. On the other hand, power spectrum analysis could be applied to recognize different firing modes during the odorants detection. Specific peaks in the power spectrum reflected the periodic activities in olfactory system (Fig. 4.6b), which might indicate certain odor-dependent firing modes [11].

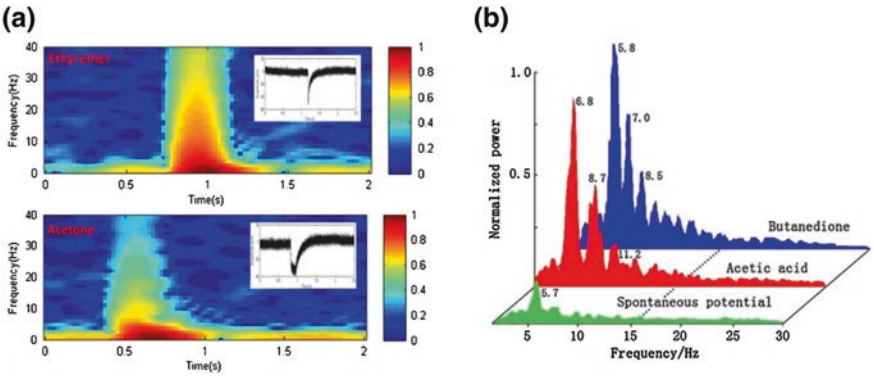

Fig. 4.6 **a** Sonogram spectrum analysis for different odorants. (Reproduced with permission from Ref. [18]. Copyright 2012 Springer). **b** Power spectrum analysis of response signals in different odorants detection. (Reproduced with permission from Ref. [11]. Copyright 2010 Elsevier)

4.4.3 Spatio-Temporal Analysis and Odorants Discrimination

If the action potentials were recorded from multiple neurons over a long time but simultaneously and with precision in the microsecond range, the analysis of the synchronicity, or co-occurrence of action potentials at different sites of the epitheliums can be obtained [29]. For olfactory epithelium-based biosensors, based on multi-channel recording performance of MEAs and structural and functional integrality of native olfactory epitheliums, spatiotemporal analysis can also be carried out to study the extracellular activity patterns of neurons in the tissues [11, 17]. Take a 16-channel olfactory epithelium-based biosensor as an example, recording of synchronous activities and pattern on all channels from the extracellular potentials could be determined by signal processing such as differentiation, square, slipped window integral, threshold selection ,and peak detection, indicating time offset characteristics of the multi-channel signals (Fig. 4.7a). As a typical spatiotemporal pattern of olfactory epithelium signals on MEAs, a 2-D time offset map could also be a helpful tool to study the internal relations and spreading of signals intuitionally (Fig. 4.7b). The spatiotemporal pattern allowed the visualization of signal spread across the epithelium and illuminated the signal propagation both in the transversal and longitudinal directions. Besides, cross-correlation coefficient sequences between any two channels could be calculated from intact spontaneous signals (Fig. 4.7c) and represented in heat pattern (Fig. 4.7d), implying the change of excitation centers. With signal specific firing mode of olfactory receptor cells elicited by certain odor stimuli, exploration on the synchronism of cell networks by spatiotemporal pattern analysis is helpful to obtain direct and global olfactory information to odorants in the olfactory epitheliums [17].

In the studies of traditional electronic noses, many typical pattern recognition methods have been used to realize the odors classification [5, 6]. Olfactory

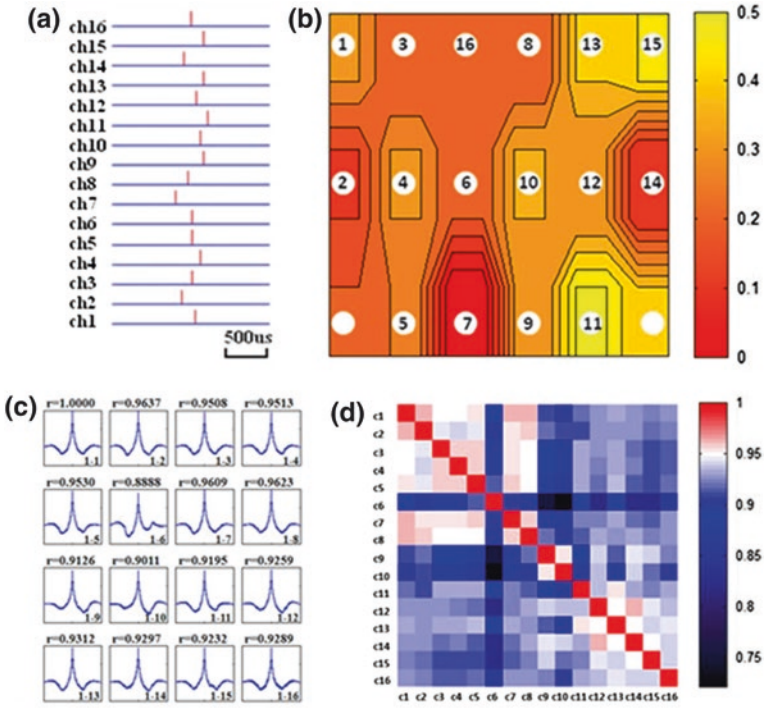

Fig. 4.7 Typical spatiotemporal patterns for multi-channel signals. **a** The peak locations of 16-channel extracellular signals. **b** 2-D time offset map based on the peak time. **c** The cross-correlation coefficient sequences between two channels of MEAs. **d** Heat pattern based on all of the coefficients. (Reproduced with permission from Ref. [17]. Copyright 2011 Elsevier)

pattern classification can visualize discrete neuronal network states, which can be a utility method in the future investigation [43]. Spatiotemporal analysis methods mentioned above were of course a useful tool for the classification. Besides, a 3-D pattern sensed by olfactory epithelium-based biosensors can also be applied in odorant discriminations (Fig. 4.8). 3-D pattern was similar to a smell sensory map, which reflects an orderly arrangement of neurons related with certain features of the environment. According to characteristics in terms of amplitude, duration, and firing rate, the response signals could be clustered into several regions. The signals located in the same main region contained similar signal features. With basic temporal characteristics of response signals, the 3-D pattern reflected the distribution of the odor response signals, and maps of the regions activated by one of chemical stimuli could be visualized with special pattern recognition. As a result, the 3-D pattern and spatiotemporal analyses could reveal the basic olfactory perception patterns and realize visual discriminations of response signals to different olfactory stimuli [18].

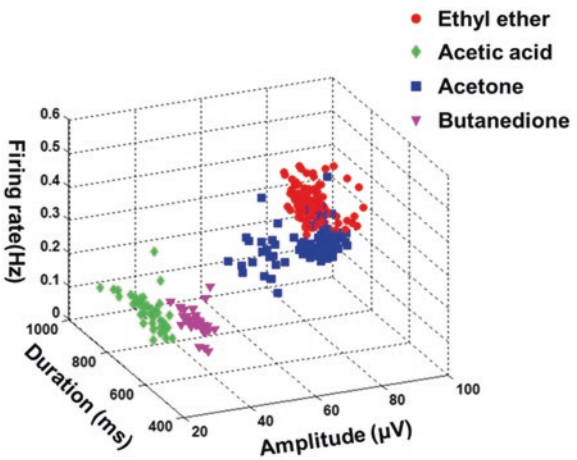

Fig. 4.8 3-D recognition pattern sensed by olfactory epithelium-based biosensors under the stimulation of different odorants. (Reproduced with permission from Ref. [18]. Copyright 2012 Springer)

4.4.4 Development and Applications of Olfactory Epithelium-Based Smell Sensors

For olfactory epithelium-based biosensors, there were two major requirements for the accurate recording of extracellular potentials of the epitheliums. One was to ensure a tight seal between epitheliums and microelectrodes so as to minimize signal loss to the bath medium. The other was to achieve low impedance across the cell-electrode interfaces to increase signal collection efficiency [44]. As an extracellular recording method, the MEAs manufactured by the micro-fabrication technology were non-invasive and allowed long-term and multiplexed measurements which, however, suffer from significantly reduced signal strength and quality.

Especially with the rapid development of nanotechnology, there was a strong tendency to change the microelectrodes of MEAs into nanoscale shapes such as nanopillars (Fig. 4.9a, b), which can significantly improve the signal strength of recorded action potentials and even can analyze one ion channels. Moreover, materials of microelectrodes can be modified by or instead of new materials, such as carbon nanotubes (CNTs) (Fig. 4.9c, d), for additional electrochemical properties or better attachment abilities with olfactory epitheliums [45].

And, it has been known that the detection limit of bioelectronic noses depends not only on measurement technique and detecting instruments, but also on signals obtained from biological elements [46–48]. Thus, enhancing original biological signals will also give improvement to olfactory epithelium-based biosensors. Integrated with nanoparticles such as zinc nanoparticles [49], the olfactory epithelium-based biosensors could detect increasing response signals, which reflected improvements of sensitivities and signal-to-noise ratio, indicating more active electrophysiological activities of the epitheliums and new directions to develop this kind of bioelectronic noses.

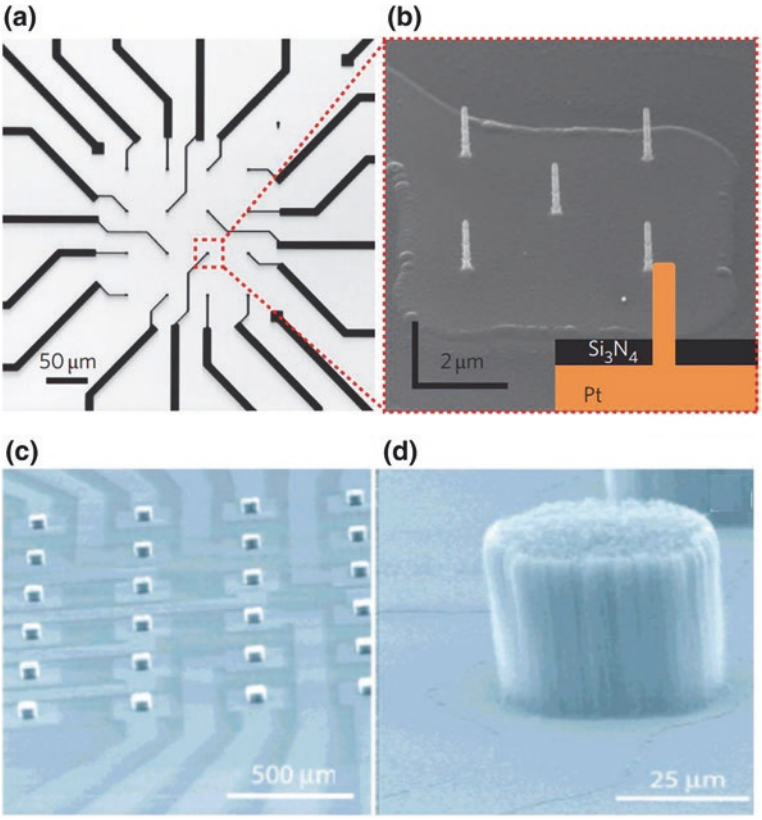

Fig. 4.9 **a** Optical image of a nanopillar electrode device with a four-by-four array of platinum pads. **b** SEM image of an array of five vertical nanopillar electrodes on one of the platinum pads. (Reproduced with permission from Ref. [44]. Copyright 2012 Nature Publishing Group). **c** A 6 × 6 CNT pillar microelectrode array of 30 × 30 µm. **d** A 50-µm-diameter CNT pillar microelectrode. (Reproduced with permission from Ref. [45]. Copyright 2006 American Chemical Society)

Progress in cell culture and micro-fabrication technologies has contributed to the development of the bio-hybrid systems for the functional characterization and detection of drugs, pathogens, toxicants, and odorants [2, 3]. Previous studies of correlation analysis for cultured neuron networks on MEAs have found that the homogeneous chains of subpopulations are connected by synapse with spatially neighboring sites, which could be recorded by the MEAs [39, 40]. As olfactory epitheliums maybe expresses only a subset of the entire receptor repertoire, the multi-channel recordings by MEAs were useful for this kind of compartmentalized anatomically and functionally discrete units of topographically localized receptors. At the same time, intact epithelium studies have found that although olfactory potentials primarily arise from the receptor cells, support cells are also possible to

contribute to the potentials directly [4, 7]. Thus, olfactory epithelium-based biosensors, whose olfactory epithelium well preserves natural state of cell populations and their extracellular compartments presented in vivo, can be benefit to study the mechanisms of the olfactory system as a convenient in vitro recording method. Besides, these biosensors can be constructed to distinguish the different types of chemicals with only a slight difference [18]. Combined with nanotechnologies, the olfactory epithelium-based smell sensors will potentially be incorporated into portable systems for environmental monitoring, food administration, and health management in the future.

4.5 Summary

In this chapter, a kind of bioelectronic noses which applied intact olfactory epitheliums as bioactive units are introduced. With well-preserved cell populations and functional receptor units, these biosensors can imitate real biological olfactory system and response to odorants. Under the help of quantitative instruments such as MEAs, the biosensors are suitable for detecting and discriminating odorants specifically binding to receptors in the olfactory epitheliums. With the rapid development of tissue culture and micro- and nano-fabrication technologies, the olfactory epithelium-based smell sensors will be a powerful tool for detecting and discriminating various odors.

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Chapter 5
Smell Sensors with Insect Antenna

Chunsheng Wu, Liping Du and Ling Zou

5.1 Introduction

Biologically inspired sensors utilize highly developed biological olfactory functional components as sensitive elements for the detection of specific chemical compounds. Biological olfactory functional components, including olfactory tissues, olfactory cells, and olfactory receptors, are evolutionary tuned for the detection of environmental chemical compounds. After millions of years of natural evolution process, insect antennae have become special chemical sensing organs with extreme high sensitivity and specificity, which are complex biochemical detection system for the detection of specific volatile organic compounds even at the trace level. The chemical signals sensed by insect antennae provide essential information about the surround environments for the insects to find hosts and mating partners as well as to avoid the enemies and competitors. The unique sensing capability of insect antennae is much higher than almost all current available analytical instruments and devices. The excellent performances of insect antennae for chemical sensing make them ideal candidates to be used as sensitive elements for the development of insect antenna-based biomimetic smell biosensors, which have many potential applications in a wide range fields such as plant protection, agricultural production, environmental monitoring, and fire detection. Due to their promising prospects and potential applications, insect antenna-based smell sensors have attracted more and more interests and have become one of the most active areas of analytical research. On the one hand, biological functional components originated from the insets, which include isolated insect antenna, olfactory sensory

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neurons, and olfactory receptors, can be utilized as sensing components in smell sensors. Upon exposure to the specific volatile organic compounds, these biological functional components can generate corresponding responses. It is thus important to get better understanding on the signal transduction mechanisms of insect antenna for chemical sensing, which can greatly contribute to the development of insect antenna-based smell sensors. For example, the knowledge on the selectivity and sensitivity pattern of insect antennae for chemical sensing allows the better design of biomimetic smell sensors by the combination of amazing capability of insect antennae with various reliable transducers. On the other hand, the fast advancements in micro fabrication technologies and nano technologies also promote the research of insect antenna-based smell sensors by providing novel transducers and coupling technologies to enhance the performances of chemical sensing applications. The reliable coupling and high-efficient signal transduction provided by the novel transducers and devices are crucial for the performances of insect antenna-based smell sensors.

Insect antenna-based smell sensors that combine the insect antennae with technical transduction devices have a vast range of possible designs and applications due to the large number of insect species equipped with diverse functional antennae for various chemical compounds. The natural olfactory systems provide sufficient chemical diversity for the development of biomimetic smell sensors for different applications that are usually related to the detection and monitoring of specific chemical compounds in the surround environments or during agricultural and industrial production. In addition, the ecological interactions between insect antennae and the environmental chemical compounds allow novel biomimetic approaches for the design and construction of insect antenna-based smell sensor systems that can be applied in the fields of agriculture production. For instance, antennae from jewel beetles have been used as sensitive elements for the development of biomimetic smell sensors for the detection of smoke, which could be applied in the fire detection of storehouses and public buildings as well as for in early warning detection systems for forest fires. In addition, the fast advancements in the nano- and biotechnologies also provide novel strategies for the development of insect antenna-based smell sensors, which contribute great to the performance enhancement and practical applications of biomimetic smell biosensors. For example, the insect antenna-based smell sensors developed on the basis of integration of insect antennae with field-effect transistor (FET) are able to detect the volatile organic compounds in a trace level that is as low as ppb range.

Insect antenna-based smell sensors have achieved significant progress and shown promising prospects and applications in the recent two decades. In this chapter, the basic mechanisms and recent progress in the development of insect antenna-based smell sensors will be reviewed in detail. In the first section, the theories of antenna-based smell sensors will be introduced in the following three main parts: the structure and electrophysiology of the insect antenna, tissue electrophysiological recording, and modeling of insect antenna electrophysiological recording. In the second part, the design of insect antenna-based smell sensors will be discussed, which include the preparation of transducers, the isolation and

fixation of insect antennae, and sensor system and data processing. Finally, the applications of insect antenna-based smell sensors in a wide range of fields will be summarized, which include the many fields of agricultural, industrial, and social fields. The development trends and the current main challenges of insect antenna-based smell sensors will also be proposed and discussed.

5.2 Fundamental of Smell Sensors with Insect Antenna

5.2.1 Structure and Electrophysiology of Insect Antennae

The sensitive and specific detection capability of insect for volatile organic compounds are mainly due to their highly evolved and specialized sensory organ, the antennae. Insect uses antennae to detect chemical volatiles in their surrounding environments in order to communicate with other individuals and identifies the nutrition sources, navigation, or avoid the dangerous [1]. The sensilla are distributed on the surface of antennae and palp, which contain the olfactory receptors that can interact with molecules of target volatiles [2]. There are various types of sensilla that can be distinguished under scanning electron microscopy (Fig. 5.1), which function as different sensing unit for different surrounding signals such as chemical signals, mechanical signals, thermo signals, and humid signals [3]. The special types of sensilla for olfaction and taste are single-pore sensilla and multi-pore sensilla, respectively. When insects are exposed to stimulus molecules, the olfactory sensilla localized on the antenna allow the stimulus molecules reaching inside the sensilla through the pore tubules to initiate the olfaction process. Figure 5.1e is the scanning electron microscope image of sensilla basal part, which clearly shows the perforation cover of the entire surface. These pore tubules allows the chemical volatiles arriving at the sensilla lymph (Fig. 5.1f), where the chemical volatile molecules can be captured by the odorant binding proteins (OBPs) to form a hydrophobic volatiles soluble to avoid the degrading of volatiles by enzymes and transported through the aqueous sensillum lymph and arrived at the outer membrane of dendrites (Fig. 5.1g). OBPs that bind with volatile molecules can interact directly with the olfactory receptors located in the cellular membrane or release the volatile molecules near olfactory receptors to allow the direct interactions between volatile molecules and olfactory receptors [4]. The specific interactions can thus initiate a cascade of intracellular biochemical reactions, which is not completely understood [5]. These intracellular biochemical reactions will result in the opening of ion channels or ion-channel-like chaperones located on the cellular membrane. This will lead to the cell membrane potential changes. If there have sufficient chemical volatile molecules to make enough ion channels open at the same time, the membrane potential changes could exceed the threshold of the cells and ultimately leading to the generation of action potentials [6]. By the following, the volatile molecules is released from the olfactory receptors and degraded by the special enzyme in the sensilla to avoid the repeatable and

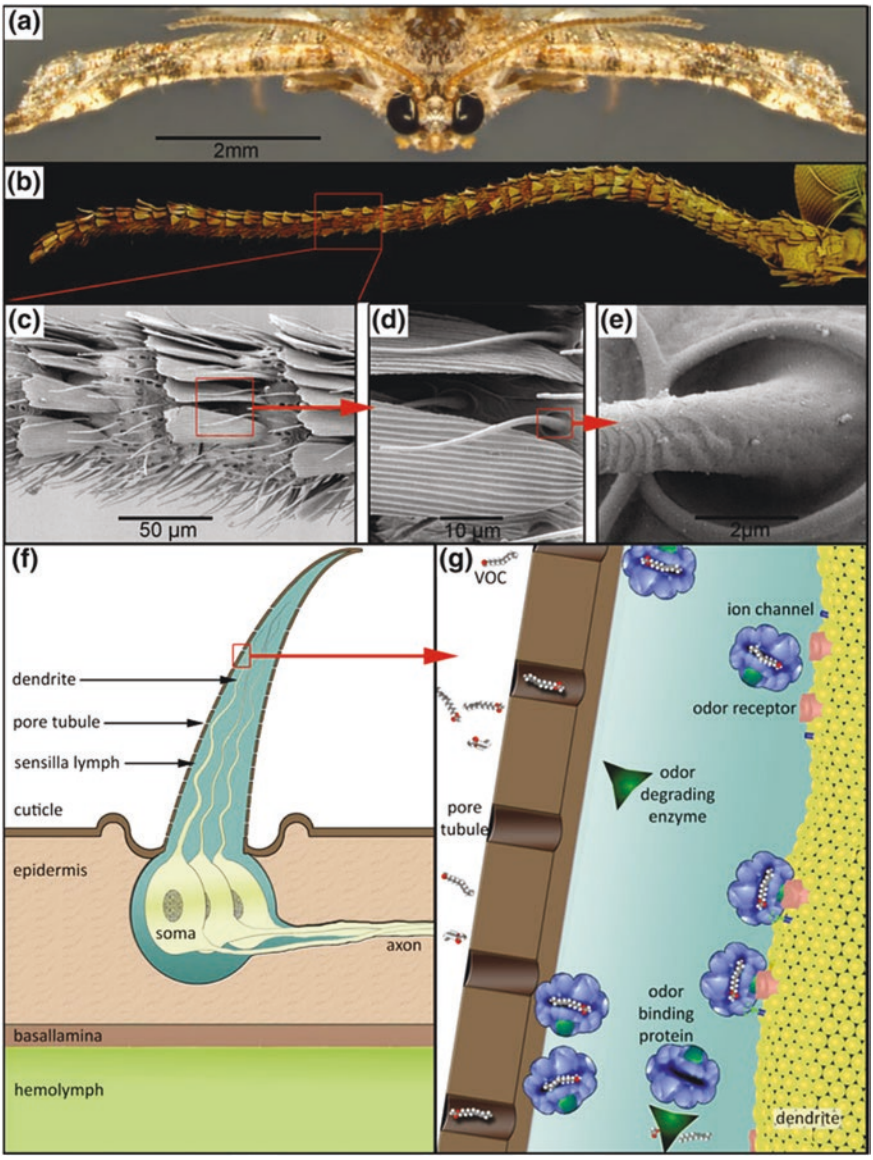

Fig. 5.1 Structure and signal transduction mechanisms of the insect antennae. **a** An image of the male *Lobesia botrana*. **b** Structure of the *L. botrana* antenna shown by combined and pseudocolored scanning electron microscopy image. **c**–**e** The structure of sensilla with pore tubule openings on the surface. **f** Schematic diagram showing the structure of a sensillum, with the receptor neurons consisting of dendrite, soma and axon that reach to the antennal lobe. **g** Schematics of signal transduction mechanisms of volatile reception at the dendrite membrane with the associated proteins and enzymes [8] (Reproduced with permission from Ref. [8]. Copyright 2013 Springer)

continuous stimulation of olfactory receptors by the same volatile molecules. With these signal transduction process, the chemical signals are converted into electrical signals generated by membrane potential changes of olfactory sensory neurons. The cellular electrical signals are then sent to the primary processing center of nervous systems via the axons of olfactory sensory neurons, where the olfactory signals receive primary process and then transmitted to the brain for further processing and decoding to achieve the final olfaction and generate a behavioral reaction [7].

The number of dendrites in the individual sensilla is usually from 1 to 5 [3]. But some exceptions like the hymenopteran *Sceliphon spirifex* contain 140 olfactory sensory neurons in one sensillum [3, 9]. In addition, the number of chemical compound that can elicit the response of insect antenna also varied among the insect species, which is mainly depended on their living mode. For instance, Moths can only respond to only one specific chemical compounds and show no response to other chemical compounds even in a very high concentration, which is known to live in a simple life mode [10]. On the contrary, the olfactory receptors of flies are usually responsible to several chemical compounds, where single-chemical compound can be perceived by pattern recognition [10]. The honeybee can also responds to many different chemical compounds, which is known to feed on many different plants and live in an environment with changing chemical compounds. This means that the specificity of insect antenna-based smell sensors is mainly depended on the types of insect antennae used as the sensitive elements for chemical sensing. The extreme high sensitivity of some insect antennae for the detection of specific chemical volatile compounds makes them suitable to be employed as sensitive elements for the development of antenna-based smell sensors. For instance, the black jewel beetle M. acuminate is able to detect the forest fires from several kilometers away, which is mainly attributed to their amazing high sensitivity of antennae for guaiacol compounds at the very low concentrations [11].

5.2.2 Electrophysiological Recording from Insect Antenna with Micro Devices

With the better understating on the signal transduction mechanisms of insect antennae, more and more details on the cellular events involved in the detection of chemical volatile compounds have been revealed. This also advances the development of antenna-based smell sensors. The extreme capabilities of insect antennae for chemical sensing have been used as sensitive elements in many ways such as the whole insect antennae in vivo or in vitro, olfactory sensory neurons, and olfactory receptors. All these strategies require the conversion of chemical signals into readable signals, for example, the electrical signals and fluorescent signals. The most direct and convenient strategies are usually involved in the recording of

electrical signals from antennae or olfactory sensory neurons representing the cellular responses to the specific chemical compounds. In this section, electrophysiological recording from insect antennae with different methods using various micro devices will be introduced, which are mainly divided into two categories: electroantennogram (EAG) measurements and FET-based methods.

EAG has become one of the most commonly used and well-established methods for the measurement of insect antennae in response to the chemical volatile compounds since its first report in 1955 [12, 13]. There are mainly two types of EAG measurement modes. The one is using two electrodes to connect the insect antennae to measure the responses of antennae between two electrodes when exposed to the chemical volatile compounds. Basically, the membrane potential changes generated on the dendrite surface usually are on the level of nano voltage, which require the further amplification by the peripheral circuits. Saline solutions (e.g., KOH or Insect Ringer's solution) or gels are often used for the coupling of electrodes with antennae, which can contribute to the maintaining of antenna functions and allow the measurement to be carried out in a longer term. The other type EAG measurement can be used to record the smell electrical signals from the antennae of dissected heads or whole insects. This method also uses two electrodes, but only one electrode is connected to tip of insect antennae and the other indifferent electrode is connected to the antennal suture or insect neck, which require the insect to be fixed to avoid the influences of motor neuronal signals on the measurement of smell signals [14, 15]. The position of electrodes placed on the insect is crucial to avoid the influences from other nerves. This type EAG measurement is suitable and favorable to be applied in the measurement on the antennae that can only survive for a short time after isolation from insects. Both type of EAG measurement can also be performed in the liquid phase, for example, the antenna from the water beetle *Dytiscus marginalis* show similar sensing capability both in the gas phase and liquid phase [16]. EAG is able to record the responses of insect antennae to the specific chemical compounds in vivo as well as in vitro, which is very useful and convenient for the recording of electrical signals from insect antennae representing the smell responding signals. However, there also some disadvantages that hamper the applications of EAG. Than main disadvantage is the EAG record almost all the responses of olfactory sensory neurons in antennae, which is usually the sum responses and it is difficult to discriminate or isolate the pure olfactory responses from individual olfactory sensory neurons. In addition, isomerism is sometimes required to be considered in order to interpret the recording signals of insect antennae by EAG.

FET has also been widely applied in the development of insect antenna-based smell sensors, where the antennae can be directly coupled with the gate surface of transducer. FET can efficiently record the electrical signals of insect antennae in response to the specific chemical volatile molecules. In addition, due to the high-input impedance, the using of FET as a transducer to combine with biological functional components to develop biosensor is very convenient, which is also referred as a BioFET [17, 18]. In order to utilize the intact insect antennae as sensitive elements for the detection of chemical volatile compound, the

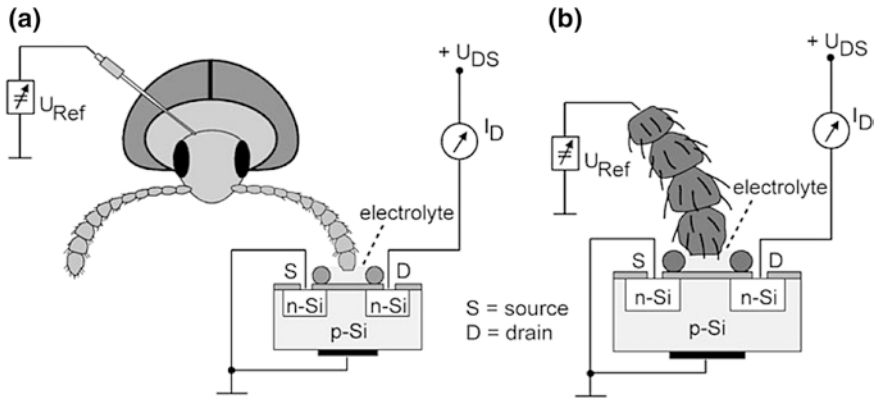

Fig. 5.2 Schematic diagram of insect antenna-based smell sensors using FET device as transducer via an electrolyte solution. **a** Intact pyrophilic beetle was coupled with FET to serve as sensitive elements for the detection of specific chemical volatile compounds. **b** Isolated insect antenna was couple to the similar FET measurement setup. (Reproduced with permission from Ref. [21]. Copyright 2013 Springer)

reliable junction with stable mechanical and electrical stability are highly essential, which can not only influence the electrical signal recording from insect antennae, but also responsible for maintaining the natural structure and functions of insect antennae. As shown in Fig. 5.2, FET can provide a particular reliable joint between an insect antenna and transducer to meet the special requirements on the electrical coupling and mechanical stability [19–21]. Figure 5.2a shows the "whole-beetle" setup, the gate of FET was mounted with an electrolyte solution to allow the direct coupling of beetle antenna with FET without isolation of antenna from insect. On the contrary, the "isolated antenna" setup usually includes the isolated antenna to couple with the FET gate surface via the electrolyte solution (Fig. 5.2b). Upon exposure to the specific chemical volatile molecules, smell sensory neurons located inside the insect antenna will generate the cell membrane potential changes via a cascade intracellular biochemical reactions and finally leading to the formation of a dipole potential over the whole antenna. This potential changes will couple to the gate of FET via the electrolyte solution and modulates the channel conductance between the FET source and drain electrodes, which can be measured by recording the changes in the drain current that is dependent on the concentration of specific chemical volatile compounds. The decisive advantages of insect antenna and FET hybrid configuration are related to their high potential in the miniaturization of the whole sensor systems. Due to the small size of insect antenna and microelectronic FET devices and circuits, the whole measurement setup can be developed in a portable sensor system that can be used to detect the specific chemical compounds in fields, for example, the detection of smoke in the building or the forest.

5.2.3 Modeling of Insect Antenna Electrophysiological Recording

Insect antenna can respond to a single or a blend of a few chemical volatile molecules with very high sensitivity and specificity. The sensed chemical signals by insect antenna are then converted into the electrical signals, which are originated from the membrane potential changes of smell sensory neurons located in the insect antenna. Each kind of chemical volatile molecules can be detected by a particular type of smell sensory neurons that are specially tuned by the expression of defined types of olfactory receptors and can generate responsive electrical signals upon the exposure of specific chemical volatiles. The whole process of chemical signal transduction to electrical signals starts from the specific interactions between chemical compounds and olfactory receptors, which will ultimately result in the generation of action potentials. The electrophysiological recording of insect antennae are mainly originated from the cellular electrical responsive to the specific chemical volatile molecules. The investigation on the electrophysiological recording of insect antennae usually involved in the building of models in order to better interpret and understand the electrophysiological recording from insect antennae.

In general, upon the stimulation of the specific chemical compounds, olfactory sensory neurons can generate a negative deflection of the transepithelial potential as compared with the resting state [22]. The recorded electrical signals are around 20–30 mV and last a few seconds. The responsive cellular electrical signals are thought to be triggered by the specific stimulus that can decrease the cellular membrane resistance, which allow the propagation of membrane potential changes along the whole cell. This is the model that provides the explanation on the opposite polarity of the membrane potential changes. Equivalent circuit diagrams of the sensillum trichodeum of *Antheraea polyphemus* has been used to investigate the generation mechanisms of responsive electrical signals of smell sensory neurons, in which two main current paths were proposed from the hair tip toward the hemolymph space. One pathway begins at the sensillum lymph space and cross the folded apical membranes of the trichogen and tormogen cells, and then enters these accessory cells and continues through their basolateral membranes into the hemolymph. The other one begin at the dendritic membrane of olfactory sensory neurons and leaves from the soma region. A computer model of distributed resistances and voltage sources was used to simulate static responses of the sensillum and thereby to infer possible contributions of some sensillar elements. Based on the computer model, the electrophysiological recording are compatible with the idea that the stimulus does indeed affect the dendritic resistance.

The complete model for the sensillum of insect antennae includes most of the sources of membrane potential changes. The model of sensillum function needs to consider the unusual ionic conditions, especially around the outer dendritic segment. For instance, the potassium pump located in the cellular membrane contributes to the transepithelial potential and should be included in the complete model

of sensillum. In addition, the electrogenic pump could be the origination of nonlinear voltage-current relationship that was recorded across the sensillum of insect antennae. Olfactory sensory neurons usually respond to a large number of chemical volatile compounds and the responsive amplitudes are proportional to the concentration of stimulus [23, 24]. Some features of the dose-dependent responses have been well explained by a model that includes the specific binding between olfactory receptors and chemical compounds, activation of receptors, and membrane potential changes [25]. This model indicated that the relationship between membrane potential changes and stimulus concentration is a typical S-shape curve in a semilog plot with a slope of 1 for lower stimulus concentrations in the double-log plot. This model demonstrated that the relative height of membrane potential changes is not directly proportional to the fraction of activated olfactory receptors.

5.3 Design of Insect Antenna-Based Smell Sensors

The basic idea of the insect antenna-based smell sensors is to employ the extreme high capability of insect antennae for the detection of specific chemical volatile compounds. The combination of a highly specialized insect antenna with a transducer can generate potential devices and instruments that can be used for highly sensitive detection of chemical compounds in a trace level in the atmosphere. For this purpose, it is necessary to achieve functional insect antennae that still maintain their natural structure and functions to serve as the sensitive elements for chemical sensing. And then the proper transducers should be built to convert the chemical signals sensed by the insect antennae into the readable signals. For the development of insect antenna-based smell sensors, micro electrodes and FET are the most commonly used two types of transducers. Both of them can record the electrical responsive signals from insect antennae upon exposure of chemical volatiles. Finally, the related measurement setup and peripheral circuits are required for the construction of the sensor systems to realize the detection of specific chemical volatile compounds. For some sensor systems, the data processing is usually necessary for the recognition of special chemical compounds in complex environmental conditions. In this section, we will introduce the design of insect antenna-based biosensors according to the development process which includes the isolation and fixation of insect antennae, preparation of the micro devices, and sensor systems and data processing.

5.3.1 Isolation and Fixation of Insect Antennae

Insect antennae play important roles in the communications between insects as well as between insects and plants. The generation and detection of chemical volatiles are basic living capabilities of insects which usually require the consumption

of energy. After million years of evolution, insects are able to realize this communication process with extreme high efficiency by generation of lowest amount of chemical compounds and detection of chemical compounds at a trace level. This can not only save a lot of energy, but also avoid the enemies to read the chemical signals. Therefore, insect antennae can detect the specific chemical compounds with very high sensitivity and specificity. It is indicated that the insect antennae can detect the specific chemical volatiles with the detection limit lower than that of most advanced devices and instruments that are usually developed on the basis of gas sensors or gas chromatography (GC)-mass spectrometry (MS) [5]. In addition, the specificity of insect antennae for chemical sensing is extreme high, where even the isomers of the same substance only with minor differences in spatial configuration can generate the completely different responses such as functioning as attractants or repellents [26]. One of the most typical examples isomer-specific reception in insect antennae is gypsy moth (*Lymantria dispar*) and the nun moth (*Lymantria monacha*), which are repelled by (-)-disparlure and attracted by (+)-disparlure, respectively [27, 28]. All these features make insect antennae very attractive and promising to be utilized as sensitive elements for the development of insect antenna-based smell sensors. For this purpose, it is critical and highly required to achieve functional insect antennae to realize the detection of specific chemical volatile as well as the conversion of sensed chemical signals into readable signals that can be detect by the proper transducers.

The most commonly used insect antenna for the development of smell sensors is an antenna of the Colorado potato beetle (*Leptinotarsa decemlineata Say*) [19, 29, 30]. The beetles whose antenna are utilized for the development of insect antenna-based smell sensors are reared on 4-week-old potato plants at 20–25 °C, 50–70 % relative humidity, and 16 h daylight with 5000–10 000 lx. The beetles (male or female) can be available during the whole year, which are usually utilized for the preparation of antenna 4–24 days after emerging from pupae. Furthermore, the wild type beetles are introduced into the reared beetle population once a year in order to avoid the genetic degeneration. Basically, the beetle antennae can be directly connected with transducers via the hemolymph Ringer solution [14]. There are two types of antennae preparation methods have been applied in the development of insect antenna-based smell sensors, which can be referred as "whole beetle methods" and "isolated antennae methods," respectively. As for the "whole beetle methods," the insect antennae is still kept in the body of insect and fixed on the surface of transducers where the hemolymph Ringer solution is filled between antennae and transducers to make a connection between them. The reference electrode is usually make from platinum wire and placed into the site between the neck and head of the beetle. On the contrary, the "isolated antennae methods" usually need to remove the antennae from the inset body and fixed on the surface of transducers via the hemolymph Ringer solution. The reference electrode is directly connected with the insect antennae via the electrolyte solution.

After the preparation and fixation of insect antennae, the carrier air loaded with the specific plant odorant (Z-3-hexen-1-ol) can be applied to the antenna, which can generate the depolarization responses that can be detected by the transducers.

If FET is used as the transducer, the depolarization responses of antennae will lead to the corresponding changes of the drain current that can be measured as the sensor output signals. Based on the measurement of using FET as transducer, it is demonstrated that both insect antennae preparation methods can achieve efficient coupling between the antennae and transducer, which can respond to the specific plant odorant (Z-3-hexen-1-ol) with very high sensitivity and specificity. This suggests a potential application of insect antenna-based smell sensors is to be applied in the detection of plant damages, which can be used as a valuable tool for the plant protection. In addition, different kinds of insects can provide different types of antennae for the development of various antenna-based smell sensors for the detection of a variety of chemical compounds. The highly sensitive and quick detection of specific chemical compounds combined with their potentials in miniaturization make it possible to develop the insect antenna-based smell sensors that can realize the in-field measurement, which can greatly facilitate and expand their practical applications. However, in order to achieve practical applicable biosensors, the coupling between antenna and transducers is still need to be optimized in order to improve the stability and reproducibility of the antenna-based smell sensors.

5.3.2 Preparation of Micro Devices

Benefit from the fast advancements in the micro fabrication process, more and more special-designed precise micro devices can be fabricated, which are available to be used as transducers for the development of insect antenna-based smell sensors. The main function of transducers is related to the detection of antennae responsive signals upon the exposure of specific chemical compounds and transmission of detected responsive signals to the peripheral circuits for further processing such as signal amplification, data collection, data storage, and data display. Therefore, the transducers for antenna-based smell sensors require the high-efficient coupling with antennae, which is not only referred to the physical connection, but also more importantly referred to the signal transduction. There are two main typical transducers have been applied in the development of insect antenna-based smell sensors, which are micro electrodes and FET devices, respectively [19, 29–31]. Both of them have been successfully applied in the detection of specific-responsive signals from insect antennae, which proves their potential capability of being used as transducers in insect antenna-based smell sensors. By the following, the preparation process of micro electrodes and FET devices that are used as transducers in insect antenna-based biosensors will be briefly introduced.

Micro electrodes have been successfully applied in the development of insect antenna-based biosensors, where the micro electrode can not only realize the electrical detection of antennae responses to the chemical compounds, but also realize the mechanical fixation of antennae in the measurement setup [31]. Due to the small size of insect antennae as well as the movable property of antennae as

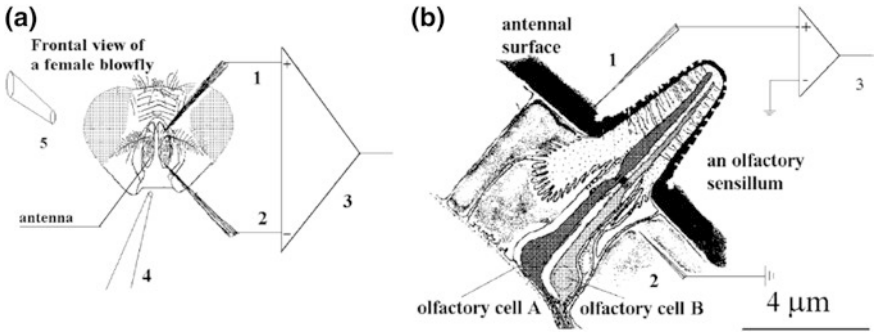

Fig. 5.3 Schematic diagrams of biomimetic smell sensor based on micro electrodes. **a** *Frontal view* of the head of a female blowfly when measurements are taken. **b** An olfactory sensillum and its two sensor cells (A and B), and electrodes as in (**a**) (Reproduced with permission from Ref. [31]. Copyright 2000 Elsevier)

a living olfactory organ, it is highly essential to hold the antennae in a mechanical and electrical stable manner in order to utilize the antennae as the sensitive elements for chemical sensing. The micro electrodes usually made from a tungsten wire 0.25 mm in diameter by electrolytical etching to make the mental micro electrodes with a tip diameter of 1–2 mm, which make them suitable to be connected with antennae of blowflies (*Calliphora vicina*). The typical anatomic and morphological structure of blowfly antennae and olfactory sensillum is shown in Fig. 5.3. Olfactory sensory neurons that extend to the tip of antennae locate in the sensillum of antennae, which can respond to the specific chemical compounds and generate the responsive electrical signal via membrane potential changes. Usually, the female blowflies have large antenna that is suitable for electrical recordings via micro electrodes. The antennae of blowflies have seven pairs of large pits on both sides that ate surrounded by several sensilla. There are many kinds of olfactory sensory neurons located on the surface of antennae. On the different region of antennae surface, there have different types of olfactory sensory neurons that can respond to different chemical compounds. However, during the measurement, it is impossible to determine the location of specific classification of olfactory sensory neurons that are responsive to a defined chemical compounds by the light microscope due to its low resolution. The major drawback of micro electrodes for the recording of antennae responsive signals is that the recorded signals are from all the olfactory sensory neurons that are measured at the same time. It is difficult to record the responsive signals from single-olfactory sensory neuron. Figure 5.3 also shows the typical measurement location of the micro electrodes in order to measure the electrical responses of antennae to the specific chemical compounds. The chemical compounds used as olfactory stimulator are introduced to the insect antennae via a micropipette at a certain concentration and defined exposure duration.

The FET devices are another type of commonly used transducers for the development of insect antenna-based smell sensors [19, 29, 30]. Benefit from the fast development of micro fabrication technologies, FET devices can be fabricated on the basis of silicon wafer via the standard micro fabrication process. An insulator layer with proper thickness is usually thermally grown on the gate of FET devices and followed by the deposition of a Si_3N_4 layer through plasma enhanced chemical vapor deposition (PECVD) process. The FET devices are then passivated using a thin layer of polyimide and fixed with a printed circuit board. The electrical connections are realized using the ultrasonic bond with the aluminum pads. Then, the FET devices are encapsulated with a conventional epoxy resin. The gate surface of FET devices is exposure to the hemolymph Ringer solution that is used to connect the insect antennae with FET devices. Based on this method, the electrical responses of antennae to the specific chemical compounds can be coupled to the gate electrode of FET and then converted into the output signals of FET. The transduced signals can then be output via the source and drain electrodes of FET, which make it possible for the signals to be further amplified and processed by the peripheral circuits.

5.3.3 Sensor System and Data Processing

The configuration of sensor system for the development of insect antenna-based biosensors is mainly depended on the transducers that are used for the recording of responsive signals from the insect antennae. The main function of sensor system is to amplify and process the signals from the output of transducers. Another important function of sensor systems is to provide the proper approaches for the introduction of specific chemical compounds to the insect antennae to elicit the measurable responses by the transducers. Other functions of sensors systems include the data collection, data processing, and data display. Here, we briefly introduce two different sensor systems and data processing of insect antenna-based smell sensors, which are developed on the micro electrodes and FET devices, respectively.

The sensor systems developed for the micro electrodes to measure the responsive signals from insect antennae are shown in Fig. 5.4, which mainly include the odor exposure setup and the signal processing and analysis components. In this setup, the insect antennae are fixed to avoid the movement of living olfactory organs. Two microelectrodes are used as the measurement and reference electrodes, respectively. The movement of the measurement micro electrode can be controlled by a piezotranslator. The output signals from the micro electrodes are transmitted to an amplifier, which is a low noise, differential AC and DC amplifier with independent and simultaneous ac and dc outputs. The beginning frequency of dc is 0.01 Hz without attenuation. These features make this system possible to record the responsive electrical signals from insect antennae as well as the action potentials of olfactory sensory neurons. In addition, the higher frequency

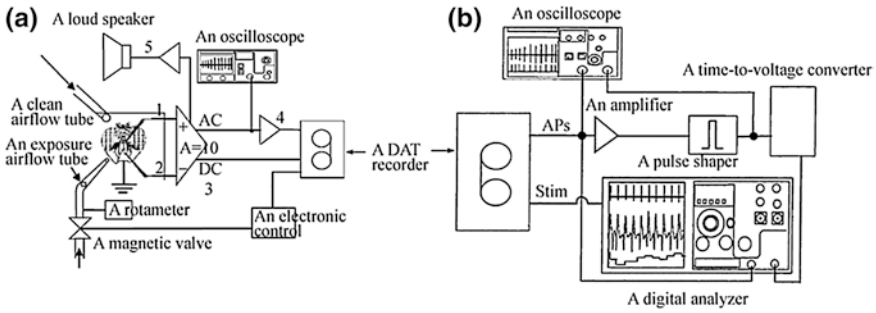

Fig. 5.4 Schematic diagrams showing the sensor system of insect antenna-based smell sensors using micro electrodes. **a** The setup for the introduction of specific chemical compounds and the configuration of sensor measurement system. *1*, a measurement micro electrode; *2*, a reference micro electrode; *3*, a micro electrode amplifier; *4*, an instrumentation amplifier; *5*, an audio amplifier. **b** The procedure and instruments for the analysis of responsive action potentials recorded by the micro electrodes from insect antennae. Aps, Action potentials; Stim, exposure timing (Reproduced with permission from Ref. [31]. Copyright 2000 Elsevier)

action potentials can be isolated from the dc output by the using of a spike filter in the filter bank, which can be further used for the electronic counting of the action potential rate. The output signals of the amplifier can be monitored using an oscilloscope. During the exposure of chemical compounds, the recorded responsive signals as well as the action potentials are stored on the tape using a digital audio tape, which can be further analyzed using the time-to-voltage conversion technique. Also, the audio amplifier and the loudspeaker are used to generate the popping sound during the exposure of specific chemical compounds to indicate the electrode-antennae contact that can generate a typical noise in the loudspeaker output. In this setup, the specific chemical compounds at the gas phase are introduced to the antennae via a glass tube that is controlled by a magnetic valve in a repeated manner with certain intervals at the proper flow rate measured by a rotameter. During the intervals, the clean air flow is used to refresh the tube systems for chemical compound introduction.

In a standard sensor system for insect antenna-based smell sensors, it is essential to provide a steady stream of cleaned and humidified air to the insect antennae in order to achieve a standardized background and comparable responsive signals. During measurement, the blank air should be introduced to the antennae to evaluate the background signals ant then the air containing the specific chemical compounds can be introduced to the antennae to observe the responsive signals. However, the responsive capability of insect antennae could decrease over repeatable stimulations due to the adaptive properties of biological olfactory systems. Therefore, in order to compare the measurement data recorded from one antennae, the background of the insect antennae under blank air should be evaluated at a regular intervals to make it possible to normalize the recorded responsive signals [32]. There are several methods have been applied in order to feed

the test substances into the air stream, for instance, by placing a defined amount of substance on a permeable filter paper, on a rubber septum, or in a drop of paraffin oil [32–34].

The data processing of responsive signals recorded by the micro electrodes from insect antennae is time-consuming and laborious due to the high frequency of action potential that is typically higher than thousands of pulses per second. In order to address this issue, a time-to-voltage conversion method is employed to analyze the recorded action potentials. The analysis procedure of this method is shown in Fig. 5.4 together with the corresponding instruments. The recorded action potentials are replayed and amplified by an instrument amplifier. Then, the amplified action potentials are converted into a sharp-edged constant amplitude pulses that can be used to trigger the time-to-voltage converter. The converter can generate the corresponding signals that can result in the memory of a signal analyzer for the counting of pulse rate. In addition, the action potential rate can be further integrated with the response time analysis. This makes it possible to judge if the reliable action potential rate can be achieved by this time-to-voltage conversion method.

The sensor systems for another kind of insect antenna-based smell sensors are commonly developed based on the using of FET devices as transducers [19, 29, 30]. The configuration of the sensor systems for FET devices to record the responsive electrical signals from insect antennae are similar to that of micro electrodes, which consists of the introduction setup for chemical compound exposure in the gas phase and the peripheral circuits for the signal processing and analysis. The chemical compound introduction setup can generate a constant flow across the insect antennae using a suction pump. As shown in Fig. 5.5a, the introduction air that is used as carrier of stimulation chemical compounds is first filtered by a charcoal filter in order to remove the stimulating components in the air. While the odorant containing the specific chemical compounds at a certain concentration introduced to the antennae using a syringe. Figure 5.5b shows the coupling of insect antennae with the gate surface of FET devices via the electrolyte solution. Upon the exposure of specific chemical compounds, the insect antennae can generate the corresponding electrical signals and coupled to the gate surface of FET via the electrolyte solution. A reference electrode is also put into the electrolyte solution. Based on this measurement setup, the responses of insect antennae to the specific chemical compounds can be measured by recording the output of FET signals. The drain current is one of the most commonly used parameters as the output signals of FET devices, which can then be amplified and recorded by the peripheral circuits. When different concentrations of chemical compounds are introduced to the insect antennae, the FET device can record the corresponding electrical responses of antennae. Figure 5.6 shows the typical recording of the FET drain current in the measurement of insect antennae in response to different concentrations of (Z)-3-hexen-1-ol gas. It is indicated that the insect antenna-based smell sensors can respond to different concentration of (Z)-3-hexen-1-ol gas in a dose-dependent manner. In addition, it is proved that the using of charcoal filter can greatly decrease the influences of ambient

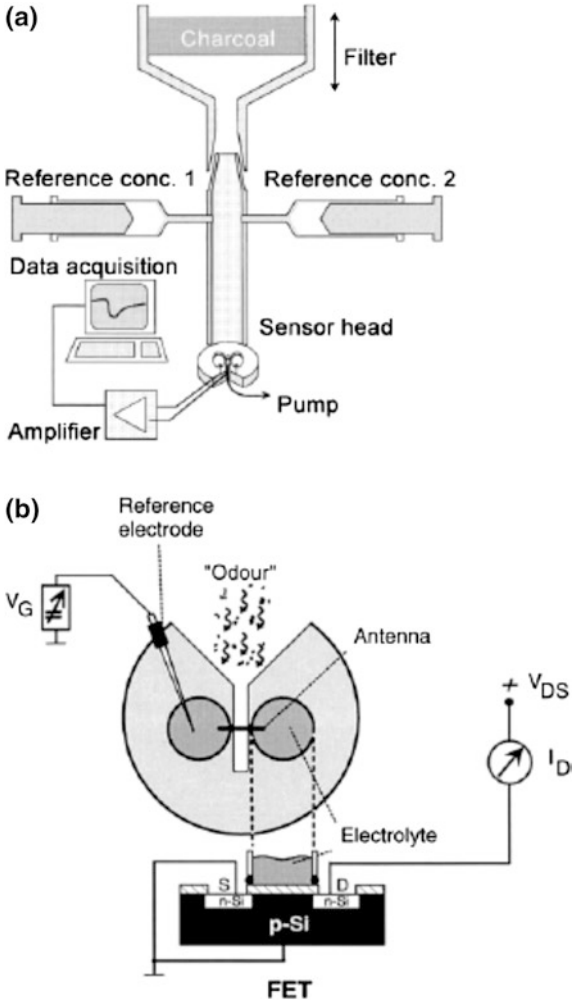

Fig. 5.5 Schematic diagrams of insect antenna-based smell sensor using FET devices as transducers. **a** The setup for the introduction of specific chemical compounds in gas phase; **b** the coupling of antenna with FET devices via an electrolyte solution (Reproduced with permission from Ref. [30]. Copyright 2000 Elsevier)

air on the electrical recordings of insect antennae in response to the specific chemical compounds. Because even the clean air contains some chemical compounds that can stimulate the insect antennae to generate electrical responsive signals. This can also be observed in Fig. 5.6, when the dry air is applied, a spike can be recorded that is similar to that of upon chemical compound exposure but with smaller amplitude. All these indicate that in the importance of gas introduction setup in the sensor systems for the development of insect antenna-based smell sensors.

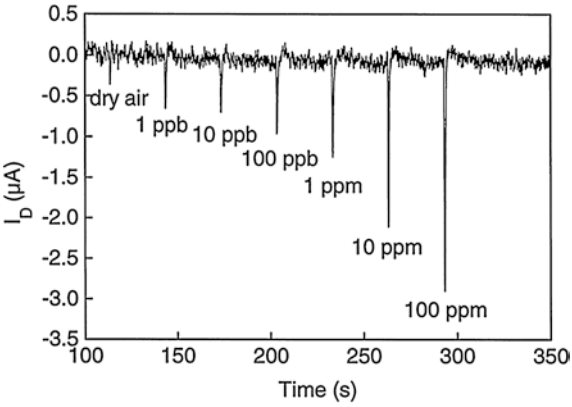

Fig. 5.6 Typical responses of an insect antenna-based smell sensor using FET devices to different concentrations of (Z)-3-hexen-1-ol gas as indicated by the differences in the drain current of FET (Reproduced with permission from Ref. [30]. Copyright 2000 Elsevier)

5.4 Application of Smell Sensors with Insect Antenna

Insect antenna-based smell sensors are characterized with high sensitivity, high specificity, and fast responses for the detection of specific chemical compounds due to the unconventional using of insect antennae as the sensitive elements, which hold the unique capability for the detection of specific chemical compounds. In addition, the insect antenna-based smell sensors are flexible, which can be tuned for the detection of specific chemical compounds by changing the types of insect antennae. It is cheap and easy to reproduce the insect antennae that are suitable to be used as sensitive elements, which can be achieved in an engineered manner. Insect antenna-based smell sensors have shown promising prospects and potential applications in many fields. By the following, we briefly introduce some typical applications of insect antenna-based biosensors, which include the recording of electrophysiological signals, plant protection, and smoke detection.

5.4.1 Recording of Electrophysiological Signals

Insect antenna-based biosensors using micro electrodes have been applied in the recording of electrophysiological signals from olfactory sensory neurons in response to the specific chemical compounds [31]. In this measurement setup, action potentials of olfactory sensory neurons located in the antenna of blowfly were recorded by the micro electrodes and amplified by a high-impedance amplifier. The recorded action potentials were further processed and analyzed based on a time-to voltage converter method. The responses of olfactory sensory neurons to 1,4-diaminobutane, 1-hexanol, and butanoic acid, were investigated. It is demonstrated that the olfactory sensory neurons located in the antenna of blowfly can respond to the specific chemical compounds with high sensitivity, which include 1,4-diaminobutane, 1-hexanol, and butanoic acid. But the measured olfactory

sensory neurons show no response to other chemical compounds tested. Upon the exposure of the specific chemical compounds, the action potential rate of olfactory sensory neurons shows a significant increase as compared with spontaneous state. But upon the repeated exposure of the same chemical compounds, olfactory sensory neurons show distinct responsive electrical signals as indicated by the action potential rate changes. As shown in Fig. 5.7, the recording of micro electrodes indicates that olfactory sensory neurons show different responsive signals upon the first exposure, second exposure, and continuous exposure of 1,4-diaminobutane. Upon the second exposure and continuous exposure, a decrease in the action potential rate was observed as compared with the first exposure. This is probably due to the decrease in the emission of 1,4-diaminobutane from the filter paper with the increase in the number of repeated exposures, which leads to the decrease in the action potential rates of olfactory sensory neurons. Both the pulse rate and the odor concentration in air decrease exponentially as a function of the number of exposures [35]. When 1-hexanol and butanoic acid were applied to stimulate the antenna, action potential rate of olfactory sensory neurons also showed a similar decrease to that of 1,4-diaminobutane. In addition, it is proved that different concentrations of chemical compounds can result in differences in the action potential rates. This makes it possible to evaluate the dose-dependent responses of olfactory sensory neurons to the specific chemical compounds. It is indicated that higher concentration of chemical compounds can lead to the cessation of action potential generation due to the adaptation and overloading of the sensor as well as the saturation of the corresponding olfactory sensory neurons.

5.4.2 Plant Protection

Insect antenna-based smell sensors using FET devices have been explored to be applied in the detection of plant damage in the glasshouse [19, 29, 30]. Many insects can recognize the special chemical compounds released by the plants with extreme high sensitivity and specificity. It is well-known that the insects have the unique capability to detect the special chemical volatiles that are released from the damaged plants, which can provide useful information for the insects to find the food sources or the mating partners. This makes it possible and attractive to directly utilize the insect antenna as sensitive elements for the development smell biosensors for the detection of plant damage. In addition, the highly developed insect antennae are able to be reproduced and combined with the transducers for the purposes of target chemical compound detection.

The insect antennae of Colorado potato beetle were combined with the gate surface of FET devices via the electrolyte for the development of insect antenna-based smell sensors that were able to detect (Z)-3-hexen-1-ol gas which is the specific chemical compound released by the damaged plants. In addition, the intact antenna can be integrated with FET devices in a miniaturisable manner. This allows the whole sensor systems can be integrated as a portable measurement

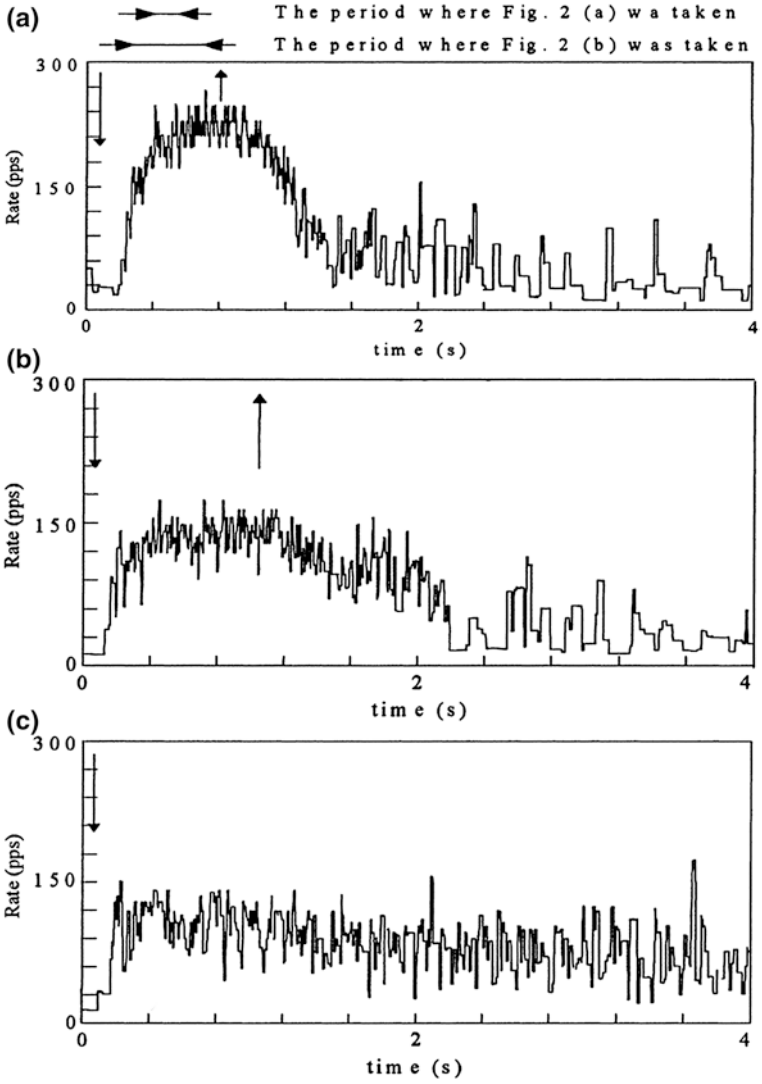

Fig. 5.7 The action potential rates of olfactory sensory neurons recorded by the micro electrodes upon **a** first exposure, **b** second exposure, and **c** continuous exposure of 1,4-diaminobutane (Reproduced with permission from Ref. [31]. Copyright 2000 Elsevier)

setup, which makes the in-field detection possible. Furthermore, the functional and spatially integration also contribute to the mechanical and electrical stability of the insect antenna-based smell sensors for chemical sensing. This integration also avoids the damage to the insect antenna, which is a kind of non-invasive coupling with FET devices. Consequently, the lifetime of the insect antenna-based smell sensors could be greatly improved. The insect antenna-based smell sensors

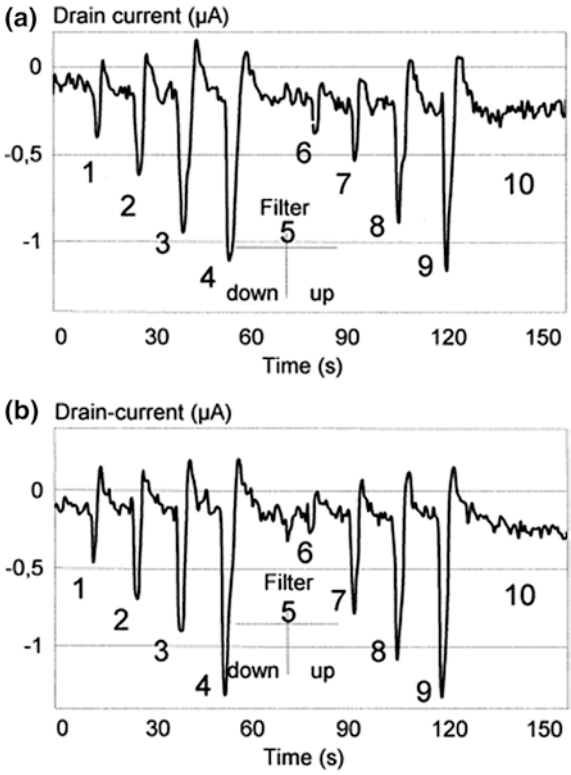

Fig. 5.8 Responses of insect antenna-based smell sensor to the same calibration steps using different concentrations of (Z)-3-hexen-1-ol gas measured **a** in a glasshouse containing only undamaged potato plants and **b** in a glasshouse containing three beetle-infested potato plants (Reproduced with permission from Ref. [30]. Copyright 2000 Elsevier)

using FET devices have been successfully applied in the detection of plant damage in a glasshouse under real-world conditions. As shown in Fig. 5.8, the responses of insect antenna-based smell sensor to the same calibration steps using different concentrations of (Z)-3-hexen-1-ol gas measured (a) in the a glasshouse containing only undamaged potato plants and (b) in a glasshouse containing three beetle-infested potato plants. It can be observed that when the ambient air in the glasshouse with damaged potato plants was introduced to the insect antenna-based biosensors, the calibration responses were significant smaller than that of measured in the glasshouse without damaged potato plants. It is indicated that (Z)-3-hexen-1-ol gas can be detected at very low concentrations with very high selectivity and fast response time, which make the insect antenna-based biosensors able to distinguish single mechanically or beetle-damaged plants in background emissions of 1000 undamaged plants in the glasshouse.

The insect antenna-based smell sensors can be a valuable tool for the plant protection to detect the pathogen infestation as well as for the stored food protection to control the food quality. Early detection of plant pathogen infestation plays an important role in the agriculture and food industry, which can greatly reduce the plant and food damage by pathogens and thus improve the efficiency and quality of plant protection and food storage. It has been demonstrated that different types

of plant damage can elicit different chemical compounds, which can be used as the marker for the detection of plant damage by the biosensors or other chemical sensing instruments [18, 36, 37]. Therefore, the insect antenna-based smell sensors have great potentials to be applied in the damage detection of different types of plants as well as the detection of different kinds of pathogens that infect plants. However, in order to develop a practical applicable insect antenna-based smell sensor for the detection of plant damage, the sensor systems still ne to be further optimized and improved. For instance, the life time of the insect antenna is still not enough for the in-field measurement, especially for the development of a pre-alarm system to monitor the plant damage. The coupling between insect antenna and transducers are still need to be enhanced in order to achieve more stable responses and higher performances for the detection of specific chemical compounds in the practical applications.

5.4.3 Smoke Detection

Insect antenna-based smell sensors using freshly antennae isolated from *Melanophila* as sensitive elements have also been applied in the smoke detection [11]. It has been demonstrated that the jewel beetles of the genus *Melanophila* can approach forest fires as far as 50 km away in order to make their larvae develop in the wood of trees freshly killed by fire [38, 39]. This is partial due to the unique capability of highly sensitive infrared receptors that are useful for the detection of forest fires [40–42]. *Melanophila* has been suggested to be able to detect the smoke [43], but the behavioral research fails to prove that *Melanophila* is able ot approach the smoke sources [38]. However, the antennae of jewel beetles have been demonstrated to be able to detect the specific chemical compounds in the smoke released from the burning wood [11].

Freshly prepared antennae of *Melanophila acuminate* were connected to a gas chromatograph. In the measurement setup, two types of detectors were employed for the detection of oxidizable compound and the electrophysiological recording of insect antennae, which are flame-ionization detectors (FID) and electroantennographic detectors (EAD), respectively. Smoke that was generated by smoldering splint wood from *Pinus sylvestris* was collected and introduced to the measurement setup. As shown in Fig. 5.9, the measurement results indicate that there are several chemical compounds existed in the smoke can elicit the response of insect antennae. Most of the chemical compounds that can elicit the greatest antenna responses are phenolic compounds, which are derivatives of 2-methoxyphenol (guaiacol) released by the incomplete combustion of lignin and have been identified as the atmospheric markers of wood smoke [44]. In addition, the fires of different species of trees can generate the phenolic compounds with different chemical structures [45]. This makes it possible to develop an insect antenna-based biosensor for the detection of remote forest fires by the using of antennae isolated from *Melanophila* as the sensitive elements. Moreover, with further

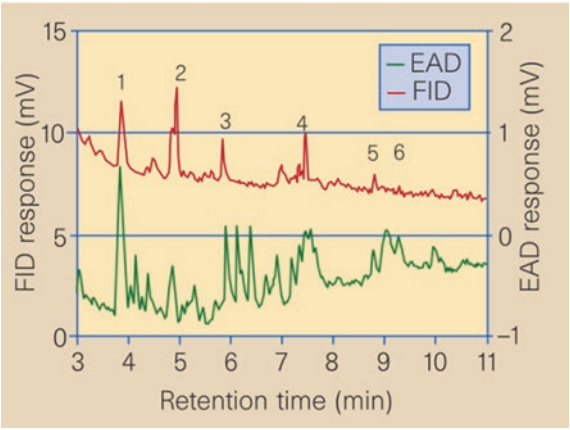

Fig. 5.9 Typical responses of gas chromatograms using two different types of detectors, which are flame-ionization detector (FID) and electro-antennographic detector (EAD), respectively. An isolated antenna of *Melanophila acuminate* was used as sensitive elements of EAD for chemical sensing. FID responses indicate any oxidizable compound. EAD responses is the electrophysiological recording of the antenna upon the exposures to various chemical compounds, which include *1*: a-pinene; *2*: carene; *3*: 2-methoxy-phenol (guaiacol); *4*: 2-methoxy-4-methyl-phenol (4-methyl-guaiacol); *5*: 4-acetyl-guaiacol; *6*: 4-formyl-guaiacol (Reproduced with permission from Ref. [11]. Copyright 1999 Nature Publishing Group)

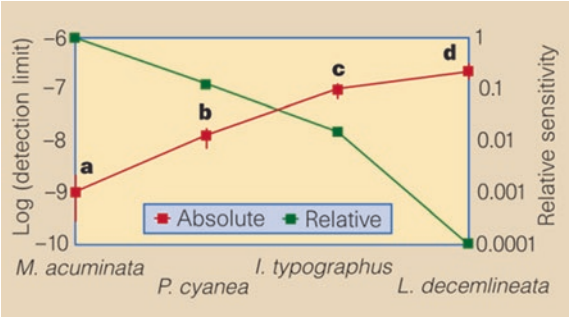

Fig. 5.10 Absolute and relative sensitivities of pyrophilic and non-pyrophilic insects to guaiacol (Reproduced with permission from Ref. [11]. Copyright 1999 Nature Publishing Group)

development and improvement, the species of tree is also possible to be identified using the insect antenna-based biosensors by the detection of special pattern of chemical compounds in the smoke.

The sensitivity of insect antenna-based smell sensors for the detection of guaiacol derivatives is very high. As shown in Fig. 5.10, guaiacol derivatives as low as a few parts per billion (p.p.b.) can be detected by the insect antenna-based biosensors using antenna isolated from four different types of insects. These insect antenna-based biosensors show similar performances for the detection of smoke,

which are estimated to be able to detect a single pine tree 30 cm in diameter that has smoldering bark to a height of 2 m and a bark depth of 1 cm, releasing about 7 g of guaiacol in an hour under light wind conditions (0.3 m/s), from a distance of more than 1 km [46]. The insect antenna-based biosensors provide a useful and promising tool for the detection of forest fires as well as fires in the storehouse and public buildings, which can also be further developed into the early warning systems for the forest fires.

5.5 Summary

Various insect antenna-based smell sensors have been developed by the combination of insect antennae with proper transducers, which have been applied in many fields and shown promising prospects. Compared with functional components originated from mammalian olfactory systems, biosensors using insect antennae as sensitive elements have advantages that are not able to be achieved with those made with mammalian. Insects are naturally to some chemical compounds that are usually considered as the marker of special application, for example, the detection of forest fires. This makes it possible to directly utilize insect antennae as the sensitive elements for the development of smell biosensors that can be used in the detection of forest fires. In addition, the large number of insect species can provide sufficient chemical sensing spaces for the development of insect antenna-based smell sensors in order to detect different desired chemical compounds. The fast advancement in the micro fabrication and nano technologies also provide novel approaches for the design and fabricate next generation of insect antenna-based smell sensors. In this chapter, we first introduced the signal transduction mechanisms of insect antennae and the basic principle of insect antenna-based smell sensors. Then, the preparation of insect antenna, the fabrication of micro devices used for the development of insect antenna-based smell sensors, and the coupling of insect antenna and micro devices were introduced in detail. The insect antenna-based biosensors using micro electrodes and FET devices were introduced. The sensor system and data processing of insect antenna-based smell sensors were introduced as well. By the following, we summarize the applications of insect antenna-based smell sensors in three aspects, which include the electrophysiological recording, plant protection, and smoke detection.

In the future, the development trends of insect antenna-based smell sensors are based on the novel designs to better utilize the unique capability of insect antenna for chemical sensing. For example, the design and fabrication of novel transducers that can be used to record the responses of insect antennae in a more efficient and reliable manner. However, there are also some challenges in the further development of insect antenna-based smell sensors. For instance, how to maintain the function of insect antenna with a longer time might be one of the main challenges for the practical applications, especially for the development of monitoring or early alarm systems. On the other hand, with the fast development of

biotechnologies, the antennae isolated from the genetic modified insects might provide novel desired function for the development of smell sensors with special functions. In order to achieve in-field measurement, the sensor systems for insect antenna-based smell sensors should have higher level of integration to realize further miniaturization. Benefit from the fast development of nano- and biotechnologies, the next generation of insect antenna-based smell sensors are expected to be more powerful, more efficient, and even with intelligence.

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Chapter 6
Smell Sensors Based on Olfactory Receptor

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6.1 Introduction

The discovery of olfactory receptors (ORs) multigene superfamily promotes the research in the field of olfactory molecular mechanisms and signal transduction pathways greatly [1]. The gene family is comprised of more than 1,000 different genes encoding an equivalent number of OR types. These ORs are located restrictively on the olfactory sensory neurons (OSNs), which occupy a small area in the upper part of the nasal epithelium. Each OSN possesses only one type of OR, and each OR can detect a limited number of odorant molecules. Therefore, each OSN is highly specialized for a few odorants. When inhaled odorant molecules are exposed to the nasal cavity, they will be detected by ORs selectively, triggering the intracellular signal transduction cascade and inducing the depolarization of OSNs. The chemical information of odorants is thus transformed into the electric signal of OSNs. The OSNs containing the same type of OR send thin nerve processes directly to the same glomerulus of the olfactory bulb, afterward the information will be relayed from these glomeruli of the olfactory bulb to the primary olfactory area of the brain cortex for further processing [2]. Since the first OR was matched to a volatile ligand [3], more and more ORs were deorphaned using the ligand-binding assays [4, 5], which have a significant impact on the understanding of olfactory system. Besides, another type of olfactory proteins, odorant-binding proteins (OBPs), has also been found in the mucus fluid surrounding dendrites and cilia of OSNs. They are water-soluble small proteins and produced by glands

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within the nasal cavity. It's found that OBPs can enhance the access of hydrophobic odorant molecules to receptor recognition sites in an aqueous environment [6]. To provide convenience for the research of ORs, an olfactory receptor database has been established by Yale University (http://senselab.med.yale.edu/ordb), which includes a large amount of OR information.

The biological olfactory system is known as the most excellent and intelligent gas sensory system in the world, which can recognize and remember about 10,000 different odorants at nanomolar and even low concentrations in a complex environment. This inspires engineers to develop biomimetic olfactory biosensors with the collaborations of biologists [7, 8]. The research of the olfactory biological mechanisms has promoted the development of biomimetic olfactory biosensors and provided the potential theoretical basis. Since Göpel first put forward the concept of the "bioelectronic nose" [9, 10], lots of biological components originating from the olfactory system have been used as the sensing elements in olfactory-based biosensors, such as whole animals, olfactory tissues, olfactory cells, and olfactory-related proteins. Animals have been used for the public health risk assessment for centuries, such as dogs and cats, which played important roles in aiding the early identification of food contamination, environmental pollution, infectious disease transmission, medical diagnosis, and even bioterrorism or chemical terrorism events [11, 12]. In recent years, more and more tissues, cells, and even protein molecules from biological olfactory system are employed to develop olfactory-based biosensors for the detection and discrimination of specific odorants. By utilizing living olfactory tissues and cells as the sensitive elements, related olfactory biosensors are able to detect odorants as well as provide a new platform to investigate the performance of the olfactory system in vivo and in vitro. However, one significant shortcoming of these sensors is that olfactory tissues and cells need the rigorous culture environment, which restricts their specific in-field applications. On the other hand, olfactory receptors and olfactory-related proteins have increasingly become a new and promising source of sensitive elements in the field of olfactory-based biosensors [13, 14]. They offer some important advantages over olfactory tissues and cells in many aspects, such as stability, long-term activity, and the maintenance conditions. Various OR-based biosensors have been studied intensively in the past two decades, employing specific ORs or partially purified ORs as the sensitive materials [15–17].

In this chapter, OR-based biosensors will be introduced systematically. First, theories of OR-based biosensors will be presented, including the biological structure and function mechanisms of ORs, production methods, as well as the efficient maintenance of their natural structure and function. Second, various OR-based biosensors will be described, mainly focusing on their designing principles and characteristics, secondary transducers and the coupling of ORs. After that, applications of OR-based biosensors will be summarized. Finally, a summary will be made by discussing the critical issues and the future challenges in this field.

6.2 Theories of Olfactory Receptor-Based Smell Sensors

6.2.1 Biological Structure and Function of Olfactory Receptors

The biological structure of olfactory system is complex, and it is generally divided into the main olfactory system (MOS) and the accessory olfactory system (AOS). The main olfactory system plays important roles in the odorant detection and discrimination, and the accessory olfactory system is mainly used to detect pheromones, chemical messengers that carry information between individuals of the same species. The previous genetic studies revealed that the accessory olfactory system does not exist in some primates and human beings. The main olfactory system includes the main olfactory epithelium (MOE), the main olfactory bulb (MOB), and the brain cortex connected with the main olfactory bulb. The main olfactory epithelium consists of olfactory sensory neurons (OSNs), microvillar cells, supporting cells, and progenitor cells. Olfactory sensory neurons are the main functional cells, which can transform the odorant chemical information into the cellular action potential and send the electrical signal to the main olfactory bulb for further processing. The specific function of microvillar cells is still not clear. Supporting cells play roles in keeping the structure of olfactory epithelium, secreting mucous, and insulating olfactory sensory neurons. Progenitor cells are stem cells having the potential to differentiate into olfactory sensory neurons in order to maintain the regeneration [18].

The structure and mechanism of the human olfactory system are illustrated in Fig. 6.1. The olfactory epithelium, indicated as the olfactory region in Fig. 6.1, is a specialized mucous epithelium tissue inside the nasal cavity. OSNs are located in the olfactory epithelium and expressed with ORs, therefore they are also named as olfactory receptor cells. OSNs are biopolar neurons with cilia, in which ORs are expressed. Cilia project into mucus above the surface of olfactory epithelium, which increase the contact surface area between ORs and odorants, as well as the olfactory sensitivity. Once odorant molecules are exposed to the nasal cavity, OBPs in the mucus will accommodate odorant molecules to access the cilia and bind with ORs. The interaction between ORs and odorant molecules will initiate the intracellular signal transduction pathways and induce the depolarization of OSNs. The action potential will be transmitted into olfactory bulb via the other end of OSNs for further information processing [18].

Prior to the discovery of ORs, researchers found several findings supporting a G protein-coupled, cAMP-mediated transduction mechanism for odorant detection in the main olfactory system. The studies strongly suggested that ORs would belong to the GPCR superfamily. The large repertoire of odorant molecules detected by the main olfactory system also implicate a multigene family of ORs. Buck and Axel confirmed that the ORs are part of a large, multigene family in 1991, and they were awarded the Nobel prize for their landmark discovery. Genome sequencing has also confirmed Buck and Axel's conclusion.

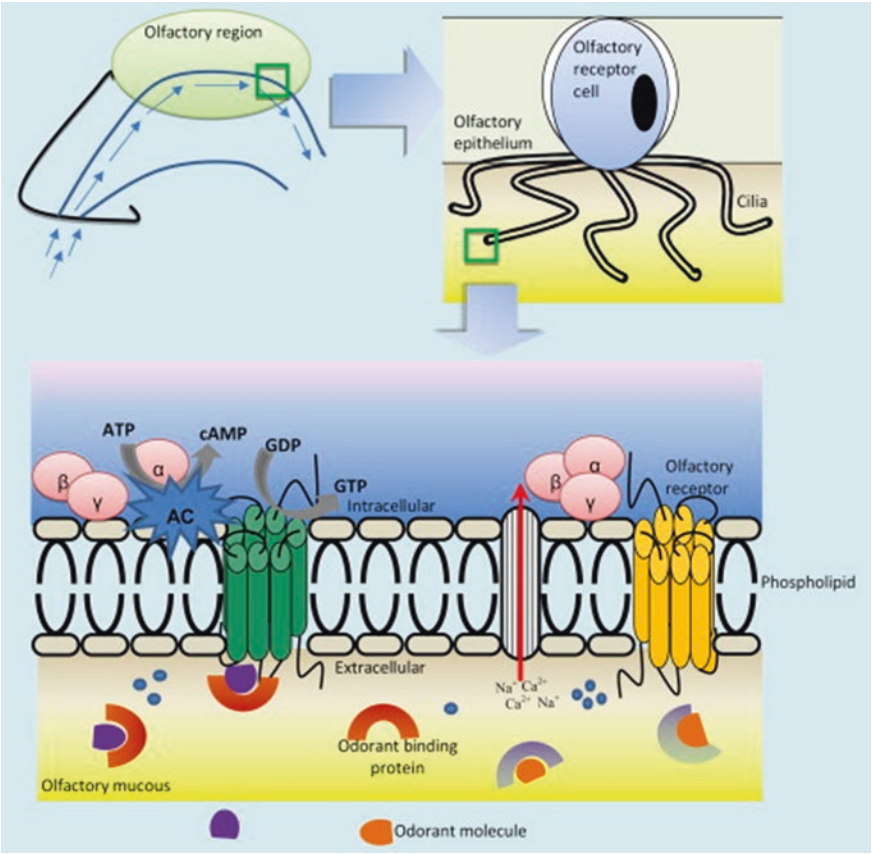

Fig. 6.1 Schematic diagram showing the organization and signal transduction mechanism of human olfactory system [19]. (Reproduced with permission from Ref. [19]. Copyright 2012 Elsevier)

It is estimated that humans have about 350 functional ORs, while rodents express more than 1,000. ORs are expressed specifically onto the cilia of OSNs. The N terminal is located outside the neuron, and C terminal inside the neuron. There are some conserved sequences and residues in the OR superfamily, which could ensure the right folding of ORs. While the variable parts are located in the extracellular domains, indicating that the specific binding sites of odorants are also located in the extracellular domains. In recent years, the computer modeling as well as the experimental data have predicted that the binding site of odorants should be the pocket structure composed by nine aminos of transmembrane domains. Knowledge of ORs not only provided invaluable tools for elucidating the organization and function of the main olfactory system, but also probed the potential direction for the further application of ORs in the odorant sensing of smell sensors.

The odorants' specific binding with ORs will trigger the G protein-coupled, cAMP-mediated intracellular signal transduction, as shown in the lower part of Fig. 6.1. The odorant molecules are bound with the specific OR under the assistance of OBPs. The binding event activates a G protein, which in turn activates an adenylyl cyclase (AC). The intracellular ATP molecules therefore are converted into the second messengers, cyclic AMP (cAMP), which bind to the intracellular domain of the cyclic nucleotide-gated (CNG) channels. The opening of CNG channel enables the conduction of cations such as Na^+ and Ca^{2+}. The influx of Na^+ and Ca^{2+} ions triggers the depolarization and generates an action potential. Therefore the biochemical reaction transduces the chemical information of odorants into action potential of OSNs [18]. In addition to the cAMP-mediated signal transduction, other signal transduction mechanisms have also been found in different biological olfactory systems, such as cGMP- and IP_3-mediated pathway.

6.2.2 Production Techniques of Olfactory Receptors

In the development of OR-based biosensors, the activity of functional ORs will directly influence the performance of biosensors such as sensitivity, specificity, and stability. Therefore, the production techniques of functional ORs are very crucial and fundamental. The following requirements should be met: first, maintain the natural structures and native functions of ORs to recognize their natural ligands; second, low production costs; third, ease of storage and long shelf life. Many methods have been reported, but each having its own advantages and disadvantages. To date no single method can meet all the requirements mentioned above.

Due to their hydrophobicity and dependence on a lipid bilayer environment, membrane proteins represent challenging targets in the structural biology and drug discovery. They play key roles in diverse cellular processes including the signal transduction, the cell division, the growth and differentiation, often as multimers or complex assemblies. As one kind of membrane proteins, ORs have the same difficult problem in the production and structural analysis [20].

Extracting ORs from living olfactory tissues and sensory cells is the most direct and convenient method for utilizing them as sensing elements of biosensors. For example, Wu et al. isolated ORs from bullfrogs and utilized them for the detection of distinct volatile organic compounds by coating them onto a sensor array [21]. Houtari directly employed insect OSNs in vitro to detect odorants by recording action potential responses with a microelectrode [22]. Liu and Wu cultured rat OSNs onto the sensor surface and investigated cellular responses to the odorant stimulus [23, 24]. In the early stage of smell sensor, this method was used widely. Its most remarkable advantage is that ORs' natural structures and neuronal connections are well maintained, preserving the natural functions of ORs to recognize their natural ligands. Another merit is the biological olfaction encoding based on these natural structures and innate cellular connections, which is also the most fundamental factor of the olfactory system for the powerful

odorant discrimination. Therefore, tissue- and cell-based smell sensors acquire encoded olfaction response signals. Signal processing methods and olfaction decoding theories will help interpreting the complex odor information; therefore functional bioelectric noses would be realized. However, there are still many great obstacles. The major one is that it's hard to achieve the specific sensitive elements with desired ORs. Moreover, it is very expensive and inefficient to purify specific type of ORs from living olfactory tissue and sensory cells. Additionally, living tissues and cells need a specific culture environment to stay alive and maintain the functions, which restricts the potential applications in commercial biosensors and under in-field conditions.

With advances in biotechnology, cell-based OR production methods provide new solutions, in which ORs can be expressed heterologously in cell lines, and then extracted to be applied into the development of biosensors. As illustrated in Fig. 6.2a, expression vectors are engineered by inserting the gene of a specific OR to transfect expression cell lines, which will result in the cellular expression of ORs. In the research of OR-based biosensors, the most commonly used ORs are usually extracted from rat and mouse, and the most widely utilized cell expression systems are human embryonic kidney (HEK) cells [25, 26] and yeast [27–30]. There are some similarities between the signal transduction cascades of OSNs and expression systems that ensure natural structures and functions of expressed

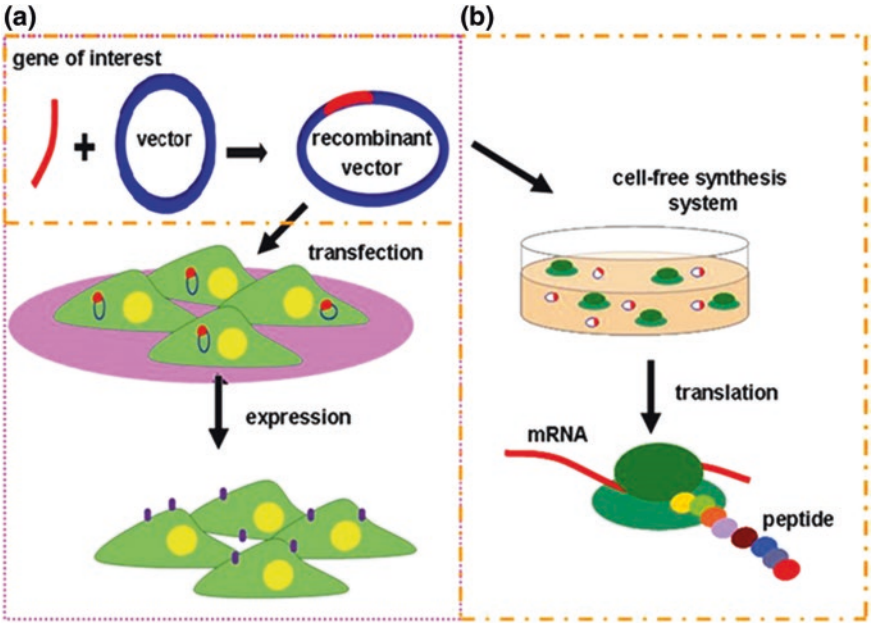

Fig. 6.2 Schematics of OR production techniques based on heterologous cell expression systems (**a**) and cell-free protein synthesis methods (**b**) [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

ORs well preserved. For example, the human OR (hORI7-4) [31] and zebra fish OR (OR131-2) [32] have been successfully expressed in HEK-293S cells. The extracted membrane proteins containing the expressed ORs are utilized as sensing elements for biosensors. One advantage of this production method is that only one desired type of OR is specifically expressed on cell plasma membrane, at the same time native structure of ORs could be well preserved. Moreover, it allows the grafting of tags in the terminal of expressed ORs, which can help the efficient immobilization of ORs and improve the specificity of the OR-based biosensor. However, this method is relatively time consuming, labor intensive and inefficient. Furthermore, irrelevant proteins could be mixed with the product protein. Therefore additional purification processes will also be required. As ORs undergo continuous internalization and recycling in heterologous cells, a method is also needed to maintain the cell surface localization.

On the other hand, cell-free protein synthesis is a valuable and promising candidate to produce ORs with high efficiency and low cost. It has attracted so many attentions and been an alternative tool for the recombinant production of GPCRs, because it only needs a few hours to finish a whole expression process. This system just tries to mimic the natural cytoplasmic environmentbut it doesn't require living cells. It would circumvent cellular toxic effects known in a conventional cell-based expression systems, which are caused by membrane incorporation of recombinant membrane proteins and incompatibility of membrane protein function with cell physiology. Figure 6.2b shows how the cell-free protein expression method exploits the cellular protein synthesis machinery to produce proteins directly using exogenous messenger RNA (mRNA) or DNA as template without intact cells. The synthesis system contains all the necessary substances for protein synthesis, including an exogenous supply of essential amino acids, nucleotides, salts and energy-generating factors [33]. Compared to traditional cell-based expression methods, it offers several advantages, such as easy modification of reaction conditions to help the right protein folding, decreased sensitivity to product toxicity, and suitability for high-throughput strategies. Besides, some effective detergents (digitonin, Brij58, and Brij78) are used and optimized, in order to avoid the formation of large protein–detergent micelles and possible expression inhibition. Cell-free production of a human OR (hORI7-4) has been reported and used in a biosensor for odorant assays successfully [34].

As a complementary method, chemical synthesis is also reported to produce olfactory peptides and proteins in the development of smell sensors. In general, specific binding domains of ORs and short olfactory peptides could be synthesized using this method. Computational simulations can help to predict the binding domains of ORs and determine the binding affinity as well as orientation to selected ligands. Once simulations determine the sequence of an OR binding site (polypeptide), ORs will be synthesized chemically by amino acids reactions and can be used as sensing materials for odor detection [35, 36]. The synthetic peptides provides lots of merits when using as sensing materials of biosensors, such as good stability and reproducibility, high selectivity (similar to protein), and

relatively low production cost [35, 36]. Furthermore, synthesized peptides can be easily modified in specific sites, for example, tagging a thiol on one terminal of peptides, which is very useful for the later immobilization on a transducer.

6.2.3 Natural Structure Maintenance of Olfactory Receptors

ORs are located on the membrane of OSNs and belong to G protein-coupled receptor (GPCR) family. The right folding and structural maintenance of seven transmembrane domains will affect the function directly as well as the binding interaction between ORs and corresponding odorants. Therefore, maintaining the natural structures of ORs is a fundamental and crucial issue in the development of OR-based biosensors. Researches in this area require not only the biological knowledge to understand the nature of these ORs, but also the ability to use and modify them, or their synthetic mimics, in a manner which utilizes them to detect chemical odorants, and the suitable sensing mechanism to detect these biological recognition events. In the following part, we will discuss the natural structure maintenance of ORs combing with different production methods in the development of biosensors.

For those actual ORs obtained from the natural olfactory sensory tissue or cells, they are in the native structures and have the environment to keep it. The natural lipid membranes could help sustain the seven transmembrane domains. For these receptors, if they are used in the whole cells, their function will be kept in the most possibility. However, it is not practical and applicable in many applications, because one has to keep the natural tissue or cells alive in the biosensors, which will increase the complexity of the whole system. If extracted from the natural tissues or cells, even though the receptors are same, the natural lipid bilayer and the environment will be changed more or less. Currently, there is no effective method to evaluate the function and structure of extracted olfactory receptors used for the biosensor. These main shortcomings restrict the usage olfactory receptors from the natural source and at the same time drive the research to find the alternatives, which maintain the structure and sensitivity inherent in whole organisms, and are worthy of the commercial research founding in terms of performing a required application.

For cell-based OR synthesis method, the problem of natural structure should be of concern from the beginning of the synthesis. The production of membrane proteins in cellular systems have several problems due to the hydrophobic nature of ORs, which often causes protein misfolding, aggregation and even cytotoxicity, resulting in low yields of functional proteins. Like the natural receptors, ORs produced cell-based are localized onto the cellular membranes. Once folding in the right manner is achieved, the natural structure will be ensured. Therefore, a great many methods are proposed during the process of cellular expression to ensure the right expression and localization of these receptors. For example, the gene of a kind of short peptide (rho-tag) was inserted in the expression vector before the

gene of OR in order to lead the synthesized OR to localize on the cellular membrane [37]. Considering the further extraction from the cells, some labels are also added, such as his_6-tag [37], which can assist the purification of the corresponding receptors and the immobilization onto the sensitive surface of sensors. Besides, the same problem persists when extracting out of the cells.

As for the cell-free OR synthesis method, keeping the natural structure seems especially necessary since there is no real cellular membrane in this system. So much attention should be paid to the stability and right folding of ORs in the synthetic system. In order to produce non-aggregated membrane proteins, it is very critical to select optimal surfactants. That will ensure the newly produced membrane proteins not only to fold correctly, but also remain soluble and biologically functional. Till date, finding an appropriate surfactant for specific membrane protein is still a kind of laborious work, because different proteins react differently to the same detergent, even highly related proteins may differ very much. So it would be advantageous if a class of simple surfactants could be used for stabilizing diverse membrane proteins. Wang et al. used peptide surfactants in the commercial *Escherichia coli* cell-free system to rapidly produce milligram quantities of soluble G protein-coupled receptors (GPCRs), including the human formyl peptide receptor, human trace amine-associated receptor, and two other ORs. These receptors expressed in the presence of the peptide surfactants were soluble and had α-helical secondary structures, which suggested that at least they were properly folded. In addition, microscale thermophoresis measurements showed that one of ORs expressed using peptide surfactants can bind its known ligand heptanal. It indicates that these short and simple peptide surfactants will be able to facilitate the rapid production of ORs, or even other membrane proteins, for the structure and function studies [38]. That provides a convenient solution for the efficient production and natural structure maintenance of ORs in the development of biosensors.

For the ORs produced by the chemical synthesis method, there is no necessity to supplement the cellular membrane. Most synthesized products are not the complete sequence of the ORs, but are the functional motif or peptides, which usually don't contain the complete seven transmembrane structures. Therefore, the structure maintenance is not as demanding as that of other methods. It provides much convenience for all the development and storage of biosensors, and has promising prospect in the future applications.

Last but not least, all the receptors, no matter extracted or synthesized, need some general storage conditions like common protein products. Usually it needs the low temperature to avoid inactivation and oxidization. As a solution, it should kept in the right pH and stabilizing agents. Besides, dry powder or crystalline form is beneficial to the preservation, which has longer shelf life.

6.3 Design of Olfactory Receptor-Based Smell Sensors

6.3.1 *Secondary Transducers for Olfactory Receptor-Based Smell Sensors*

Among various transducers used for OR-based biosensors, field effect transistor (FET) is one of the most commonly used transducer device. The typical structure of FET used for the development of OR-based biosensors is illustrated in Fig. 6.3a. In this structure, the gate area is usually covered by an additional ion- and/or charge-sensitive layer, which can generate surface electrical charge and potential changes in response to target chemical or biochemical analytes. The surface electrical charge and potential changes generated in the gate layer will modulate the FET's drain-source channel current, which allows for the detection of any kind of electrical interactions at or nearby the interface of the electrolyte such as the absorption of charged macromolecules (e.g., polyelectrolytes, proteins, DNA) and the potential changes (e.g., action potential of nerve cells, metabolic processes of bacteria or cells, ligand-receptor interactions). FET device can detect these chemical and electrical changes based on field effects [39]. One important advantage of using FET devices as transducers is their innate signal amplification function, which is crucial for the weak signal detection. As a result, FET devices have been widely utilized as the secondary transducers for the development of OR-based biosensors. For instance, the insect antenna was coupled with the gate of a FET device to develop a specific odor-sensitive devices based on the detection of voltage changes originated from the antenna responding to target odors, which have been applied in the detection of plant damage in a greenhouse under real- world conditions [39]. In another report, FET device was modified with single-wall carbon nanotube (swCNT) in order to develop a bioelectronic nose for

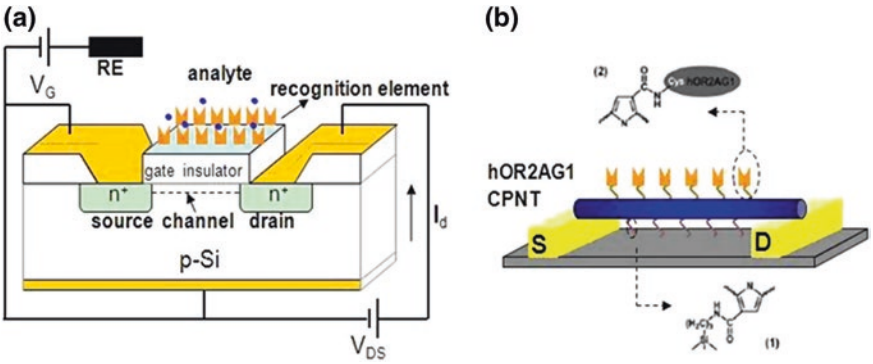

Fig. 6.3 **a** Schematics of typical structure of FET devices utilized as transducers of OR-based biosensors. **b** Schematic diagram of a CPNT-FET device, (*1*) CPNTs were covalently bind to the electrode substrate and (*2*) ORs were covalently immobilized on the nanotubes [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

the detection of specific odorant molecules [25]. The surface of swCNT-FET was coated by a layer of human OR protein (hOR2AG1), which can generate a conformational equilibrium between inactive and active biophysical states of the OR. Upon the exposure of a specific odorant, hOR2AG1 can bind to the odorant molecules and the equilibrium shifted toward active receptor states, which can generate a responsive electrostatic perturbations. The measurement results indicate that this biosensor have an ultrahigh selectivity, which can discriminate two kinds of odorant molecules with only a single carbon atom difference in structure. Similarly, hOR2AG1 was coupled with a FET device modified with carboxylated-polypyrrole nanotubes (CPNTs) as shown in Fig. 6.3b [40]. hOR2AG1 was selectively and covalently immobilized on the surface of CPNTs that was coupled with a FET device. The interactions between hOR2AG1 and its target odorant molecules can result in the changes in the source-drain current of FET device. This OR-based biosensor can detect the cognate ligand at concentrations down to 40 fM.

In the recent decades, electrochemical impedance spectroscopy (EIS) has attracted more and more attentions in the development of OR-based biosensors due to its capability of label-free and highly sensitive detection of molecular interactions. The EIS measurement systems usually employ a three-electrode arrangement which consists of a working, a reference, and a counter electrode. The surface of working electrode is usually functionalized with a layer of ORs for the detection of odorants. As shown in Fig. 6.4a, the exposure of target odorants will generate the structure rearrangement of OR, which can be detected via EIS measurements. For example, a gold electrode was functionalized with the rat OR (ORI7) for quantitative odorant detections via EIS measurements. The ORI7 protein was immobilized on the surface of gold electrode which maintained its natural function to recognize its target ligands, heptanal and octanal. On the other hand, interdigitated electrodes were functionalized by the immobilization of yeast cells expressing the human OR (ORI7-40) for odorant detection via measurement of conductometric changes that originated from the specific interactions between ORs and target odorant ligand molecules [29]. It is demonstrated that this OR-based biosensor has high sensitivity (threshold 10^{-14} M) and specificity. Similarly, the yeast cells co-expressed ORI7-40 with α-subunit of G_{olf} protein were utilized as sensitive elements for odorant detection based on impedimetric measurement [27]. The results show that the co-expression of ORI7-40 with GRP-γ-S can enhance the activity of ORI7-40 by 4 times higher as indicated by detection sensitivity. In addition, polarization resistance measurement by EIS has also been employed for the detection of the differences in inactive and ligand-bound states of rat receptor OR-I7 [41]. Figure 6.4b shows the obvious difference between these two states by Nyquist plot of OR-I7 in the presence and absence of specific odorant. Furthermore, for the prediction and interpretation of electrical properties of a single sensing protein, the theoretical framework behind them was also investigated in detail [42]. It is suggested that EIS technique is not only an efficient method for OR-based biosensors toward odorant detection, but also very useful for the investigation of OR structural changes.

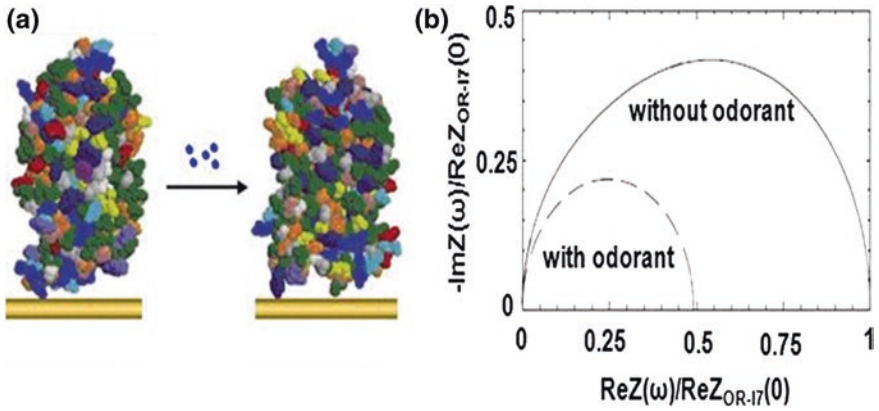

Fig. 6.4 **a** Schematics showing the conformational changes of the rat ORI7 upon the exposure of target odorant molecules. **b** Nyquist plot of rat ORI7 before and after the exposure of odorant ligands [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

Surface acoustic wave (SAW) devices are also demonstrated to be applicable for the development of OR-based biosensors, which are very sensitive to mass changes loaded on their sensitive area. Figure 6.5a is a photograph of a SAW device with an input and an output inter-digital transducer (IDT). When an electric field is applied to a confined region of a piezoelectric crystal in the acoustic wavelength range, surface acoustic waves are generated. Any minute mass changes loaded on the sensitive area of SAW device will result in the changes in the frequency of SAWs. In addition, the changes of the resonance frequency are proportional to the changed mass. The sensitive area of a SAW device was coupled with ORs in order to realize the highly sensitive functional assays of ORs for odorant detection [26]. It is demonstrated that this OR-based biosensor is able to detect the target odorants as low as 10^{-10} mM and with high specificity, which has great

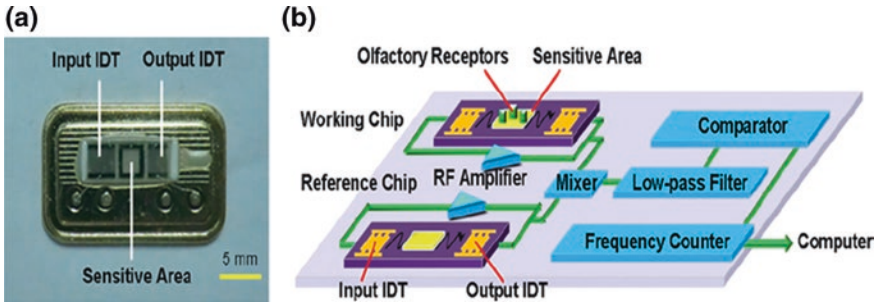

Fig. 6.5 **a** An image of a SAW device showing the input and output IDT as well as the sensitive area. **b** Schematics of the sensor system based on a pair of delay line SAW devices that are utilized as the working device and reference device, respectively [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

potential to be used for the characterization of odorant response profiles of ORs. The key advantages of this biosensor originated from the combination of the high sensitivity of SAW devices and the high specificity provided by ORs.

Quartz crystal microbalance (QCM) device is also applied in the development of OR-based biosensors, which is one of most commonly used mass-sensitive devices. Figure 6.6a shows an image of a QCM device and the schematic diagram of the QCM measurement system. QCM devices are usually developed by fabricating gold electrodes on both sides of a quartz crystal. The crystal is able to oscillate at a tuned frequency, which changes in response to the mass changes on the crystal. The binding/dissolution event occuring on the crystal will lead to the increasing or decreasing of the mass, which can consequently change the oscillation frequency. Therefore, it is possible to measure the mass changes on the crystal by monitoring the shifts of oscillation frequency in real time. QCM device has been employed as the secondary transducer for the development of OR-based biosensors for odorant detection due to its mass-sensitive property. For instance, the surface QCM device was coupled with HEK-293 cells expressing the rat ORs for the detection of specific binding between ORs and odorant molecules [43]. It is indicated that this OR-based biosensor can specifically respond to the target ligand of the ORI7 receptor, octyl aldehyde. The detection limit of this biosensor was as low as 10^{-8} mM. On the other hand, ODR-10 was expressed in *E. coli* and crude membrane extracts were coated on the QCM surface in order to detect the specific odorants [44]. The interaction between ORs and various odorant molecules was detected by monitoring the frequency shifts of QCM device before and after the odorant exposure. It was demonstrated that this biosensor can respond specifically to the natural ligand of ODR-10, diacetyl, which show highest responsive signals.

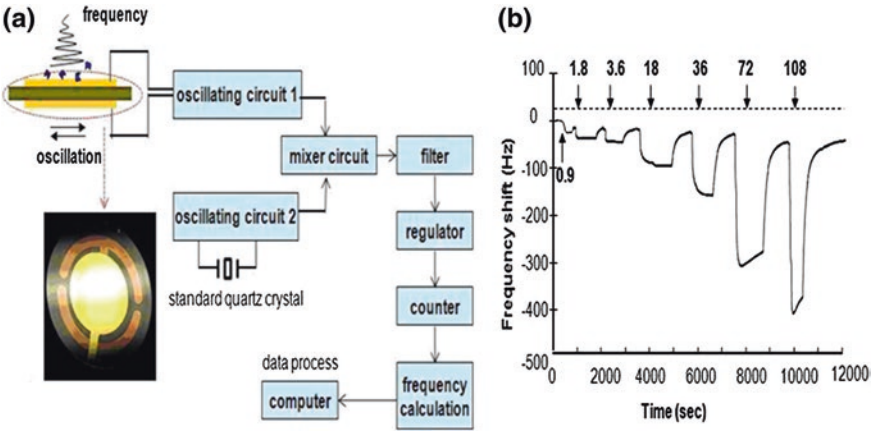

Fig. 6.6 **a** A photo of a QCM device showing the gold electrode fabricated on the surface of quartz crystal and the schematic of the sensor system. **b** Responses of an olfactory receptor-based smell sensor using QCM devices as the secondary transducer to various concentrations of acetic acid (in mg/mL) [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

In addition, an OR-based biosensor has also been developed using QCM devices as transducers for the detection of bacterial pathogens in packaged beef for food safety applications [35]. This biosensor can respond to 1-hexanol and 1-pentaol with very low detection limits, which are approximately 2–3 and 3–5 ppm, respectively. Similarly, QCM device was coupled with a chemically synthesized OBP for the detection of alcohols in low concentrations at room temperature, which show a detection limit as low as 1–3 ppm [36]. This biosensor provides a highly sensitive alcohol detection method for food safety applications. QCM devices have also been applied in a multi-array coatied with synthetic polypeptides and conducting polymers, which was able to realize the simultaneous detection and identification of VOCs [45]. It is indicated that the frequency shifts of QCM is proportional to the total weight of acetic acid adsorbed into the sensing material on the surface of crystal.

6.3.2 Coupling Techniques of Olfactory Receptors with Transducers

One key step of the development of OR-based biosensors is the coupling of functional olfactory receptors with transducers, which is crucial to the biosensor performances. It is important to realize functional immobilization of ORs on the surface of secondary transducers, which requires capturing ORs efficiently and maintaining their natural functions. Because ORs are membrane proteins, it is especially important to provide a hydrophobic environment for the maintenance of natural structures and functions of ORs. In addition, the ideal coupling requires high stability and specificity of immobilization, which are usually covalently immobilization that can greatly improve the stability, repeatability, and specificity of the biosensor. As membrane proteins, ORs could be immobilized on the transducer surface by employing the membrane protein immobilization strategies [46]. At present, the commonly used strategies employed for the immobilization of ORs onto transducers can be divided into three main categories: physical adsorption, self-assembled multilayer immobilization via antibodies, and covalent immobilization via chemical reaction. All the three strategies are illustrated in Fig. 6.7.

Physical adsorption is the simplest strategy, which only requires the evenly coating of the OR-containing solution onto the transducer surface. This strategy has been employed by many researchers due to its simplicity and convenience [21, 25, 43]. For instance, native nanovesicles containing functional ORs were immobilized on the surface of gold electrodes by physical adsorption and the immobilization procedure has been intensively investigated, which indicated that immobilization efficiency was affected by electrode hydrophobicity and the nanovesicles dimensions in suspension [47]. However, physical adsorption suffers from the lack of binding strength and the low stability. Furthermore, this strategy lacks the specificity of immobilization, which means lots of unwanted proteins

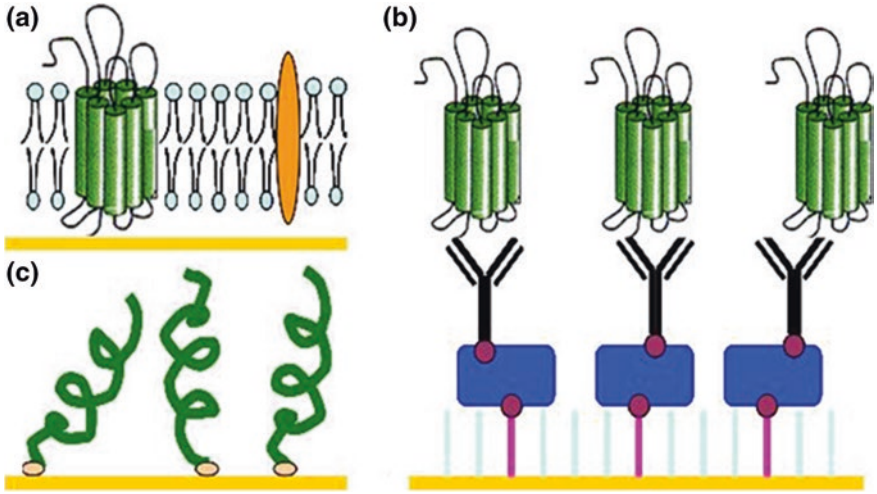

Fig. 6.7 Schematics of three types of immobilization methods for ORs coupling with transducers. **a** Physical adsorption, **b** self-assembled multilayer immobilization via antibodies, and **c** covalent immobilization [20]. (Reproduced with permission from Ref. [20]. Copyright 2013 Elsevier)

are also immobilized unless the protein solution contains only purified ORs. This could influence the performance of OR-based biosensors such as specificity and sensitivity.

Self-assembled multilayer immobilization via antibodies was developed in order to improve the specificity and stability of OR-based biosensors. In this method, ORs can be specifically recognized and immobilized by antibodies of a well-designed self-assembled multilayer. Hou et al. utilized this method for ORs immobilization, and compared the stability with Langmuir-Blodgett (LB) film's technique. The results demonstrated that the self-assembled multilayer immobilization via antibodies exhibited higher stability [48]. The schematic of this method is illustrated in Fig. 6.7b. First, a mixed self-assembled multilayer (SAM) carrying biotinyl group was formed on the gold surface of the secondary transducer via Au–S bonds (step 1); Next, neutravidin was captured on the substrate via biotinyl-neutravidin binding event (step 2); Afterward, biotinylated specific antibodies were captured via the active sites of neutravidin, and they can specifically bind the corresponding part of ORs (step 3); Finally, ORs were specifically captured via antibodies, and a specific and stable immobilization structure was realized (step 4). With the help of anti-receptor specific antibodies, the receptor proteins were immobilized with high efficiency and specificity as well as the stability. Besides, preliminary purification was obtained so that some other irrelevant proteins can be washed away. This method was employed for the immobilization of ORs in an electrochemical impedance spectroscopy (EIS)-based biosensor for odorant detection [28]. A similar method was also reported by Vidic, who employed antibodies

to specifically capture tagged ORs located in nanosomes on a transducer surface [30]. Besides, the aptamer is a kind of alternative molecule to antibodies, which can specifically bind to its ligands. Aptamers are single-stranded oligonucleic acid molecules. Compared with antibodies, they are more resistant, less expensive, and more easily modified with functional groups or tags. Therefore, Du et al. proposed a method using an aptamer-containing self-assembly monolayer to immobilize ORs for the development of smell sensors. In their design, an OR, ODR-10, was expressed in HEK293 cells with a His_6-tag on its N-terminus. The specific anti-His_6 aptamer was employed and its terminal was modified with −SH group for the direct immobilization onto the gold substrate via Au–S bonds. The results demonstrated that the purification and immobilization were realized simultaneously with the assistant of aptamers, and better performance of biosensors was achieved compared with that made by the physical adsorption method [37].

Direct covalent immobilization is also a specific and stable method, which is mainly based on Au–S self-assembly process [35, 36]. It is also simple with high stability during the fabrication process. It is especially suitable for the immobilization of highly purified synthesized peptides only if containing rich cysteines to provide –SH group. As illustrated in Fig. 6.7c, olfactory peptides containing a cysteine molecule on one terminus provided thiols to the covalent immobilization. Theoretically, all the ORs can be covalently immobilized onto any transducer surface only considering the possible covalent reaction between the protein and the substrate surface. If needed, the surface of transducers can be modified with some functional groups. Sankaran et al. utilized this method to immobilize chemically synthesized ORs onto the gold substrate of QCM biosensors [35, 36].

In addition to methods mentioned above, Vidic et al. also utilized a kind of carboxy-methyl modified dextran polymers to link the membrane nanosomes with the gold surface of the SPR sensor. They produced a kind of OR-containing membrane nanosomes by co-expressing mammalian ORs and an appropriate G protein in *Saccharmyces cerevisiae* [49]. The surface plasma resonance technique was employed in order to detect the interaction between ORs and odorants. This kind of adhering technique reduced the risk of receptor alteration and activity loss, just required relative low concentration of ORs, and kept the receptor binding sites accessible and functional. Therefore the measurements of receptor activity became much easier. The bioactivity of ORs was quantitatively analyzed in the immobilized membrane nanosomes.

6.3.3 Sensor System and Data Processing

Various transducers have been applied in the development of OR-based biosensors. Therefore, different sensor systems and data processing methods have been employed correspondingly for the efficient recording of the responses of ORs to the specific odorant molecules. In an OR-based biosensor, the configuration of sensor system and data processing are specifically designed and developed based

on the special transducer utilized for the transduction of responsive signals of ORs. Because the FET devices and EIS devices are very commonly used in the development of biosensors and have been introduced in other chapters of this book. Here, we briefly introduce two typical sensor systems and data processing methods that have been applied in the development of OR-based biosensors, which are based on SAW devices, and QCM devices.

SAW devices can detect the changes of mass loading on their sensitive area by monitoring the the resonance frequency shifts of SAW. In OR-based biosensors, the sensitive area of a SAW device is coupled with ORs and the specific binding between ORs and target odorants will result in the changes of mass loading on the SAW devices and consequently, lead to the shifts of SAW resonance frequency [26]. Figure 6.5b shows the schematic diagram of a dual delay line SAW system, in which two SAW devices were utilized as the working chip and the reference chip, respectively. Only the sensing area of working chip was immobilized with a heterologously expressed OR, ODR-10, and used as sensing elements. In addition to a pair of SAW devices, RF amplifiers were used to drive the SAW devices. The output signals from the working SAW device and reference SAW device were mixed by a mixer, and then a low-pass filter was employed to filter the frequency difference between them. Following, a comparator would generate a square wave for the frequency counter to count frequencies. Finally, the signals were transported to a computer via RS232 port for data recording and further data processing. For odorant detection, the sensitive area of SAW devices was exposed to odorants via a sealed detection chamber with input and output pipes. Odorants at the desire concentrations can be introduced to the sensor surface using nitrogen as carrier gas.

Similarly, QCM device is also a kind of mass-sensitive devices, which can detect the mass changes loading on the surface of quartz crystal. In case of OR-based biosensors, ORs are usually immobilized on the surface of gold electrodes fabricated on the surface of quartz crystal. The responses of ORs to odorant stimuli can be monitored by recording the resonance frequency changes of QCM. Figure 6.6b shows the responsive signals of this biosensor to various concentrations of acetic acid [45]. QCM devices are usually used together with a standard quartz crystal as a reference. Oscillating circuit is employed to drive the QCM device. Then the output signals of the QCM device and stand quartz crystal are mixed by a mixer circuit and subsequently, filtered by a filter. After the regulator, the frequency can be countered by a counter and then calculated by a frequency calculation. Finally, the data is transmitted to a computer for data processing.

Both SAW and QCM devices are mature transducers for the detection of tiny mass changes, which show very high sensitivity and require relatively simply sensor systems and data processing. Both of them have attracted more and more attentions for the development of OR-based biosensors, which combine the high sensitivity of SAW and QCM devices with the high specificity of ORs. Especially, OR-based biosensors using SAW and QCM devices provide a valuable tool for the research of interactions between ORs and odorant molecules.

6.4 Applications of Olfactory Receptor-Based Smell Sensors

6.4.1 The Odorant Detection in Food Industry

The basic function of olfactory system is to detect and distinguish various odors. Therefore, the odorant detection and discrimination is one of important applications of olfactory receptor-based smell sensors. At present commercialized olfactory receptor-based smell sensors are still not available in the market, but researchers are trying to broaden its practical applications, such as in the fields of food industry, toxic detection, environment safety, and even health care.

Trimethylamine (TMA) is a volatile degradation product of nitrogenous organic material, which has been widely identified in many animal and plant tissues together with other amines. It is produced by metabolism of the precursor trimethylamine N-oxide by microorganisms and its concentration will rapidly increase in marine products after putrefaction. Therefore the detection of TMA could indicate the freshness of seafood. Besides, TMA is also used as a catalyst and an intermediate in the chemical industry, and its exposure will induce considerable risk to human health. Suska et al. developed a new approach for the detection of TMA using a recombinant cell line of *Xenopus laevis* [50]. The mouse trace amine-associated receptor 5 (mTAAR5) is a G protein-coupled receptor from the mouse olfactory epithelium and sensitive to TMA. In their research, mTAAR5 was expressed in the cell line *Xenopus laevis* and cellular responses to TMA was analyzed by absorbance measurements and cellular imaging. The results demonstrated mTAAR5 transfected Xenopus melanophores could respond to a selection of tertiary amines, including TMA. As shown in Fig. 6.8, different amines exerted varying potencies and maximal efficacies when stimulating the mTAAR5 receptor (Absi is the initial absorbance, Absf is the final absorbance). The dose–response curve showed a characteristic sigmoidal shape. The half maximal effective concentration was 4 μM.

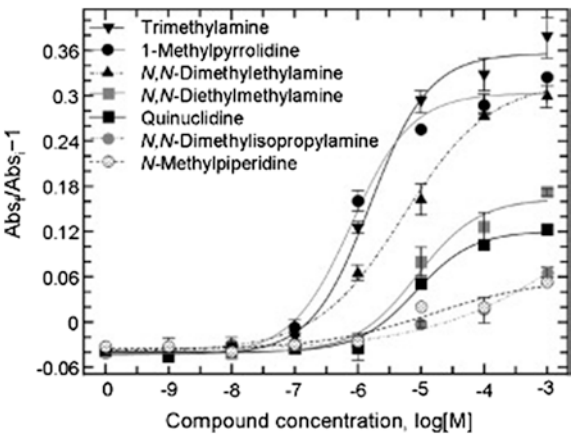

Fig. 6.8 Responses of an OR-based biosensor to various odorant molecules at different concentrations [50]. (Reproduced with permission from Ref. [50]. Copyright 2009 Elsevier)

Sankaran et al. utilized a synthetic polypeptide sequence based on LUSH OBP binding site as a sensing material in a QCM sensor to detect alcohols. The sensor was sensitive to low concentrations (with estimated detection limit of <5 ppm) of alcohols such as 3-methyl-1-butanol and 1-hexanol [35]. Sankaran et al. also developed QCM-based polypeptide sensors based on simulation studies. Tripos/Sybyl®, a molecular simulation program was used to predict the binding site of mouse olfactory receptor 774 using a modeled 3D structure. A polypeptide sequence incorporating the binding site amino acid residues was then selected and synthesized as the possible sensing material. This material was deposited on a QCM crystal to assess the sensor sensitivity to alcohols. The sensor was developed for the detection of alcohols that could be related to *Salmonella* contamination in packaged beef. It was reported that the developed sensor was sensitive to 1-pentanol and 1-hexanol with an estimated lower limit of detection of about 3–5 and 2–3 ppm, respectively [36].

6.4.2 Research of Olfactory Receptors and Ligands Interactions

Until now still lots of olfactory receptors are orphans for their unknown ligands. A high-throughput screening platform is urgently needed to monitor the interactions between olfactory receptors and ligands, as well as to evaluate the function of olfactory receptor. In this case the olfactory receptor-based smell sensor is one of good candidates.

Surface plasma resonance biosensor is a kind of label-free technology, which is sensitive enough to detect extremely small changes on its sensor chip surface. The commercial device is now available and has been combined with olfactory receptors to detect the interaction between receptors and odorants. Zhang's group produced olfactory receptor hOR17-4 using cell-free synthesis system. In order to evaluate the bioactivity of produced olfactory receptor hOR17-4, they utilized a commercial surface plasma resonance biosensor (Biacore, GE Healthcare) to monitor the response of olfactory receptor to its ligand and other odorants. The result is shown in Fig. 6.9a, a dose-dependent specific binding of undecanal, a known ligand, to the receptor captured was observed on the Biacore sensor surface. But experiments with a known nonbinder odorant of hOR17-4 did not show any interaction. Furthermore, the data could also be used to derive an affinity constant, K_D, of 22 μM (Fig. 6.9b), which is also consistent with in vitro experiments that have also shown that odorants bind to hOR17-4 with EC50 in the micromolar range [34].

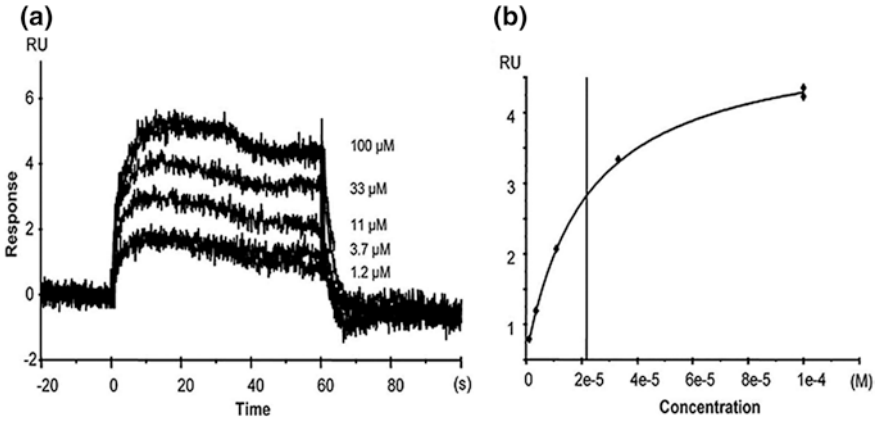

Fig. 6.9 The interaction between hOR17-4 with the known hOR17-4-binding odorant undecanal detected by the surface plasmon resonance device. **a** Responses to undecanal at 1.2, 3.7, 11, 33, and 100 μM. **b** Equilibrium binding responses plotted versus the undecanal concentration and then fitted to a simple binding isotherm to yield an affinity of ~22 μM for hOR17-4 [34]. (Reproduced with permission from Ref. [34]. Copyright 2008 National Academy of Sciences, U.S.A.)

6.4.3 Toxic and Explosive Gases Detection in Public Safety

The detection of toxic and explosive gases is one of important work in public safety monitoring, and it is also one of crucial application fields of olfactory receptor-based smell sensors. For example, one of the best-known explosives 2,4,6-Trinitrontoluene (TNT) distributed in air, water, and soil, is recognized as an environmental pollutant as well. Its exposure will result in severe adverse effects on people's health due to the toxicity. Besides, its biodegradation products are also mutagenic and harmful to the terrestrial life. Therefore, the detection of TNT and its metabolites is crucial for public security as well as the environment and human health [51]. The traditional TNT detection is based on the expensive and sophisticated laboratory instrumentation, such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS). Kim et al. developed a selective and sensitive TNT sensors using biomimetic polydiacetylene-coated FET. TNT-binding receptors (tryptophan-histidine-tryptophan: WHW) are linked to a conjugated polymer polydiacetylene (PDA) and modified onto the surface of single wall carbon nanotube (SWNT)-FET sensors. The selective binding events between WHW receptors and TNT will be detected by the sensitive SWNT-FET conductance sensors. The possible mechanism of signal transduction is depicted in Fig. 6.10f. When TNT is selectively recognized by WHW receptors, the π electrons of the TNT molecule can interact with the electron-rich π orbital environment of the three aromatic side chains of WHW as the red arrows indicated in Fig. 6.10f. Since the distance between the binding site and conjugated PDA is about 1 nm, the binding events could perturb the conjugated PDA electronic band

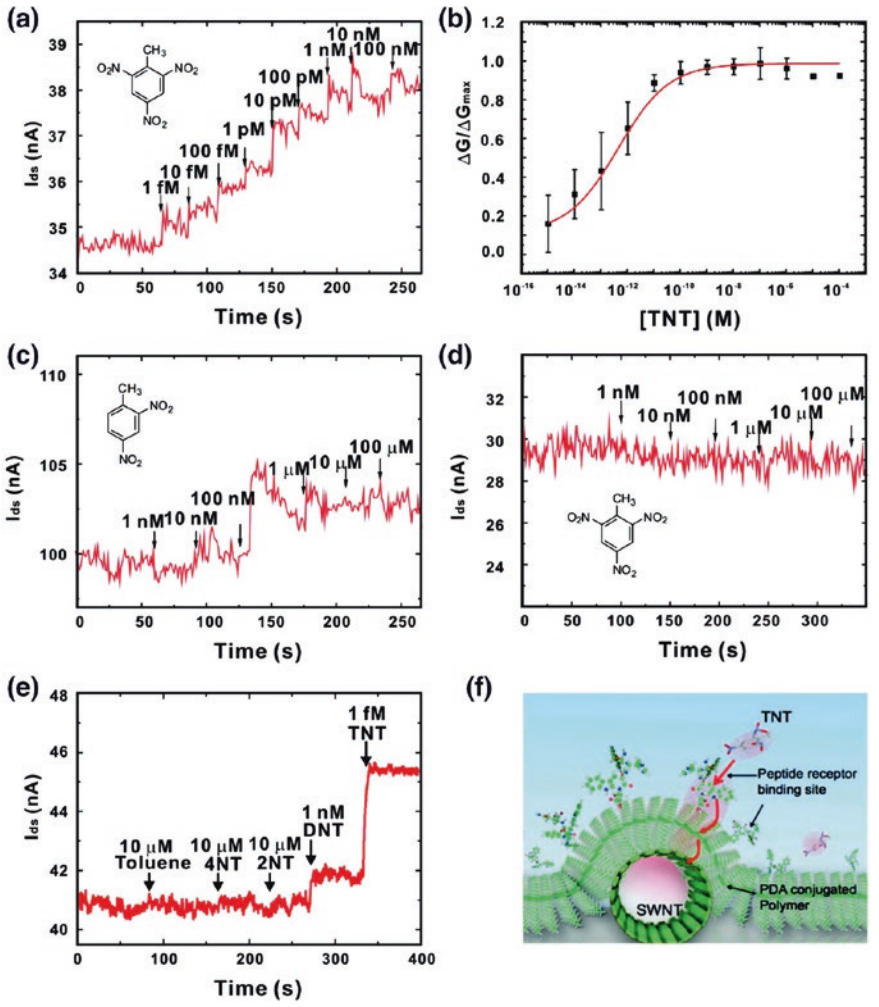

Fig. 6.10 Specific and sensitive detection of TNT using the TNT sensors based on WHW-PDA-functionalized FET [51]. (Reproduced with permission from Ref. [51]. Copyright 2011 American Chemical Society)

structure and cause a chromic shift. The electronic perturbation can be further detected through the electric conductance signals. To detect the target TNT and other control chemicals, the source-drain currents of WHW-PDA functionalized FETs are monitored. As shown in Fig. 6.10a, the electrical conductance showed significant increase in response to TNT from 1 fM to 1 nM, and then gradually came to saturation from 10 nM. A calibration curve shown in Fig. 6.10b, in which the normalization of conductance increased with the concentration of TNT and came to the saturation gradually. The addition of DNT caused a conductance

increase of FET only in high concentration (Fig. 6.10c). When the whole sensor system doesn't contain WHW receptors, the conductance showed no change to the addition of TNT or DNT at a concentration from 1 nM to 100 μM (Fig. 6.10d). The response to TNT in mixed solution of toluene, 4NT, 2NT, and DNT is shown in Fig. 6.10e. All the results demonstrated that this biosensor could distinguish TNT selectively and sensitively.

Radhika et al. engineered a *Saccharomyces cerevisiae* strain, in which olfactory receptor signaling is coupled to green fluorescent protein expression. Olfactory receptors were screened to report the presence of an explosive residue mimic 2,4-dinitrotoluene. Using this approach, they have identified the novel rat olfactory receptor Olfr226 as a 2,4-dinitrotoluene—responsive receptor [52].

6.5 Summary

OR-based biosensors are very promising due to the great prospects and commercial potential. Only in the past two decades, much progress have been achieved. In this chapter, some common issues about OR-based biosensors were discussed, and the critical technical problems such as the production of ORs and various immobilization methods were emphasized in detail. In terms of the significant advantages of high sensitivity and specificity, OR-based biosensors are promising and attractive for the odorant detection for various applications such as environmental safety monitoring, food security, drug screening, and agricultural diseases [35, 53, 54]. In addition, it could also be used for the basic research of olfactory receptors, for example, high-throughput screening ligands for orphan ORs.

The natural characteristics make ORs very appropriate for the development of smell sensors and the detection of numerous odorants. Therefore these special features can be used to design a bioelectronic nose in order to mimic the olfactory system. Till date, much efforts have been devoted to the design and development of the bioelectronic nose. Significant improvements have also been made in this field. However, some challenging problems are still in presence on the way. The first one, only a few cognate ligands exist in the currently available odorant repertoire in mammal's OR. So various OR genes have to be cloned and expressed in order to produce the desired OR. Second, the low expression yield of recombinant ORs in heterologous cell systems is still a bottleneck. Many attempts using numerous expression systems have been made to increase the level of expression. Adequate expression systems for the functional expression of ORs are still required and the introduction of additional components like chaperones may allow for sufficient levels of OR expression on the cell membrane. Third, concerning the anchoring of ORs on the solid surface of sensor, a variety of approaches have been reported, but this is still an important factor in the field of biosensor development. Since OR proteins have to remain in a lipophilic environment to retain their structure and function, the procedures used to immobilize OR proteins or cells expressing OR on solid surfaces have to be conducted under mild ambient

conditions such as at the proper pH, temperature, etc. Also, a better understanding of the mechanism of OR-odorant binding on the molecular level is required for the development of a more efficient bioelectronic nose. The binding between ORs and odorants immobilized on solid surfaces causes a conformational change of the ORs or the signal transduction of olfactory cells. Efficient immobilization of ORs or cells on solid surfaces can allow one to more reliably and reproducibly monitor binding.

Currently, there is almost no commercialized OR-based biosensor available since most OR-based biosensors are in the experimental stage and not mature enough for practical applications. The major obstacles to develop a commercial biosensor include fragility of the biological components used for odorant sensing as well as the lack of small, portable signal transduction systems. On the other hand, the development of more stable sensing materials is still one of the key research topics concerning smell sensors in future. More specifically, proteins and peptides are likely to substitute tissues and cells as sensing elements in biosensors due to their higher stability and reliability. In addition, further understanding of odorant binding sites will facilitate the development of alternative/synthetic OR substitutes for odorant detections in OR-based biosensors. Additionally, the development of microfluidic technologies will greatly contribute to the miniaturization of OR-based biosensors due to the much smaller size of devices as well as the dramatic reduction of sensing materials and sample volumes. Benefits from these advantages and the integration of OR-based biosensors with microfluidic devices would also be a promising research direction, which could make biosensors more suitable for real-world applications. In summary, OR-based biosensors are the research products of multiple disciplines, which involve biology, protein engineering, materials science, electric technology, and information processing, etc. With the development of microfabrication techniques and multidisciplinary cooperation, mature OR-based biosensors suitable for practical applications will undoubtedly emerge and find promising prospects in many fields.

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Chapter 7
Smell Sensors Based on Odorant Binding Proteins

Yanli Lu, Yao Yao and Qingjun Liu

7.1 Introduction

Odorant binding proteins (OBPs) belong to one of the most abundant class of proteins found in the olfactory apparatus, which play important roles in binding the small hydrophobic molecules to enhance their aqueous solubility and transport them to specific olfactory receptors (ORs). As the extracellular proteins, OBPs of insects and vertebrates were indentified almost at the same time and have a number of similarities [1]. They are all small soluble polypeptides of about 10–30 kDa and are highly concentrated in the nasal mucus of vertebrates and in the lymph of chemosensilla in insects [2–5]. Both possess a hydrophobic cavity that is the main place for odorants binding. Additionally, OBPs have a relatively broad specificity to various volatile hydrophobic molecules, and the dissociation constants are near submicromolar. However, their structures have their own characteristics. Vertebrate OBPs belong to a subclass of lipocalins and share a conserved folding pattern, such as eight antiparallel β-sheet and an α-helix at the C-terminal end [6, 7]. Meanwhile, vertebrate OBPs exist as monomer or homodimer depending on OBPs. For example, bovine OBP is a homodimer, while porcine OBP is a monomer [8–10]. For OBPs of insects, they present a completely different three-dimensional structure, which is constituted by six α-helical domains arranged in a very compact structure.

Compared to membrane protein ORs, OBPs are easier to be isolated and purified in the process of production. Additionally, OBPs are robust enough to stand up to

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wide ranges of pH and temperatures (even to 80–100 °C) for substantial mistreatments, without denaturing and losing their binding properties [2–5]. All of these will greatly enhance the practicability of those materials used in sensors. Therefore, OBPs are good candidates as biological elements in the development of smell sensors.

Taking OBPs of mammals or insects as the sensing elements, different sensors, such as surface acoustic wave (SAW), quartz crystal microbalance (QCM), electrochemical impedance spectroscopy (EIS), surface plasmon resonance (SPR), and cantilever sensors, have already been developed [7, 11–17]. For mammal OBPs, such as bovine OBP and porcine OBP, they have already been studied in biosensors for odor detections, such as ethanol, octenol, and carvone, with high specificity and sensitivity [5, 7, 17–19]. For insects, studies show that OBPs of honeybee and fruit fly could be used to detect floral odors, pheromones, and semiochemicals, in vitro [11, 14, 15, 20]. Excitingly, recent studies show that peptide sequences derived from OBPs of Drosophila and honeybee (*Apismellifera*), and some mutant OBPs have also been used to detect chemicals, such as alcohols and trinitrotoluene (TNT) [11, 20]. Moreover, with the help of X-ray, nuclear magnetic resonance (NMR), and computer modeling, OBPs conformation changes and the most related amino acids can be inferred. It offers great help in designing specific biosensors for odorant detections. With odorants, even that did not occur in the insects' natural environment, such as volatile compounds emitted from cancer cells [21, 22], have been successfully detected, OBPs-based biosensors provide promising approaches for chemical molecular sensing, as well as for precise binding functions investigating this kind of special olfactory protein [15].

Based on our previous studies of OBPs [14, 15], this chapter will give a detailed illustration about the development of OBPs-based biosensors, including how to isolate and purify OBPs, analysis of electrical characteristics of OBPs, the development of OBPs-based biosensors, and their promising applications.

7.2 Theories of Odorant Binding Proteins-Based Smell Sensors

7.2.1 Molecular Features of Odorant Binding Proteins

The OBP family includes two kinds of proteins, the phoneme binding protein (PBP) and the general protein (GOBP) [23]. Both have low molecular weight, and generally low isoelectric point. Although the exact function of OBPs remains unclear, studies summarize them as follows: acting as transporters of hydrophobic odorant molecules that protect the odors from degradation before activation of the receptors, the essential cofactors in activating ORs, and scavenger or deactivator by removing odorants from the sensillum lymph [24] (Fig. 7.1).

Although there are differences between structures of vertebrate OBP and insect OBP, the tertiary structure of these OBPs shares a common point, a central hydrophobic pocket. There are a variety of chemical groups exposed in the cavity, which

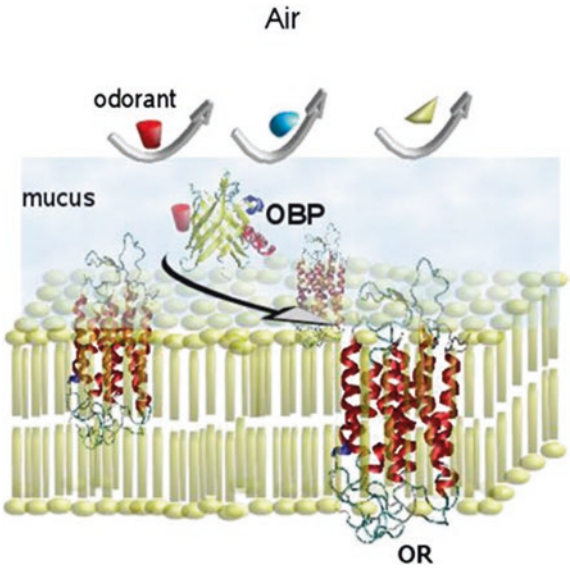

Fig. 7.1 Odorant binding proteins (OBPs) in the nasal mucus, capture odorants to putatively transport them to olfactory receptors (ORs) [25] (Reproduced with permission from Ref. [25]. Copyright 2012 Wiley)

provide an optimal environment for the interactions between odorants and the protein. When different odorants are bound in the cavity of OBPs, they form a specific conformation that might be stabilized by hydrophobic, polar, electrostatic, and π-stacking interactions. The structures of OBPs could encode different binding properties for the wide spectrum of odorant molecules, such as aromatic molecules and aliphatic compounds. Moreover, as selective peripheral signal filters to the actual receptor proteins, OBPs are robust enough to withstand wide ranges of pH and temperatures [2–5].

Differing from vertebrates, insects like honeybees are more dependent on olfaction to distinguish, comprehend, and estimate the overall situation, which is essential for their survival, reproduction, and social communication within the hive; whereas vertebrate animals use their highly developed vision to define the outer world [26, 27]. While their behavior heavily relies on olfactory cues, insects have remarkable olfaction ability to recognize signals around the habitat environment.

7.2.2 Electrical Properties of the Odorant Binding Proteins

The interactions between OBPs and their odorants can cause the conformation changes of proteins, especially the backbone and enhanced protein internal dynamics at the odorant entry site [23, 28]. These conformation changes have a close relationship with the protein's electrical properties. Therefore, electrical testing has the potential to study the odorant binding process and OBPs' conformation changes.

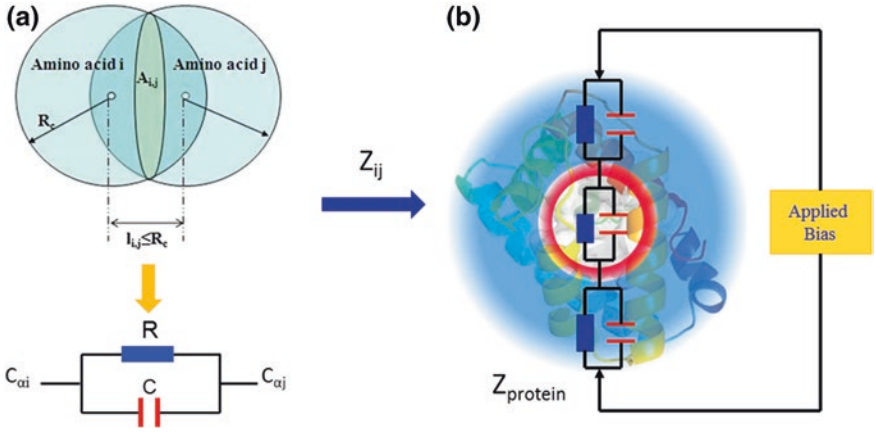

Fig. 7.2 Electrical properties and the theoretical impedance model of OBPs. **a** The link between amino acids and its equivalent impedance elementary RC circuit. (Reproduced with permission from Ref. [15]. Copyright 2014 Elsevier) **b** Cartoon of the protein backbone and cavity with the equivalent circuits. (Reproduced with permission from Ref. [31]. Copyright 2013 Elsevier)

In order to study the electrical properties of OBPs, some theoretical models have been established [29, 30]. In these models, each amino acid was taken as a node and replaced with a sphere, whose position coincided with its C_α atom. Each couple of nodes was connected with a link, when their distance $l_{i,j}$ was less than the assigned interacting radius R_c. The optimal value of R_c should be in the range of 5–15 Å. To the protein that was the network of amino acids, it can be equaled to a single R-C parallel circuit [31]. The predominantly hydrophobic internal cavity of OBPs provided additional possible interactions for odorant binding. Therefore, an independent R-C parallel element can be used to represent the properties of the pocket. Finally, the whole impedance of OBPs can be represented by Z_{protein} (Fig. 7.2). Thus, the impedance can be measured by electrochemical sensors.

7.2.3 Modeling of the Odorant Binding Proteins to Ligands

Generally, structures of proteins could be investigated by X-ray or nuclear magnetic resonance (NMR). However, the apparatuses were expensive and the detection process was time-consuming. Hence, the computer-assisted calculation was getting greater attention. Studies show that using the reported amino acid sequences, the tertiary structure of OBP can be modeled by I-TASSER server [32]. To predict the binding modes of different ligands to OBPs, the odorants could be docked into the binding site of OBPs using the molegro virtual docker (MVD). The binding pocket covers a site with a user-defined origin and a radius of 15 Å. MolDock SE was used as the searching algorithm and MolDock Score was used

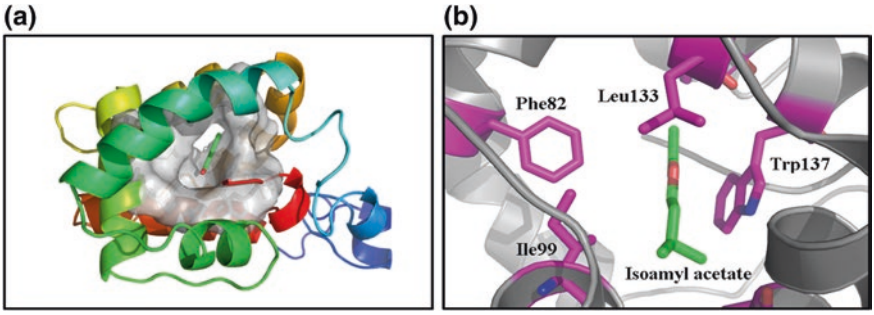

Fig. 7.3 Molecular docking of the odorants binding to OBPs. **a** Structure of BdorOBP2-odorant (isoamyl acetate) complex. **b** Isoamylacetate in the binding pocket of the OBP. (Reproduced with permission from Ref. [14]. Copyright 2015 Elsevier)

for the energetic evaluations. With the molecular docking models, the most related amino acid sequences, and the correlations between the conformational changes of protein and biosensors can be discussed. The detailed processes have been discussed in our previous papers [14, 15].

For instance, OBP of oriental fruit fly (*Bactroceradorsalis*), BdorOBP2, is illustrated in Fig. 7.3. BdorOBP2 consists of six α helices. These helices compose a large binding cavity, which is highly hydrophobic and can accommodate various odorants. To evaluate the binding mode and potency, isoamyl acetate was docked into the binding cavity. For the BdorOBP2-isoamyl acetate complex, isoamyl acetate lay exactly in the hydrophobic middle of several hydrophobic groups, such as benzene rings of Phe82 and Trp137. At the same time, electrostatic interactions between isoamly acetate and the negative charged side chain of Ile99 and Leu133 were also important in maintaining the stable structure. From the docking results, it can be inferred that four amino acid residues in the cavity, Phe82, Trp137, Ile99, and Leu133, play important roles in forming the BdorOBP2-isoamyl acetate complex.

7.3 Design of Odorant Binding Proteins-Based Smell Sensors

7.3.1 Preparation of Microelectrodes

In order to detect the interactions between OBPs and odorants sensitively, the applied sensors should be made small, and even into micro devices. The most widely used sensors include SAW, QCM, EIS, SPR, and cantilever sensors. Based on semiconductor technology and microelectromechanical systems, these electrode and electrode arrays can be easily fabricated. This part takes interdigitated electrodes as an example to illustrate the prepared process.

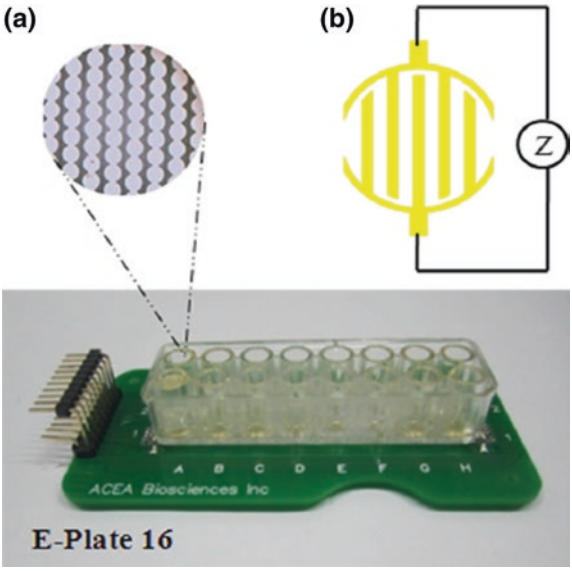

Fig. 7.4 E-Plate 16 device of the OBPs-based biosensor system. **a** Circle-on-line interdigitated electrodes on the bottom of a well. **b** Sketch map of the impedance measurement of the electrodes. (Reproduced with permission from Ref. [31]. Copyright 2015 Elsevier)

Briefly, a sterilized Pyrex glass 7,740 was chosen as the insulating substrate. Then, after sputtering a layer of titanium-tungsten (TiW, 20 nm thick) on the glass, a layer of Au (300 nm thick) was sputtered with the same method. Subsequently, polymer photochemistry was used to protect the layer of Au and then photolithography engraved a pattern in the polymer. Later, the sensing chip was packaged with PCB board. Perspex with round holes was used as the impedance detecting well in the experiment. Finally, adhere the perspex on the board with liquid adhesive and make the round holes aligned with the electrodes. With the mature technology, the interdigitated electrodes have already been commercialized products. For instance, the E-Plate 16 device produced by ACEA Biosciences Inc, has been successfully used to develop smell sensors [31] (Fig. 7.4).

7.3.2 Production and Fixation of Odorant Binding Proteins

As shown in Fig. 7.1, ORs belong to G protein-coupled receptors (GPCRs), characterized by a seven-transmembrane domain. Therefore, it makes ORs more difficult to produce than soluble proteins, OBPs. Although cell-free expression system show potential to solve this problem, it was still difficult to produce functional receptors and stabilize them for a sufficient period of time [33]. For OBPs, however, the recombinant OBPs, such as rat OBP, porcine OBP, and insect OBPs, have been expressed in bacteria as well as in yeast in high yields, making their production simple and economical [14, 15, 34–36].

Here, we will take Acer-ASP2, OBP of *Apisceranacerana*, as an example to show the expression and purification process. Acer-ASP2 was cloned from the

full-length cDNA of adult worker bees, Apisceranacerana [37]. Briefly, using reverse transcription-polymerase chain reaction (RT-PCR) and pET-30a (+)/BL21 (DE3) prokaryotic expression system, Acer-ASP2 can be cloned and expressed by being transformed into *Escherichia coli* BL21 competent cells. The bacteria were induced by isopropyl â-D-1-thiogalactopyranoside (Merck, Darmstadt, Germany) to initiate the expression of recombinant proteins. After harvesting the bacterial cells, the crude cell extracts, pellet, and supernatant were analyzed by 15 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to check if the recombinant proteins were expressed. Afterward, the inclusion body of Acer-ASP2 was severely precipitated in 1.5 M urea in ddH_2O and finally freeze-dried (Fig. 7.5). The detailed process can be referred to in our previous papers [15].

In order to establish OBPs-based biosensors, one key factor is to develop an immobilization method for binding biomolecules on the surface of the transducer. The immobilization technique should not alter the activity, structure, and function of the biological component and should assure long-term stability of the active layer of the biosensor. The principal methods of immobilization include physical adsorption and chemical self-assembly.

In this part, we will introduce two methods for protein immobilization on the gold surface of the sensors. The first method was utilizing the binding affinities between OBPs and nitrocellulose membrane. After carefully cleaning the interdigitated electrodes, nitrocellulose membrane dissolved in methanol was added into plate wells until electrodes were completely coated. After methanol evaporated off, the OBPs solution was evenly injected on the nitrocellulose membrane. A 2 h waiting period or more was needed to guarantee that the protein molecules were embedded in the nitrocellulose membrane [15]. The residual solution was then drained out with PBS. The second method was about chemical self-assembly. After removing the organic residues off the substrate and then drying in nitrogen, α-thio-ω-carboxy poly(ethylene glycol) (COOH-PEG-SH in short) solution was added to the electrodes for approximately 24 h to form stable Au–S covalent bonds. Then a self-assembled monolayer (SAM) of PEG was established on the

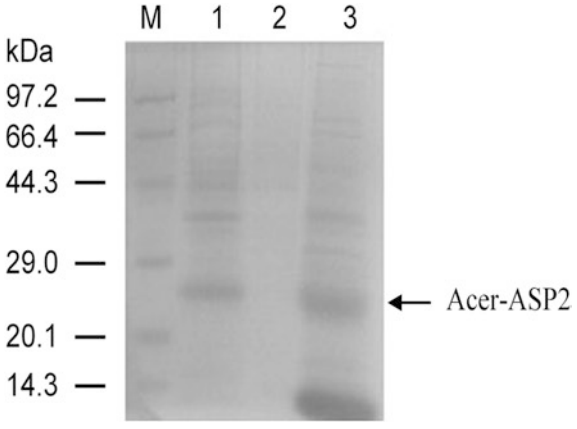

Fig. 7.5 Expression and purification of recombinant Acer-ASP2. Electrophoretic analysis of expression products from *E. coli* BL21 (DE3) cells. The protein is shown as black arrows. (Reproduced with permission from Ref. [15]. Copyright 2014 Elsevier)

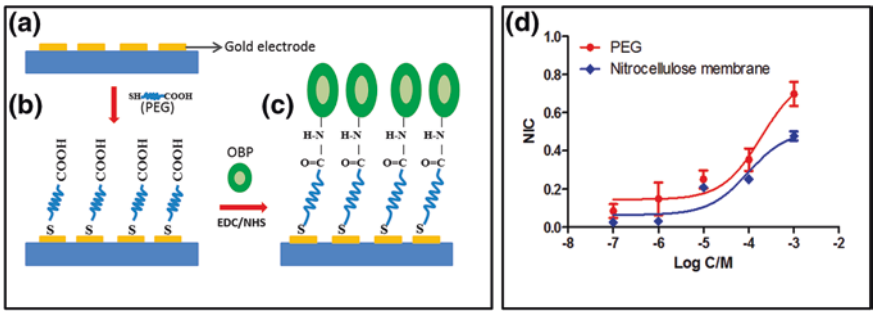

Fig. 7.6 OBPs immobilized on PEG modified interdigitated electrodes. **a** Sketch map of the interdigitated electrodes. **b** COOH-PEG-SH forms Au–S bonds with gold electrodes. **c** OBPs forms covalent amino bonds with PEG-electrodes by EDC/NHS coupling. **d** Normalized impedance changes (NICs) of OBPs interacting with isoamyl acetate. The red curve represents OBPs immobilized on electrode by PEG. The blue curve represents OBPs immobilized on electrode by nitrocellulose membrane. (Reproduced with permission from Ref. 14. Copyright 2015 Elsevier)

electrodes after washing away the unbound COOH-PEG-SH with ultrapure water (Fig. 7.6b). Via EDC/NHS coupling, OBPs was immobilized to PEG-electrode by amido bond (Fig. 7.6c).

As one of the most widely used inert and biocompatible linkers in surface engineering [38, 39], PEG could form densely packed self-assembled monolayer on gold electrode, which comprised a uniform morphology with the ethylene glycol units in a helical conformation and oriented predominantly perpendicular to the surface [40, 41]. When OBPs was immobilized by PEG, the impedance changes caused by conformationally altered proteins could be easily detected as they were all arranged orderliness through amido bonds, which provided an effective method for semiochemicals detection. For instance, under the same condition, the sensitivity of PEG modified electrodes seemed to have better sensing properties than that of nitrocellulose membrane(Fig. 7.6d).

7.3.3 Sensor System and Data Processing

Different sensors have their own detecting systems, such as SPR signals should be measured by optical system, while QCM is tested by quality testing equipment. In this part, we will introduce a traditional impedance sensing system [14, 15, 31]. For impedance sensing, Zahner ZENNIUM electrochemical workstation (ZahnerElektrik, Germany) and CHI660E electrochemical measurement system (Shanghai Chenhua Device Company, China) were widely used. Generally, the tested frequency was set from 1 Hz to 100 kHz with a 5 mV alternating voltage. Odorants at different concentrations were added to the plate wells containing 5 mM$K_4[Fe(CN)_6]$/$K_3[Fe(CN)_6]$ (1:1). All of impedance detecting was performed at room temperature (18–25 °C).

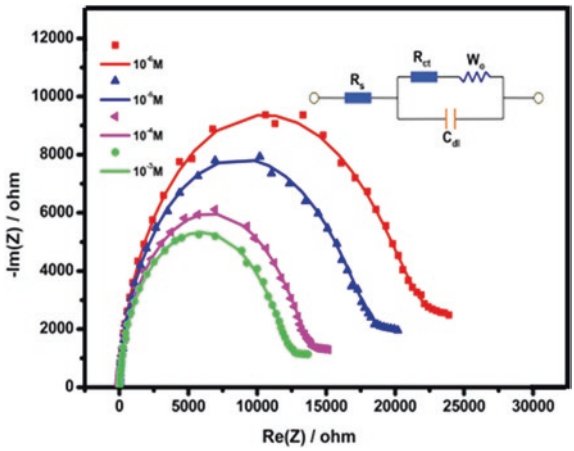

Fig. 7.7 Nyquist plots of impedance spectra of 4-allylveratrole at 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M. The symbols are the experimental data and the lines represent the simulated spectra. Randles cell is shown in the inset. The equaled circuit consists of four elements: the solution resistance *Rs*; the constant phase element CPE; the Warburg impedance Zw; and the charge transfer resistance *Rct*. (Reproduced with permission from Ref. [15]. Copyright 2014 Elsevier)

For the situation of electrodes in contact with the electrolyte, the Randles circuit could be used to simplify the complex system, shown in the inset of Fig. 7.7. Rs and Zw represent bulk properties of the electrolyte solution, respectively. CPE and Rct both depend on the dielectric and insulating features at the electrode/electrolyte interface, and they were affected by the property changes occurring at the interface. Normally, Rctwas chosen as the sensing parameter and it was capable of monitoring the protein–ligand interaction [42, 43]. With OBPs immobilized on the electrodes, Rct decreased apparently under the treatment of odorants. The Nyquist plots (Zre vs. -Zim) recorded in the electrochemical experiment can be simulated by Zview (Scribner Associates Inc., US), to visualize and determine Rct. Figure 7.7 displays impedance spectra of 4-allylveratrole as a typical Rct recording example. The diameter of the semicircle part of the Nyquist plot was equal to Rct. Obviously, the higher the concentration was, the larger the Rct decreased in ligand sensing.

7.4 Applications of Odorant Binding Proteins-Based Biosensors

7.4.1 Specific and Sensitive Odorants Detection

Up until now, approximately 3,500 kinds of odorants, which is also called semiochemical, have been identified [44]. How to detect and discriminate these semiochemicals is essential for animals, especially insects, to survive. Insects are known

for their remarkable olfaction ability, which is crucial for them to distinguish, comprehend, and estimate the overall situation and even for their survival, reproduction, and social communication [26, 45]. Therefore, semiochemicals of plants and animals can be detected by insects at low concentrations over a long distance, even dozens of meters away [46]. However, as the first filter of olfaction, there are not many kinds of OBPs to specifically sense these semiochemials. Therefore, it might tell us that OBPs could encode different binding properties for the wide spectrum of odorant molecules, such as aromatic molecules and aliphatic compounds. Studies of OBPs also suggest that one kind of OBP can bind several odorants, and has its own binding specificities, while the exact binding properties and coding schemes were not discovered.

From the impedance sensor of our studies [14, 15, 31], we can infer that Acer-ASP2 show relatively similar binding affinities to isoamyl acetate and methy-p-hydroxyl benzoate (Fig. 7.8). It also indicated that the two odorants have comparative affinities to Acer-ASP2. For butanedione, however, the interaction between Acer-ASP2 and butanedione was very small. The results of the impedance biosensor show that the affinities of Acer-ASP2 to isoamyl acetate and methy-p-hydroxyl benzoate were much higher than butanedione.

7.4.2 Odorant Detection and Analysis

For impedance sensing, the Randles impedance circuit described above was used to analyze the impedance changes. This decrease of Rct should indicate a recovery in the efficiency of the mass transfer phenomenon and the difference in the dielectric or conductive properties of proteins on the electrodes. Through simulating every spectrum of the odorants, we could find that the normalized impedance change (NIC) of Rct was linear for the logarithm of ligand concentrations. The NIC was described as NIC $= (Z_b - Z_a)/Z_b$, where Z_b and Z_a represent Rct before and after the injection of the ligands.

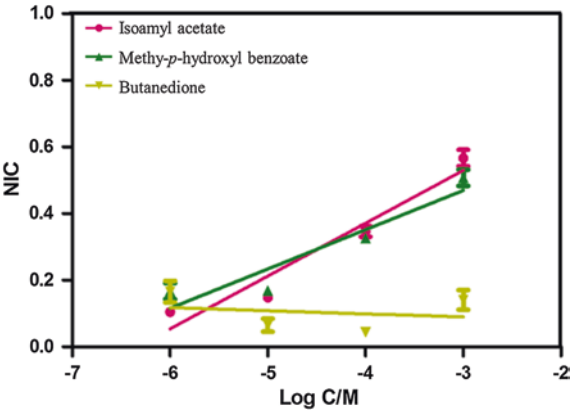

Fig. 7.8 Impedance changes of Acer-ASP2 to isoamyl acetate, methy-p-hydroxyl benzoate, and butanedione. (Reproduced with permission from Ref. [15]. Copyright 2014 Elsevier)

Apart from Acer-ASP2, another insect OBP, BdorOBP2 was also used for testing odorants, including isoamyl acetate, β-ionone, and benzaldehyde. These odorants are mainly the scents of banana, tomato, and cherry (Fig. 7.9a). As the most widely distributed fruity scents in nature, these three odorants show excellent affinities to BdorOBP2 (Fig. 7.9 b, c, and d). The sensitive test range was from 10^{-7} to 10^{-4} M, which was wider than that of Acer-ASP2-based biosensor. It indicated that BdorOBP2-based biosensor showed a relatively higher sensitivity and affinity toward odorants in impedance sensing. Moreover, the biosensor had reserved the biofunction of OBPs, and it can be used to detect odorants selectively and quantitatively. The broad substrate odorant specificity to floral odors, semiochemicals, and pheromones was in accord with properties of OBPs [37, 47].

For further analyzing the interactions between OBPs and odorants, we should use molecular docking. With a cavity prediction algorithm, the fast and accurate identification of potential binding modes and poses could be obtained by molecular docking [48]. The energetic evaluations of the protein-ligand complexes were implemented with MolDock Score, which contain electrostatic force, van der Waals force, hydrophobic interaction, hydrogen bond, and other noncovalent bond (π-stacking or cation–π interaction et al.). It could reflect the affinity between ligands and OBPs. The larger the negative MolDock score was, the stronger was the binding interaction. The detailed MolDock score between Acer-ASP2 and different odorants showed in our previous paper [15].

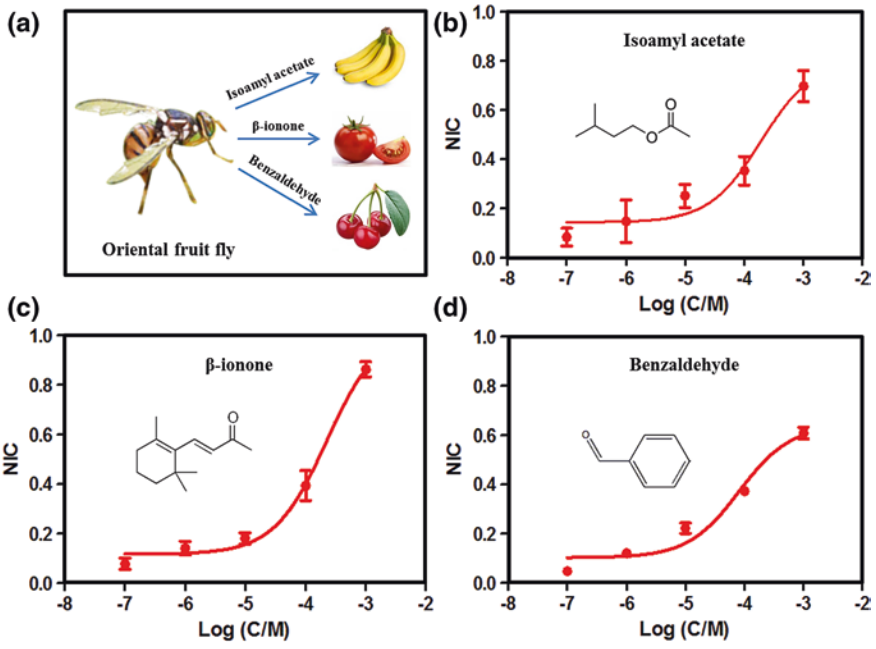

Fig. 7.9 Normalized impedance changes (NICs) of BdorOBP2 from oriental fruit fly interacting with different semiochemicals (**a**) isoamyl acetate (**b**); β-ionone (**c**); benzaldehyde (**d**). (Reproduced with permission from Ref. [14]. Copyright 2015 Elsevier)

7.4.3 Application of the Odorant Binding Proteins-Based Smell Sensors

For analysis of chemicals binding with the olfactory molecules in vitro, the most important thing is to obtain the active recombinant proteins. Compared with G protein-coupled receptors with seven-transmembrane domains, OBPs can be easy expressed and purified, which has been described previously. Moreover, through calculation, the three-dimensional models of proteins and the exact function groups of the protein to different chemicals can be modeled [23]. Based on molecular docking, fast and accurate identification of potential binding modes were obtained, which can help to figure out the most important amino acid residues in the binding process. All of these interesting characteristics of OBPs making them ideal tools for biosensors that can successfully detect different odorants.

Generally speaking, there are only a few ways of detecting binding of a ligand to immobilized proteins that do not have catalytic activity. Taking OBPs of mammals or insects as the sensing elements, however, researchers have developed many kinds of biosensors for odorant detection, such as EIS, cantilever, SAW, QCM, and SPR, based on different principles. In an impedance biosensor, the binding can modify electrical properties of a protein, which can be part of a transistor or a capacitor. For QCM, the extra mass of the odorant bound to the protein can be measured with a quartz microbalance. Meanwhile, the ligand binding can modify the refractive index of OBPs, which can be tested by SPR. In some cases, calorimetry can be used to measure changing thermal properties when odorants bind to the protein. The performances of different sensors are summarized in Table 7.1. From the results, we can infer that there were differences between the specificities of different OBPs.

According to the studies mentioned above, where odorants can be successfully detected by OBPs-based biosensors, we can infer that biosensors in vitro could achieve the first step in odorant detection. Detecting and discriminating odorants that act as semiochemicals was important for research on chemical communications in chemical ecology. Moreover, by directly targeting the sense of smell, interactions between OBPs and different chemicals could advance the progress in the understanding of chemical communication systems of vertebrates and insects, which showed strong potential for applications in many fields, such as control of odorant pollution, insect vector-borne diseases, olfactory-based insect control technology, affinity columns for analysis and purification, and even cancer diagnosis [41, 48, 49]. Studies also suggest that an OBP from honeybee, *Apismellifera*, contains a C-terminal tail fragment that has been shown to bind to pheromones and other chemical targets. Interestingly, the tetrapeptide sequence from ASP1 protein shares homology with the C-terminal end of the TNT binding dodecapeptide from a phage peptide library [20, 50]. It tells us that artificial proteins or peptide recognition elements have promising applications in specific odorant detection. Figure 7.10 summarizes some of the applications of OBPs. With more functional amino acid residues or peptides interpreted by molecular docking from

Table 7.1 The properties of OBPs-based biosensors (Reproduced with permission from Ref. [14]. Copyright 2015 Elsevier)

Type of biosensors	Biosensing elements	Odorants	References
Electrochemical impedance spectroscopy (EIS)	OBPs from honeybee (*Apisceranacerana)*	Linalool, Geraniol, β-ionone, 4-allylveratrole, Phenylacetaldehyde, Dibutyl phthalate, Isoamyl acetate, Methy-p-hydroxyl benzoate	[15]
	OBPs from oriental fruit fly (*Bactroceradorsalis*)	Isoamy acetate, β-ionone, Benzaldehyde	[14]
	Rat OBP-1F	Isoamyl acetate	[5]
	Porcine OBP (pOBP)	Ethanol	[19]
Cantilever	Porcine OBPs	2-isobutyl-3-methoxypyrazine	[13]
Surface acoustic wave (SAW)	Bovine OBPs	Octenol, Carvone	[7, 16, 17]
Quartz crystal microbalance (QCM)	OBPs from Drosophilidae (*Drosophila,* LUSH)	Alcohols	[11]
Surface plasmon resonance (SPR)	OBP-1F and OR1740	Helional	[12]

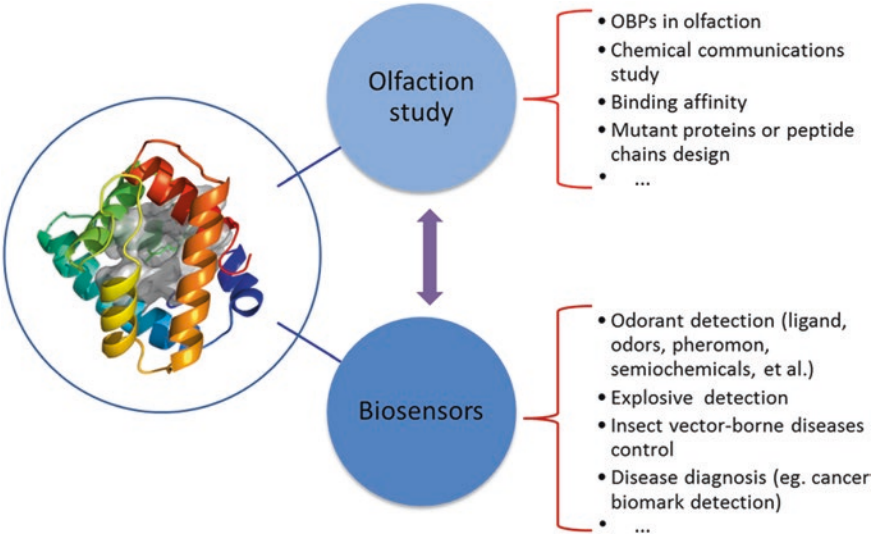

Fig. 7.10 The applications of OBPs, including the olfaction studies and researches on OBPs-based biosensors

the natural sequences of OBPs, we believe that along with OBPs, mutant proteins or peptide chains could be synthesized to be used as promising recognition elements in the future biosensors for practical applications.

7.5 Summary

Utilizing the conformation changes of OBPs when binding to different odorants and chemicals, various sensors have already been established for sensitively and selectively detecting and recognizing odorants, such as SAW, QCM, SPR,EIS, and cantilever sensors. In addition, the large structural information on these proteins allows easy design and synthesis of mutants, which could be used to improve their sensitivity and specificity. The design of the OBPs-based biosensors provides a platform, which could apply olfactory proteins to detecting semiochemicals, such as pheromones and floral odors, selectively and quantitatively.

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Chapter 8
DNA-Decorated Devices as Smell Sensors

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8.1 Introduction

Biomimetic smell sensors have achieved significant progress in the recent two decades, which have shown promising prospects and potential applications in many fields such as biomedicine, environmental protection, and drug discovery [1, 2]. More and more efforts have been devoted to the development of novel biomimetic smell sensors by use of biological originated materials as sensitive elements, which include the olfactory receptors, smell sensory neurons, and olfactory epithelium. Due to the extreme high performance of biological olfactory systems, biomimetic smell sensors inherit some excellent features of biological olfactory systems such as high sensitivity, rapid response, and excellent selectivity. On the other hand, these biomimetic smell sensors also suffer from use of biological olfactory materials as sensitive elements, which have greatly hampered their further development and practical applications. The current main difficulties in the biomimetic olfactory-based smell sensor studies are how to gain a great deal of functional olfactory receptors, how to effectively combine the biological materials with transducers in a more stable manner without losing their natural structures and functions. To address these issues, the new development trends of biomimetic smell sensors are gradually formed by use of novel sensing materials as well as by the integration of novel emerging technologies, for example, the biotechnologies and nanotechnologies. Among them, the deoxyribonucleic acid (DNA)-mediated biomimetic smell sensors have become one of the most attractive research directions, which provide totally new mechanisms for chemical sensing.

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DNA molecules are known as the carriers of life information, which are double-stranded helices and consist of two long biopolymers made from repeating units of nucleotides including guanine (G), adenine (A), thymine (T), and cytosine (C). The main function of DNA molecules are storage and encoding the biological information for the development and functioning of almost all known living organisms. Recently, DNA has emerged as a versatile material for constructing molecular structures and devices [3–6]. Specifically, DNA molecules have been used as sensitive elements for development of biomimetic smell sensors for detection of specific volatile compounds [7–9]. Biomimetic smell sensors have been demonstrated using single-stranded DNA (ss-DNA) as gas recognition site and single-walled carbon nanotube field-effect transistors (swCN-FETs) [7, 8] or fluorescence dye [9] as read-out component, which can respond to different volatile compounds in a sequence-dependent manner. DNA molecules are featured with high stability, potential for tremendous combinatorial diversity, and easily replicated, which make them ideal to serve as sensing materials in biomimetic smell sensors. It is possible to control the uniformity of sensing films by use of DNA molecules as sensitive elements, which contribute greatly to the repeatability of the biomimetic smell sensors. In addition, DNA molecules are easy to be engineered and replicated to provide a large combinatorial complexity of structure for diverse biomimetic smell sensor units aiming toward detection of different volatile compounds.

With the fast development of biomimetic smell sensors, there are a number of excellent literatures which reviewed the most recent progress in the smell sensors using biological originated materials as sensitive elements [10–13]. However, there is so far no literature or book chapter that deals with biomimetic smell sensors using DNA molecules as sensitive elements. In this chapter, we focus on two main categories of DNA-mediated biomimetic smell sensors, whose signal read-out mechanisms are based on swCN-FETs and fluorescence dye, respectively. The basic mechanisms of DNA-mediated biomimetic smell sensors will be briefly introduced at the beginning. By the following, the design and fabrication of DNA-mediated biomimetic smell sensors will be summarized. Finally, the typical applications and development trends of DNA-mediated biomimetic smell sensors will be discussed.

8.2 Fundamental of DNA-Decorated Devices for Chemical Sensing

8.2.1 Molecular Features of Odorant Sensitive DNA

In biological systems, DNA molecules usually exist as double-stranded helices, in which a pair of complementary ssDNA is held tightly together. This structure makes DAN molecules very suitable for storage and transmission of biological

information. The two helical chains of DNA molecules are bound together with noncovalent hydrogen bonds, which are mainly originated from the interactions of complementary nucleotides. The interaction between nucleotides of G and C, which forms three hydrogen bonds, is stronger than that between A and T which only forms two hydrogen bonds. Consequently, the higher GC-content of a DNA molecule contributes partially to the higher stability than that of DNA molecules with lower GC-content. Each strand of DNA molecule is coiled round the same axis with a pitch of 3.4 nm and a radius of 10 nm [14]. The backbone of DNA molecules is composed of alternating phosphate and sugar residues, which is pretty stable and resistant to cleavage. The special bonds between the sugars and phosphate are asymmetric, and thus make each strand of DNA molecules form a direction. This leads to the antiparallel structure in a double helix, which means the direction of one DNA strand is opposite to another strand of DNA molecules. The complementary bases that are covalently attached to the sugar lie horizontally between the two spiraling strands. The hydrogen bonds between complementary bases as well as base-stacking interactions (the conjugated π bonds of nucleotide bases align perpendicular to the axis of the DNA molecule) are two main contributions to the stabilization of DNA double helix. DNA molecules encode the biological information using the repeating unit of nucleotides, which contain the critical genetic instructions for all living organisms. Both strands of DNA molecules store the same biological information, which means that the information stored in the DNA double helix is duplicated on each strand.

DNA molecules have ideal features for storage and transmission of biological information. This is mainly due to the central feature of DNA molecules, combinatorial diversity, which can provide sufficient diverse combinations for the complex information from living organisms. Basically, each nucleotide position of one ssDNA strand can be occupied by any one of four bases. For an ssDNA strand with 20 nucleotide position, there are over trillion possible base combinations to form this DNA molecule. Each possible base combination has unique base order and structures to form DNA molecules with various base sequences. This can thus result in differences in the exact shape and surface charge distribution of different DNA molecules with different base sequences due to the different chemical structure of various bases. This means that it is possible for these trillion DNA molecules to represent a trillion different features with various chemical structures. The sequence diversity contributes greatly to the encoding of complex diverse of biological information. On the other hand, the chemical diversity of DNA molecules holds the potential for other applications, especially for development of biomimetic molecular structures and devices for chemical sensing [7–9]. The special features of DNA molecules make them ideal candidates to be used as sensing materials for chemical sensing, which can address some current critical problems encountered in the development of biomimetic smell sensors.

The ultimate goal of biomimetic smell sensors is to develop chemical sensors with high sensitivity and specificity based on the mechanisms of biological olfactory systems that can recognize and discriminate thousands of odorants at the trace level. In the biological olfactory system, smell sensory neurons expressed with

specific olfactory receptors form an odorant sensors array, which can respond to much more number of chemical compounds than the number of receptors. The sensor array system can tolerate the partial system failure and allow the flexible training, which are adaptive to the complex chemical environments. In order to build the biomimetic smell sensors that can mimic the high performance of biological olfactory systems, various biological originated materials have been applied in the development of biomimetic smell sensors that can partially inherit some advantages of biological olfactory systems such as narrowly tuned specificity. However, some critical problems still exist and need to be addressed in a novel approach, which have greatly hampered the application of biological olfactory components (e.g., olfactory tissues, smell sensory neurons, and olfactory receptors) in the development of biomimetic smell sensors. The main issue is how to achieve sufficient functional sensitive materials that can provide enough chemical diversity to mimic the combinatorial responses of biological olfactory systems; it is better if these sensitive materials can be produced and controlled in an engineered manner. This means that the sensitive materials can couple with transducers in a more stable manner without losing their natural structures and functions, which is very important to the performance enhancement of the biomimetic smell sensors. The other main issue is how to effectively readout the transduction signals originated from the responses of sensitive materials to the desired target chemical signals.

In order to address the first main issue, at present, there are generally two strategies to achieve diverse functional sensitive materials to mimic the combinatorial responsive function of olfactory receptors. The first strategy is based on the synthesis of special designed polymers, which can be endowed with desired chemical features that can specifically interact with a target chemical compound and generate the readable responsive signals such as fluorescent changes [15–17]. The second approach is to investigate the responses of currently available polymers to the desired target chemical compounds, which do not need the special design and prior knowledge on the chemical properties of polymers as well as the mechanisms of chemical sensing [18–28]. In principle, the DNA-mediated biomimetic smell sensors are developed based the second strategy, in which DNA molecules with diverse base sequences are used as sensitive materials for the detection of specific chemical compounds. The details mechanisms of interactions between DNA molecules and chemical compounds have not been completely revealed.

DNA molecules have some decisive beneficial advantages compared with commonly used polymers for the development of biomimetic smell sensors for chemical sensing, which can yield relative large number of diverse sensitive elements in a more labor efficient way. In addition to the main function of interacting with the target chemical compounds, DNA molecules have some important features that make them more ideal and attractive candidates to be used as sensitive materials. The first important property is the special sequences of DNA molecules that can provide large number of diverse candidates to generate potential combinatorial responses to the chemical compounds. The second one is that DNA molecules can be synthesized and replicated in a very efficient and convenient way benefited

from the advance technologies on chemical synthesis. This is very important for the generation a large amount of identical sensitive materials for the fabrication of chemical sensor array with good repeatable measurement results, which have great influences in the performances of the final biomimetic smell sensors. In addition, DNA molecules can be modified easily with chemical groups that can greatly facilitate the immobilization of DNA molecules on the sensor surface as well as for the special responsive signal transduction. This makes it possible to control the surface distribution and thickness of DNA molecules on the sensor surface, which is critical for the repeatability of biomimetic smell sensors. This feature also allows the use of various transducers and signal transduction strategies for the development of novel biomimetic smell sensors. Another important feature provided by DNA molecules is the possibility of screening for sensors that can respond to a large number of chemical compounds with different structures, which can provide similar sensor diversity and complexity to that of biological olfactory systems. Moreover, DNA molecules are stable in the normal living environment, which makes it easy to keep the structure and function of DNA molecules when incorporated with transducers in chemical sensors. All these features and potentials for tremendous combinatorial complexity make DNA molecules attractive candidates to be used as sensitive elements for the development of novel DNA-mediated biomimetic smell sensors.

8.2.2 DNA-Decorated Devices for Chemical Sensing

The specific features of DNA molecules make them ideal candidates for building biomimecitc smell sensors, which can interact with chemical compounds and generate combinatorial responses. As an attractive sensing material for chemical sensing, DNA molecules need to find an efficient and convenient way to convert the sensed chemical signals into readable output signals by the peripheral electronic circuits or devices. At present, there are two different strategies for signal transduction of DNA-mediated chemical sensing [7–9]. First, using single-walled carbon nanotube field effect transistors (swCN-FETs) as the electronic read-out component, which can detect the responses of ssDNA molecules to gas odors by recording the conductivity changes of ssDNA decorated single-walled carbon nanotube (swCN). In this section, signal transduction of the DNA-nanomaterials hybrid for odorant detection will be briefly introduced, although some details on the DNA chemical sensing mechanisms are still under investigation. The second strategy is based on DNA–fluorescent dye conjugates for chemical sensing, which will be introduced in the next section.

With the fast advancements on nanotechnologies, many materials with nanostructure have been found with excellent sensitive properties to the surrounding factors. For example, swCNs, which belong to a kind of semiconducting nanomaterials with one-dimensional carbon cage structure, have been found to be extremely sensitive to the surrounding electrostatic environment not only in the liquid but also

integrated with FET on a solid surface [29–31]. In the fields of gas sensors and biosensors, swCNs have been widely applied independently or combined with polymers to serve as sensitive materials for chemical sensing or target molecule detection [32–41]. The structure of swCN-FETs has been demonstrated as an attractive and promising transducer for the development of molecular sensors, which can transduce the detected molecular signals into electrical signals with high sensitivity and rapid response time. In addition, swCN-FETs can be easily integrated with nanoscale materials or array structures due to their compatibility with nanoscale fabrication technologies. Derivatized semiconductor nanowires such as swCNs have proved to be promising materials to generate sensitive elements used for the fabrication of sensors both in the gas phase and liquid phase [42–44].

DNA molecules are ideal candidates for the functionalization of swCN-FET devices, which have high affinity with swCNs originated from the π-π-stacking interaction [45]. By use of DNA-decorated swCNs as sensitive elements for chemical sensing, the performances of biomimetic smell sensors are expected to be significantly improved. The reason is because the coupling of DNA molecules with swCNs is not covalent binding, which can avoid degrading of highly sensitive electronic properties of the swCN-FET hybrid devices. In addition, the coupling of DNA molecules with swCNs is robust and reproducible due to the easily engineered capability of DNA molecules. DNA molecules also provide sufficient molecular flexibility for a wide range of chemical compounds, which makes it possible to screen the DNA molecules for chemical sensing with high throughput. All these features have motivated the researcher to explore the development of biomimetic smell sensors by using DNA molecules as sensitive elements and swCN-FET as transducers to build a hybrid nanostructure for chemical sensing [8].

The basic structure of biomimetic smell sensors based on swCN-FETs is shown in Fig. 8.1. ssDNA molecules were used to decorate individual carbon nanotube that was contacted by source and drain electrodes of FET. In this configuration, ssDNA molecules were coupled with an individual p-type semi-conducting nanotube through π-π-stacking interaction. The source-drain current (I) of FET was measured as the function of bias voltage (V_B) and the gate voltage (V_G), which can reflect the changes of carbon nanotube in conductivity. When the surface of carbon nanotubes is decorated with DNA molecules, the surrounding electrostatic environment changes accordingly. This change can influence the carriers in the carbon nanotubes and this leads to the change in conductivity. Similarly, when the ssDNA molecules coupling to the carbon nanotubes bind with chemical compounds, the conformation and charge distribution of DNA molecules can be changed accordingly. This will subsequently result in influences on the conductivity of carbon nanotubes, which can be measured by the changes in the source-drain current of FET. Figure 8.2 shows the typical changes in the source-drain current when the carbon nanotubes were functionalized with ssDNA molecules as well as at the time when ssDNA molecules were exposed to the chemical compound, trimethylamine (TMA). It is indicated that the ssDNA decoration on the carbon nanotube caused the threshold value of V_G decrease by 3–4 V, which can be explained by the decrease of carrier density in semiconducting carbon nanotube [46]. In addition,

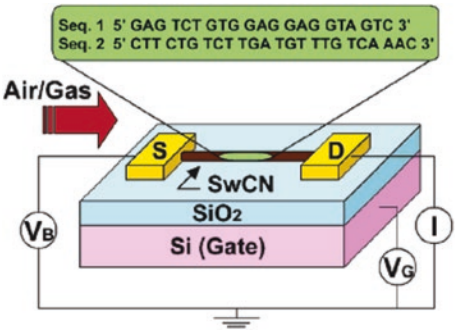

Fig. 8.1 Schematic diagram shows the structure and mechanisms of biomimetic smell sensors based on swCN-FETs, in which ssDNA molecules with specific sequences were used as sensitive materials for chemical sensing and swCN-FET was used to transduce the chemical signals into electrical signals (Reproduced with permission from Ref. [8]. Copyright 2005 American Chemical Society)

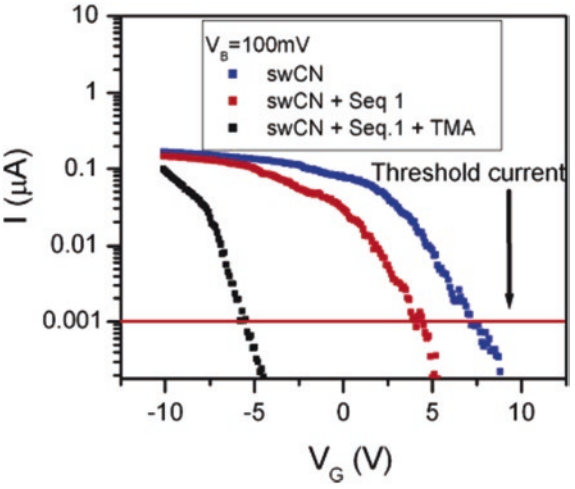

Fig. 8.2 The changes in the source-drain current (*I*) of swCN-FET device when measured as a function of back gate voltage (V_G) with a bare swCN-FET sensor (*blue*), the same device after functionalization with ss-DNA sequence 1 and exposed to air, the same ss-DNA/swCN-FET exposed to TMA vapor. Source-drain bias voltage is 100 mV (Reproduced with permission from Ref. [8]. Copyright 2005 American Chemical Society)

the exposure of ssNDA decorated carbon nanotube to the specific chemical compounds resulted in much higher threshold value decrease, which was around 10 V. It is suggested that the special nanostructure of ssDNA decorated carbon nanotube combined with FET can detect the specific chemical compounds. Based on this mechanism, it is possible to build multiple sensors to generate a gas sensor array by the combination of diverse ssDNA sequences with swCN-FETs array, which is able to achieve the combinatorial responses for chemical sensing. Due to the easily reproduction and replicated of ssDNA molecules as well as the highly sensitive property of swCN-FETs, these kinds of gas sensor arrays are able to achieve high performances for chemical sensing. Specifically, when it is utilized as the key components to be integrated in a biomimetic smell sensor in order to develop

electronic nose systems, this kind of gas sensor array has shown promising prospects and potential applications in many fields such as breath diagnosis, explosive detection, and environmental protection.

8.2.3 DNA-Fluorescent Dye Conjugates for Chemical Sensing

Another strategy for transduction of chemical signals detected by the DNA molecules is based on DNA–fluorescent dye conjugates. Basically, this strategy uses fluorescent dyes to modify the short ssDNA molecules and immobilized on a solid surface. By this method, the chemical signals detected by the DNA molecules are converted into fluorescent intensity changes, which can be detected by the peripheral fluorescent sensitive devices. This strategy can realize the conversion of chemical signals into readable output signals, which are fluorescent signals. By the following, we will briefly introduce the basic signal transduction mechanisms of DNA–fluorescent dye conjugates for chemical sensing, although some details on the molecular sensing mechanisms are still not completely understood.

DNA molecules are biopolymers suitable to be used as sensitive elements for chemical sensing, which have been considered as intriguing candidates for the development of molecular sensors for various purposes including the detection of small target molecules and the specific proteins. The decisive advantage of using DNA molecules as sensitive materials is related to their easily engineered property, which can be produced and replicated in an efficient manner. In addition, DNA molecules can be modified with various labels and chemical groups for the signal transduction as well as for the coupling with transducers. All these features can greatly facilitate the development of DNA-mediated smell sensors for chemical sensing. One typical example is recent reported research on the DNA-fluorescent dye hybrids for chemical sensing [8]. DNA molecules with various short base sequences were labeled with specific fluorescent dye and immobilized on a solid surface to screen functional ssDNA-fluorescent dye conjugates for detection of specific chemical compounds in gas phase. The screening of ssDNA-fluorescent dye conjugates was performed in a high throughput manner. It was found that the responses of ssDNA-fluorescent dye conjugates to specific chemical compounds were dependant on the base sequences of DNA molecules. When ssDNA-fluorescent dye conjugates are exposed to a particular chemical compound, fluorescent dye that is conjugated with ssDNA will generate a measureable change in the fluorescence of the intercalated dye. Although the molecular mechanisms of this responsive and signal transduction process are still not known, ssDNA-fluorescent dye conjugates have been demonstrated to be an attractive and promising synthetic polymer used for the development of biomimetic smell sensors for chemical sensing due to the tremendous combinatorial complexity provides by DNA base sequences and compatibility of fluorescent dye with bench-up

instruments for fluorescent measurement. In addition, large amount of ssDNA-fluorescent dye conjugates can be replicated using the standard synthesis methods once the ssDNA base sequences that can respond the particular chemical compounds are identified. Based on this strategy, a variety of short DNA molecules can be linked to a fluorescent dye to screen the responsive ssDNA-fluorescent dye conjugates to build a gas sensor array, which can be utilized as the key component of artificial electronic nose for the recognizing and discriminating multiple chemical compounds.

Both ssDNA and dsDNA have been investigated for chemical sensing based on the method of fluorescent dye conjugation, which were dried onto a solid plastic surface [9]. As shown in Fig. 8.3a, it is indicated that the sensors made from OliGreen Dye alone show a decrease in fluorescence when exposed to propionic acid, but show no significant change to the other chemical compounds tested. On the contrary, the sensors made from ssDNA-OliGreen Dye conjugate show obvious different responses, which is a significant increase in fluorescence on exposure of propionic acid and methanol but show no response to the other chemical compounds tested (Fig. 8.3b). All these results indicate that only ssDNA show the sequence-dependent response profiles to various chemical compounds tested, while the fluorescent dye alone shows no similar sequence-dependant responses [9]. Furthermore, the molecular libraries containing a large number of different ssDNA base sequences can be efficiently screened by this fluorescent dye conjugation strategy. The lengths of ssDNA sequences are usually from 20 to 24 bases, which were linked to fluorescent dye and dried onto solid surface. Based on the fluorescent measurement, it was found that, among 30 ssDNA-fluorescent dye conjugates with different base sequences, one ssDNA-fluorescent dye conjugate that can respond to either methanol or propionic acid was successfully identified, which show no response to other tested chemical compounds including TMA.

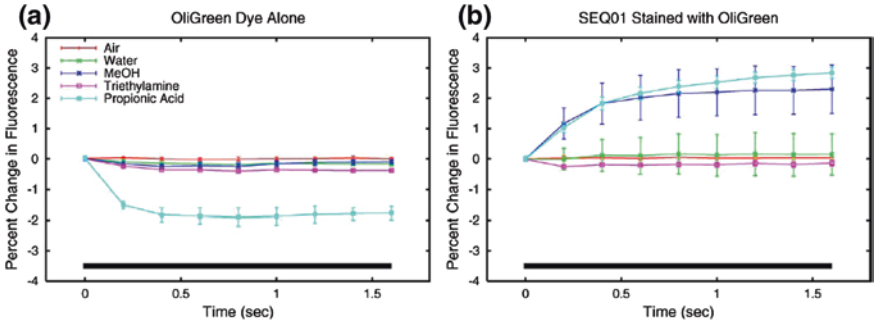

Fig. 8.3 Fluorescent changes of OliGreen Dye sensors and ssDNA-OliGreen Dye conjugate sensors in response to various chemical compounds. **a** Responses of a sensor made from OliGreen alone. **b** Responses of a sensor made from ssDNA-OliGreen Dye conjugates (Reproduced with permission from Ref. [9]. Copyright 2008 White et al.)

8.3 Design of DNA-Decorated Devices as Smell Sensors

8.3.1 Screening of Odorant Sensitive DNA

The key feature of DNA molecules to be used as sensitive materials for chemical sensing is the sufficient diversity provided by ssDNA with different base combinations, which have great potential to improve the detection resolution and accuracy of chemical compounds. However, this also put an extreme challenge to identify potent base combinations by trial and error because of the large number of possible base combinations. In order to solve this problem, it is highly desirable to develop strategies and approaches to define the base sequences of ssDNA molecules that can respond to the particular chemical compounds. For the purpose of rapid screening, the first required step is to build a large and diverse ssDNA molecular library. On the other hand, the high throughput measurement setup is also necessary, which require the well coupling and compatible signal transduction pathway between the sensitive elements and signal detection devices or instruments.

One potential strategy for the screening of odorant sensitive ssDNA have been reported based on fluorescent measurement on the ssDNA-fluorescent dye conjugates with high throughput [9]. In order to screen the ssDNA libraries for chemical sensing with high throughput, a sensor array consisting of ssDNA-fluorescent dye conjugates with different ssDNA base sequence was developed on the same solid surface. The potential sensors of chemical sensing were screened using the standard microarray methods with high throughput. In order to generate better defined ssDNA-fluorescent dye conjugates, the fluorescent dye Cy3 was utilized for signal transduction of chemical signals detected by ssDNA molecules and covalently linked with the ssDNA molecules. In addition, a special designed measurement chamber for gas test was also constructed, which makes the high-through gas-phase measurement possible with the microarray scanner. To construct the sensor array, ssDNA-fluorescent dye conjugates were spotted onto the cover slips, where each spot contains the ssDNA-fluorescent dye conjugates with different ssDNA base sequences. As a control sensor array, ssDNA-fluorescent dye conjugates with the same ssDNA base sequence were first constructed and tested. The responses of 30 ssDNA-fluorescent dye conjugates with the same ssDNA base sequence were measured and the results are shown in Fig. 8.4a. It is indicated that the responses of ssDNA-Cy3 conjugates with the same ssDNA base sequences to the same set of chemical compounds are identical. This suggests the highly correlated and reproducible responses of ssDNA-Cy3 conjugates for chemical sensing. On the contrary, ssDNA-Cy3 conjugates with different ssDNA base sequences show dramatic different responsive profiles to the same set of chemical compounds (Fig. 8.4b). Among 29 ssDNA-Cy3 conjugates with different ssDNA base sequences, at least 10 ssDNA-Cy3 conjugates were discriminated from the ssDNA-Cy3 conjugates library. In addition, the responses of ssDNA-Cy3 conjugates with different ssDNA base sequences are distinctly different from that of Cy3 dye alone and obviously dependent on the specific ssDNA base sequences.

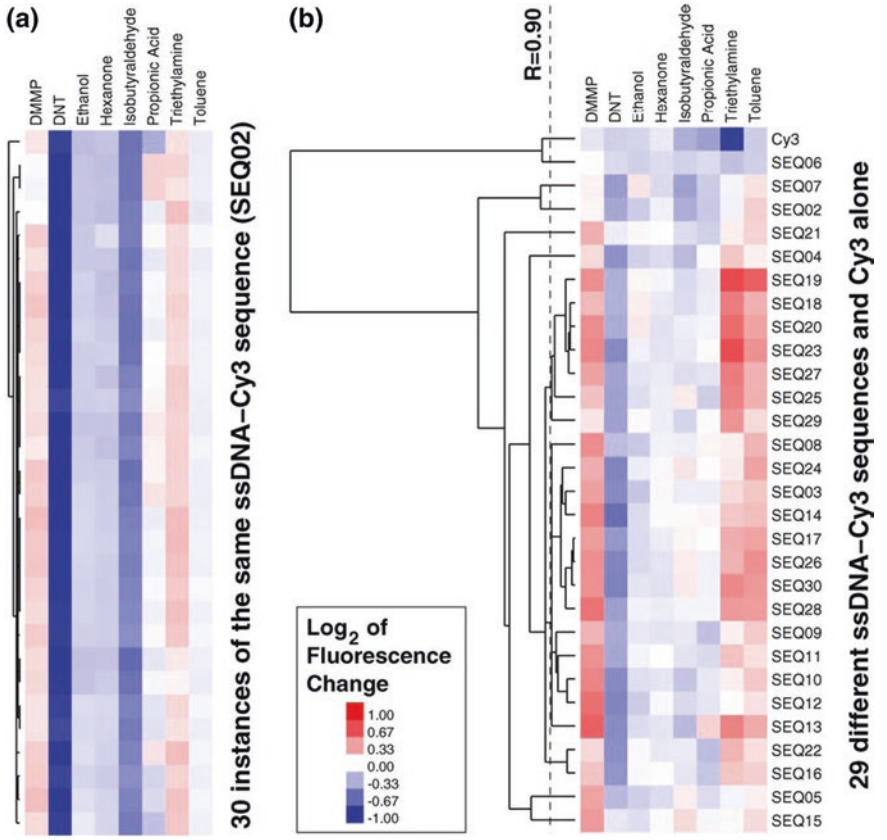

Fig. 8.4 Screening of odorant sensitive ssDNA-Cy3 conjugates with microarray scanner. **a** Sensor array made from ssDNA-Cy3 conjugates with the same ssDNA base sequences. **b** Sensor array made from ssDNA-Cy3 conjugates with 29 different ssDNA base sequences. Increases in fluorescence over baseline indicated by graded red colors and decreases indicated by graded blue colors (Reproduced with permission from Ref. [9]. Copyright 2008 White et al.)

This screening strategy makes it possible to detect the fluorescent change signals by the currently available microarray scanner, which can greatly facilitate the screening process and thus improve the screening efficiency. The responses of sensor array to a set of chemical compounds were successfully investigated. The measurement results demonstrated that large ssDNA molecular libraries can be rapidly screened for chemical compound sensing. It was indicated that there are ten groups of ssDNA sequences set among 29 groups that can respond differently to the same set of chemical compounds. This suggests that the number of different units in the sensor array among larger libraries of ssDNA molecules is probably to be enormous. In addition to the response diversity, it was also found that this DNA-mediated biomimetic smell sensors can respond to 2,4-dinitrotoluene with very high sensitivity, which has been proved to be the vapor marker of

the explosive materials, TNT. As an alternative to the specific polymers that are specially designed and synthesized for the detection of nitroaromatic compounds [15–17], ssDNA-Cy3 conjugates have decisive advantages both in the detection sensitivity and engineered capability. ssDNA-fluorescent dye conjugates provide a completely new approach for achievement of functional sensitive materials for detection of explosive materials. It is also suggested that DNA-mediated biomimetic smell sensors have great potential to be applied in the detection of explosive material in many fields such as landmine detection and airplane safety checking.

8.3.2 DNA-Decorated Field-Effect Devices as Smell Sensors

With the fast development of nanotechnologies, nanoscale smell sensors have also achieved significant progress by use of nanomaterials for development of novel nanostructures for chemical sensing. The special features of ssDNA molecules make them ideal candidates to be used as sensitive elements for development of biomimetic smell sensors for chemical sensing. These progresses motivate research on the combination of ssDNA molecules with nanomaterials to build novel nanostructures for chemical sensing. One typical example is the exploration of ss-DNA/swCN-FET hybrid nanostructure for chemical sensing, which can detect the chemical signals and convert the detected chemical signals into electrical signals [7, 8]. In this chemical sensing system, swCNs decorated with ssDNA molecules were used as sensitive materials for detection of specific chemical compounds. swCNs were grown on the surface of a SiO_2/Si substrate by standard chemical vapor deposition. The source and drain electrodes of FET were fabricated on the same substrate with electron beam lithography. The individual swCN decorated with ssDNA molecules was contacted between the source and drain electrodes of a FET, while the degenerately doped silicon substrate was used as a back gate if FET. By this configuration, FET was able to be used as the signal transduction component that can measure the conductivity changes of swCNs originated from the specific interactions between ssDNA molecules and chemical compounds. This is a new kind of nanoscale chemical sensor which uses the special chemical recognition site of ssDNA molecules and electrical readout of FET with nanostructure.

The coupling of swCNs with ssDNA molecules play a crucial role in this nanostructure for chemical sensing, which has significant influences on the performance of the DNA-mediated nanoscale smell sensors. As shown in Fig. 8.5, atomic force microscopy (AFM) images taken before and after the decoration of ssDNA molecules on swCN indicate that the decoration of ssDNA molecules leads to an increase in the diameter of swCN, which is mainly due to the formation of a nanoscale layer of ssDNA on the swCN surface [8]. In addition, the surface roughness of substrate also increases, which is originated from the unspecific absorption of ssDNA molecules on the substrate. This demonstrates that the nanostructure consisting of ssDNA molecules and swCN has been well

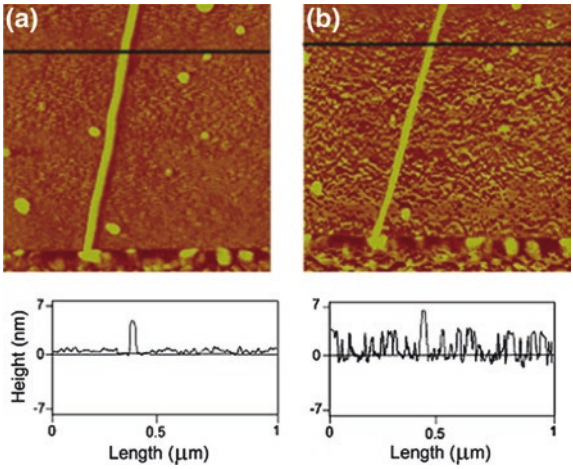

Fig. 8.5 The coupling of ssDNA molecules with swCNs was characterized by AFM. The same swCN was investigated by AFM images taken before **a** and after **b** functionalization with ss-DNA. The decoration of ssDNA molecules on swCN lead to the measured diameter of swCN increase from 5.4 to 7.2 nm (Reproduced with permission from Ref. [8]. Copyright 2005 American Chemical Society)

constructed. The combination of this nanostructure with FET allows measurement on the conductivity changes of ssDNA decorated swCN and avoids the influences of other ssDNA molecules absorbed on the substrate. The measurements on the swCN without decoration of ssDNA molecules show that no detectable conductivity change can be recorded when exposed to chemical compounds. With ssDNA decoration, this nanostructure shows distinct responses to different chemical compounds. In addition, the response profiles of this nanostructure can be tuned by changing the base sequences of ssDNA molecules. By this strategy, this ssDNA and swCN hybrid nanostructure can be used as sensitive elements for development of smell sensors for detection of a variety of chemical compounds. In addition, the regeneration of this ssDNA and swCN hybrid nanostructure has proved to be very good, which show constant responses of at least 50 cycles on exposure to chemical compounds without any sensor refreshing. All these features make this ssDNA and swCN hybrid nanostructure a promising candidate to be utilized for development of biomimetic smell sensors, which have potential applications in many fields such as breath diagnosis, environmental protection, and food industry.

8.3.3 Chemical Sensing System and Data Processing

The sensor system of DNA-mediated smell sensors is usually dependent on the signal transduction mechanisms of chemical signals into detectable electrical or fluorescent signals. At present, there are two main categories of sensor systems utilized in the development of DNA-mediated biomimetic smell sensors, which are FET-based measurement system and fluorescent-based measurement system, respectively. The main function of the sensor system is to detect the transduced chemical signals, which could be electrical signals (e.g., FET-based measurement

system) or fluorescent signals (e.g., fluorescent-based measurement system). Benefit from the fast development of science and technologies, both of measurement systems, can be realized by the currently available devices and technologies by directly employment or a certain degree of modification. For example, the configuration of FET-based measurement system can be realized by the commercially available or homemade circuit combined with microfabrication technologies. On the other hand, the fluorescent-based measurement system can utilize the commercial fluorescent devices but need some modifications on the detection chamber and the introduction systems of chemical compounds. Furthermore, it is also possible and not so difficult to realize the multiple measurements on the sensor array in order to scale-up the number of sensor units to develop array-based sensing systems, for example, the electronic nose for breath diagnosis.

The data from individual chemical sensors usually can be processed and analyzed by the general tools or the respective software. But this is commonly not enough for the processing and analysis of data from chemical sensor arrays, which contain sensor units with broad and overlapping response profiles to different chemical compounds. It is thus required substantial data analysis methods, which often relate to the pattern recognition methods. Basically, the raw data from chemical sensor arrays usually need to be preprocessed and normalized. Then the data can be processed and analyzed by the major data analysis approaches such as linear discriminant analysis (LDA), principal component analysis (PCA), principal component regression (PCR), partial least squares (PLS), cluster analysis, and computational neural networks. These data processing and analysis methods for chemical sensor arrays have been systematically described in an excellent review, in which the basis, suitability, and applications of each method was introduced in detail and compared with critiques [47]. The applicability of each data processing and analysis method not only depends on the method itself, but also on the unanticipated problems that could encounter during the period of data processing and analysis. It is thus favorable and beneficial to have a set of data processing and analysis methods and related software tools specifically for the data from chemical sensor arrays.

8.4 Applications of Smell Sensors Based on DNA-Decorated Devices

DNA-mediated smell sensors are inspired by the biological olfactory systems and developed for detection of specific chemical compounds, which have shown promising prospects and potential applications although still in the stage of infant phase compared with other commercial available products. At present, DNA-mediated smell sensors are mainly developed for the research aiming to the detection of some important chemical compounds, for example, dinitrotoluene (DNT), which is an important marker for detection of explosive materials. There are mainly two

categories of DNA-mediated smell sensors, which are fluorescent-based smell sensors and FET-based smell sensors, respectively. In the following, we will briefly introduce the application of these two types of DNA-mediated smell sensors for detection of chemical compounds.

8.4.1 Specific and Sensitive Odorant Detection

Fluorescent-based DNA-mediated smell sensors have been investigated for possible detection of various volatile chemical compounds in the vapor phase [9]. Such smell sensors utilize ssDNA-fluorescent dye conjugates as the sensitive materials for specific volatile compound detection. The response profiles of the smell sensors are mainly dependent on the specific base sequences of ssDNA molecules. By changing the base sequences of ssDNA molecules, the smell sensors could be tuned to be responsive to the specific volatile compounds. As indicated in Fig. 8.6, the smell sensors made from the ssDNA molecules with different base sequences show distinct different responses to the same set of volatile compounds, which include propionic acid, triethylamine, methanol, DNT, and dimethyl methylphosphonate (DMMP) [9]. The dynamic responses of individual smell sensors were also diverse, which were mainly dependent on the base sequences of ssDNA molecules.

In addition, different concentrations of volatile compounds were utilized to test the sensitivity of DNA-mediated smell sensors [9]. As shown in Fig. 8.6a, the smell sensors made from the ssDNA molecules with base sequence of SEQ02 have significant responses to propionic acid and triethylamine at concentrations ranging from 4 to 75 ppm. The smell sensors with SEQ02 also show different responses to methanol, DNT, and DMMP at different concentrations. On the contrary, as shown in Fig. 8.6b, the smell sensors made from ssDNA molecules with

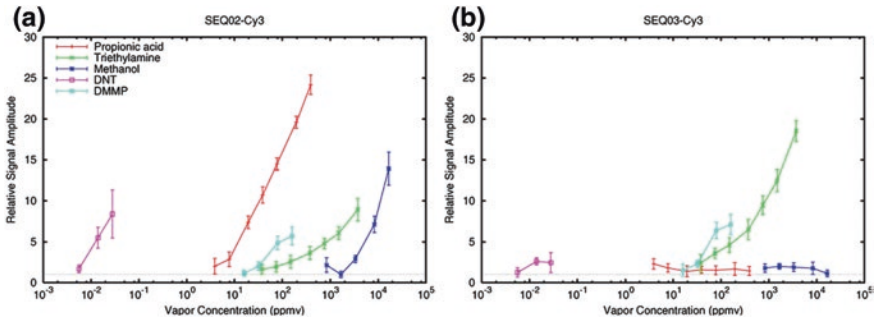

Fig. 8.6 The diverse responses of smell sensors made from ssDNA molecules with different base sequences to the same set of volatile compounds at different concentrations. **a** Responses of smell sensors made from ssDNA molecules with base sequence of *SEQ 02*; **b** Responses of smell sensors made from ssDNA molecules with base sequence of SEQ 03 (Reproduced with permission from Ref. [9]. Copyright 2008 White et al.)

base sequence of SEQ 03 show distinct responses to triethylamine and DMMP at concentrations of 75 and 30 ppm, respectively. But the smell sensors with SEQ 03 show no response to propionic acid, methanol, or DNT. It is suggested that the diverse responses of smell sensors with different ssDNA molecules are also dependent on the concentration of the volatile compounds. In the smell sensors with DNA molecules, there are some important factors that could have significant influences on the performance for detection of chemical compounds, which include the property of substrate for the deposition of ssDNA molecules, the final concentration and duration of volatile compounds for measurement, and the buffer used for the deposition of ssDNA molecules onto substrates. All these discoveries demonstrated that the smell sensors made from ssDNA molecules with diverse base sequences are able to be used for detection of specific volatile compounds at low vapor pressures and at low concentrations. DNA-mediated smell sensors have shown distinct advantages for development of electronic noses for specific and sensitive odorant detection.

8.4.2 Odorant Sensitive DNA Nanostructure

The combination of ssDNA molecules with nanomaterial has provided novel approaches for development of biomimetic smell sensors for detection of chemical compounds. ssDNA molecules with specific base sequences have been used to decorate swCNs to build a nanostructure that can respond to the specific odorants [8]. These kinds of odorant sensitive DNA nanostructures have proved to be suitable to serve as sensitive materials for the construction of biomimetic smell sensors that can detect various chemical compounds with high sensitivity and specificity. One typical example of this kind of nanostructure is the ssDNA decorated swCN coupling with FET devices to develop biomimetic smell sensors for chemical sensing. The responses of such smell sensors with nanostructures have been investigated using different volatile compounds as detection target small molecules. As shown in Fig. 8.7a, b, the responses of smell sensors with ssDNA decorated swCN indicate that ssDNA decoration is necessary and important for detection of volatile compounds including methanol and TMA. In addition, as shown in Fig. 8.7c, the diverse responses of the same smell sensors to the different volatile compounds indicate the potentials of such nanostructures for the simultaneous detection of various chemical compounds in the gas phase. It is demonstrated that the ssDNA decoration can change the response profiles of swCNs to the volatile compounds, which can increase the binding affinity of certain volatile compounds to the nanostructures. It is suggested that the ssDNA molecules have potential to be used to enhance the responses of nanostructures to the volatile compounds. On the other hand, the smell sensors also show a pretty good reproducibility. The responses of smell sensors to the volatile compounds show no significant decrease even after 50 cycles of exposures to the volatile compounds. In addition, the variation between the smell sensors with the same ssDNA decoration swCN nanostructures is small.

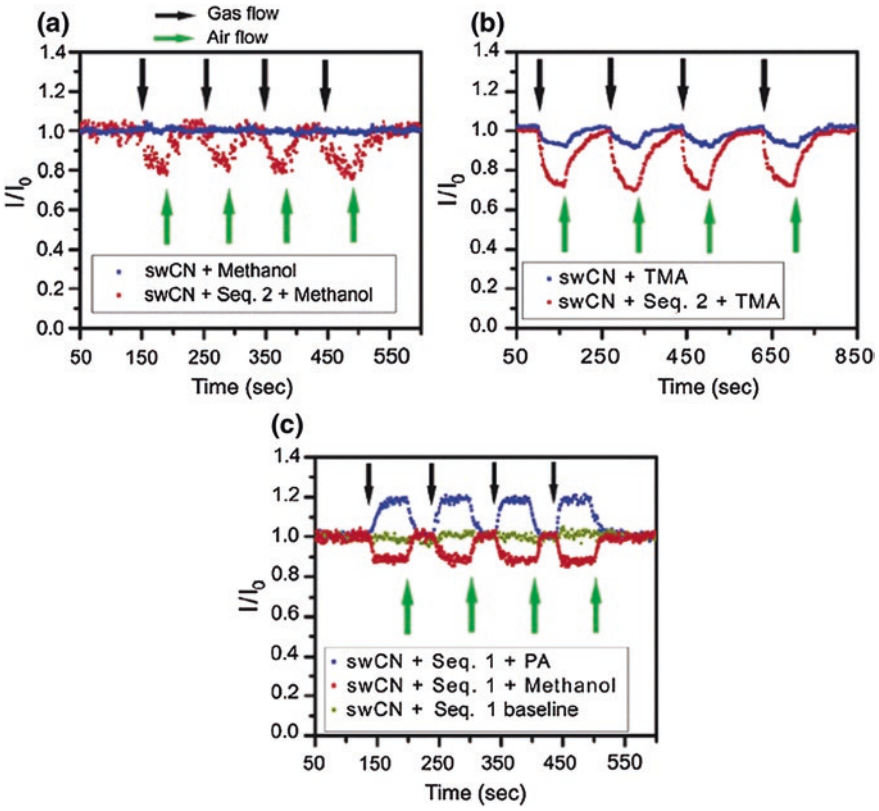

Fig. 8.7 The responses of biomimetic smell sensors with ssDNA decorated swCN nanostructures to different volatile compounds. **a** swCN-FET with and without ssDNA decoration in response to methanol. **b** swCN-FET with and without ssDNA decoration in response to TMA. **c** swCN-FET with ssDNA decoration in response to propionic acid and methanol (Reproduced with permission from Ref. [8]. Copyright 2005 American Chemical Society)

All these decisive advantages make such smell sensors favorable for development of sensor array and integrated systems such as electronic noses.

In addition to the diverse and reproducible responses of smell sensors, it is also indicated that the responses are dependent on the specific base sequences of ssDNA molecules. As shown in Fig. 8.7, smell sensors with different ssDNA base sequences show distinct response profiles to the same volatile compounds. This is mainly due to the changes in the base sequences of ssDNA molecules resulting in the response characteristics of odorant sensitive nanostructures changing accordingly, which are coupled together via the π-π stacking interaction between ssDNA molecules and swCNs. The base sequence-dependent responses were further demonstrated by the measurement of DNT and DMMP, which are specific markers for explosive materials and nerve gas, respectively. As shown in Fig. 8.8, smell sensors with different base sequences of DNA molecules were used to detect

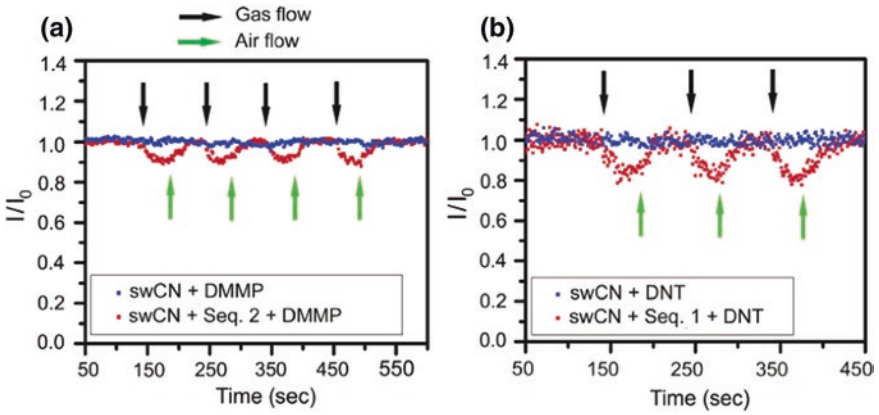

Fig. 8.8 The response of smell sensor with different base sequences of ssDNA molecules to a certain chemical compound. **a** The smell sensors with base sequence of *Seq. 2* in response to DMMP. **b** The smell sensors with base sequence of *Seq. 1* in response to DNT (Reproduced with permission from Ref. [8]. Copyright 2005 American Chemical Society)

different chemical compounds. This further indicates the base sequence-dependent responses of smell sensors to the certain chemical compounds. In addition, the smell sensors can detect DMMP and DNT at very low concentrations, which are as low as 1 ppm for DMMP and 25 ppm for DNT, respectively. Benefitting from the large diversity provided by the different combinations of ssDNA base sequences and high sensitivity originated from the nanostructures, it is possible to use ssDNA molecules with different base sequences to build a large family of smell sensors or sensor arrays that can respond to various chemical compounds with very high sensitivity and specificity, which could be used as key components for the development of electronic noses and other chemical sensing systems.

8.5 Summary

DNA-mediated biomimetic smell sensors have achieved obvious progress in the recent decade, which have shown promising prospects and potential application in many fields related to the detection of specific chemical compounds. The most important feature of DNA-mediated smell sensors is the use of DNA molecules as sensitive materials for chemical sensing, which are completely different and independent of the conventional function of DNA molecules for encoding the information of the life. The unlimited potential of combination diversity provides by the special base sequences of DNA molecules make it possible to develop sensitive materials with sufficient chemical diversity for the development of smell sensors for chemical sensing. Modern technologies for the synthesis of DNA molecules

also make it possible to generate enough diverse identical DNA molecules in an engineered manner, which can greatly facilitate the development of biomimetic smell sensors for chemical sensing. This is also the decisive advantages of using DNA molecules as sensitive materials for the development of smell sensors as compared with any other polymers. In addition, the integration of DNA molecules with nanomaterials provides novel approaches for the construction of highly sensitive nanostructures for chemical sensing. DNA-mediated biomimetic smell sensors not only provide a novel and powerful chemical sensing systems, but also highlight the novel roles of DNA molecules in addition to the function of basic life materials. In this chapter, we firstly introduced the basic theories of DNA-mediated smell sensors in two aspects, which are molecular features of odorant sensitive DNA and DNA-nanomaterials hybrid for odorant detection. Then, we summarize the approaches for the design of DNA-mediated smell sensors. First, the screening of odorant sensitive DNA was introduced in detail. Then the field effect transistor for DNA-mediated smell sensors was outlined. Finally, the sensor system and data processing of DNA mediated smell sensors were discussed. In the last section of this chapter, the most recent progress in the applications of DNA-mediated smell sensors was summarized in two aspects, which were mainly related to the detection of specific chemical compounds.

In the near future, the development trends of DNA-mediated biomimetic smell sensors are to utilize engineering approaches to gain diverse functional DNA molecules for chemical sensing, and fabricate sensor arrays and chemical sensing chips containing DNA molecules with different base sequences, which can perform dynamic and high-throughput screening or detection of chemical compounds. Inspired by the biological olfactory systems, DNA-mediated smell sensors have some decisive advantages for generation of smell sensor arrays in order to develop the electronic noses that can respond to many different chemical compounds with extreme high sensitivity and specificity. Due to the chemical diversity of DNA molecules and the progress in the synthesis of DNA engineering and advancements in nanotechnologies, significant progress in the development of DNA-mediated biomimetic smell sensors is expected to be achieved, which is expected to generate a large and diverse sensor array for electronic noses. However, there are also some challenges faced in the further development of DNA-mediated smell sensors. For example, how to define and screen the functional DNA molecules with specific base sequences, which have thousands of possible base combinations even with short base sequence ssDNA molecules. In addition, the space of chemical compounds that can be detected by the DNA-mediated smell sensors could be limited by the chemical recognition component of DNA molecules that are possible to be represented by a certain range of chemical conformations. Contributions from nanotechnologies and DNA engineering may provide novel solutions to these challenges, which could allow for the development of DNA-mediated smell sensors and sensor systems for chemical sensing characterized with miniature, integration, and intelligence.

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Chapter 9
In Vivo Bioelectronic Nose

Liujing Zhuang, Tiantian Guo and Bin Zhang

9.1 Introduction

Detection of odors has been applied to many real applications, such as quality control of food products, safety and security, environmental monitoring, medical diagnosis, and so on. These natural odors are composed of many different odorant molecules. The mammalian olfactory system can accurately recognize and discriminate a large number of olfactory stimuli [1], which has long been recognized as one of the most effective chemosensing system. Over the last decades, distinct fields such as genetic, cellular biology, biochemistry, neurophysiology, and behavior have made considerable progress in understanding how olfactory system perceives, discriminates, and recognizes odorant molecules [2]. Biological olfactory system sits between the environment and central nervous system. Animals have the ability to behaviorally detect very low concentrations of odorants ranging between 10^{-10} and 10^{-7} M in the air phase [3, 4]. The discriminatory capacity of this system derives from a series of information-processing steps that occur at distinct anatomical structures through which olfactory sensory information progresses: the olfactory epithelium of the nose, where olfactory sensory neurons detect odorous molecules [5, 6]; the olfactory bulb (OB) of the brain, to which these neurons transmit signals; and higher order structures of the brain, such as the piriform cortex, which receive information from the OB and distribute it to other regions of the brain [7]. Actually, biological olfactory system is a complex and precise smell sensing element with advantages of high sensitivity, excellent specificity, and rapid response to detect odors in the environment.

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Basic principles derived from biological olfaction, electronic nose, which comprises an array of cross-reactive chemical sensors and pattern recognition system, was developed to discriminate simple or complex odors [8]. Over the last three decades, electronic nose technologies have undergone important developments and now are useful in many areas, such as disease diagnosis [9–11]. However, its performance is still far from biological system because of limited sensitivity and receptive range [12]. With combination of cell culture and microfabrication technologies [13–17], we incorporated living olfactory cells [18–20] or tissues [21–23] as sensing element with neuron chips in vitro for biomimetic smell sensor. These biosensors provide a suitable platform with advantages of sensitivity, specificity, and rapid response for odorant detection. However, in vitro cultured condition leads to shortened cell/tissue survival. For achieving odorant response, we have to first inject odorant perfusate into the Petri dish and then wash out by standard perfusate [24]. This procedure leads to cellular damage and promotes cell death. So the working life of neuron chips combining cells could last for only a few hours. It has thus been limited to achieve long-term and repeatable odorant detection. What is more, in vitro culture will damage the intact nerve structure of olfactory system, so this technology may not define the natural pattern of neuronal activity.

In fact, some animals, e.g., dogs, play an important role in detection of a wide variety of substances, including explosives, illicit drugs, land mines [25, 26]. Recently, researchers found that sniffer dogs can recognize various types of cancer [27], though the target odors do not occur in their daily life. These applications show the remarkable ability of mammalian olfactory system for chemosensing. Although sniffer dogs are relatively easy to train, the access to signals is indirect via the animal's behavior in combination with a trainer and experimenter [28, 29]. To avoid a behavioral readout, we present a real-time direct interface between the olfactory system and artificial devices by extracting the odor information in behaving rat using multiple microelectrodes. Benefiting from the development of an novel multiple microelectrode implant technology, in vivo extracellular recordings continue for up to 18 months is possible [30, 31]. This technology uses an array of penetrating microelectrodes which are dozens of μm in diameter to implant into the nervous tissues [32, 33]. The minimal-invasive method ensures the least damage to cells and biological system. So it allows a long-term repeatable recording of extracellular action potentials from many neurons that distributed across the olfactory system in conscious animals [34, 35]. The external olfactory input can be inferred from neural activities, which is so-called neural decoding [36]. Undoubtedly, this technology allows to define the natural pattern of olfactory neuronal activity more accurately. By implanting penetrating microwire array electrode into the olfactory system of conscious rats, we specially present an in vivo bioelectronic nose system with characteristic of stability, repeatability, and high sensitivity.

9.2 Theories of In Vivo Bioelectronic Nose

9.2.1 Smell Detecting Using Whole Animals

A biosensor can be described as a biological detector or recognition element (e.g., an OR for olfactory biosensing) linked to a physical transduction system (e.g., optical, electrochemical). This definition, however, is rather broad and for smell sensors (also known as bioelectronic noses), could include the use of whole animals as the biological recognition element. The use of dogs as chemical detectors dates back to 12,000 years ago based on tomb evidence. Since World War II, dog-handler teams have been used extensively by the military to locate explosives. The civilian use of dogs began with tracking individuals and locating drugs and bombs and then has expanded to include the detection of guns, pipeline leaks, gold ore, contraband food, melanomas, gypsy moth larvae, brown tree snakes, and their use in the controversial dog-scent lineup for forensic evidence. In the last decade, dogs trained to detect flammable and ignitable liquid residues, commonly called accelerant detector dogs, have become widely utilized and their alert has proven to be admissible as evidence. The use of detector dogs has now also become widespread and routine in search and rescue, including finding the last missing person after the World Trade Center bombing, discouraging employee drug use, termite infestation inspection, and screw worm detection [37]. In addition, animals, such as dogs and cats, have been used as sentinels for public health risk assessment, such as early identification of food contamination, infectious disease transmission, environmental pollution, medical diagnosis, and even bioterrorism or chemical terrorism events [38].

In 1989, Williams et al. put forward the hypothesis that dogs may be able to detect malignant tumors by odor [39]. From then on, canine scent detection of bladder, prostate [40–42], breast, lung [28, 29], colorectal [43], and ovarian [44] cancers have been reported. Due to the remarkable scenting ability and capacity to learn how to sign differences, dogs can be trained to discriminate exhaled-breath samples from patients with cancer and control groups. According to the research published in the current issue of the *European Respiratory Journal*, trained dogs detected lung cancer with sensitivity of 90 % and specificity of 72 % [29, 45]. The stage of cancer, age, smoking, and recently eaten meal did not influence the dogs' indication. Their well-designed study involved 60 lung cancer patients and 110 healthy controls, and is novel for also including "disease controls"; 50 patients with nonmalignant lung disease. The findings of Ehmann et al. [29] corroborate the results of an earlier study of canine scent detection of lung cancer, which reported sensitivity and specificity of 99 %. Together, these two papers, which achieved high accuracy while using different dogs, trainers, and human subjects, beg the question of where this might all be leading. The high accuracy of canine scent detection of lung cancer suggests dogs might, in the future, make some modest contribution to successes in lung cancer screening and detection.

9.2.2 Signal Transduction in Mammalian Olfactory System

Olfactory coding begins with the transduction of odor information into electrical signals by olfactory receptor neurons (ORNs) (Fig. 9.1). Once the receptor has bound an odor molecule, a cascade of events is initiated that transforms the chemical energy of binding into a neural signal, that is, a change in the membrane potential of the OSN. In mammals, ORNs express only one type of receptor out of a repertoire of ~1000 [5]. Although an individual ORN expresses only one type of receptor, it can be activated by many different odorants [46]. Axons of ORNs that express the same types of receptors project into the main olfactory bulb (OB) and converge to only a few stereotyped glomeruli [47]. OB is the first olfactory processing center in the mammal brain. The principal neurons of the OB, the mitral and tufted cells (MTs), receive synaptic input from OSNs and transmit OB output to multiple higher brain areas. Within the OB, MTs are interconnected over multiple spatial scales by a variety of local, predominantly GABAergic, interneurons. Pioneering work demonstrated that odor-evoked patterns of MT activity are spatially distributed and exhibit a temporal structure that is not observed in glomerular inputs, including a slow modulation of firing rates and an oscillatory synchronization of odor-dependent MT ensembles [48]. The OB, therefore, actively reorganizes odor representations and has become a model system for studies of pattern processing by central neuronal circuits.

Odor information received by the OB is first processed and refined before being transmitted to downstream centers. The second one lies in the external plexiform layer of the OB where reciprocal dendrodendritic synapses, between dendritic spines of local interneurons and the dendrite of output neurons, are heavily distributed. The final processing occurs in higher order brain structures comprising the primary and accessory olfactory cortices. The axons of bulbar output neurons projects in the olfactory tract to higher order brain structures without contacting the thalamus. These higher centers include the anterior olfactory nucleus, which connects the two OBs through a portion of the anterior commissure, the olfactory tubercle, the piriform cortex (considered to be the primary olfactory cortex), the cortical nucleus of the amygdala, and the entorhinal area. In both the OB and higher centers, odor information seems to be encoded by activity across the entire neuronal network [2, 49].

9.2.3 Massive Parallel In Vivo Recording of Olfactory System Activity

An understanding of the mechanisms by which the mammalian olfactory system can derive behaviorally relevant information from minute concentrations of odors involves studying the spatial and temporal patterns of neural activation in the mitral and tufted cell layer of the OB. A number of tools have been used to study the first stages of

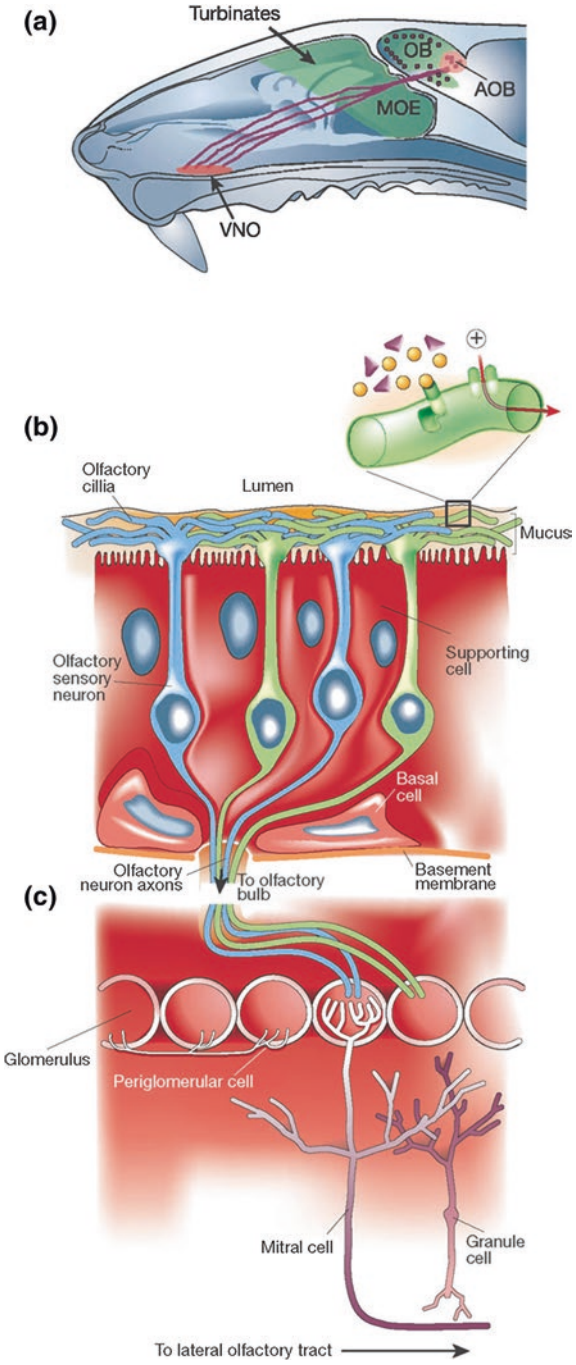

Fig. 9.1 The olfactory system in mammal **a** In a sagittal view of the rat olfactory system. **b** The olfactory epithelium consists three cell types: olfactory sensory neurons (OSNs), supporting or sustentacular cells, and a stem-cell population. **c** Wiring of the early olfactory system. (Reproduced with permission from Ref. [49]. Copyright 2001 Macmillan Magazines Ltd)

these spatiotemporal activity patterns in the mammalian OB. Optical imaging (both intrinsic and dye enhanced) and functional magnetic resonance imaging have been used to record spatial and temporal changes in pre- and postsynaptic activity in the glomerular layer of the OB, and the synaptic connections between the olfactory receptor neurons and MTs. The technique has revealed an odor- and concentration-dependent activation of specific glomeruli that is conserved across both hemispheres [50, 51]. However, limitations inherent in these techniques generally prevent direct imaging of either the MTs, or of action potentials generated by these cells.

Action potentials produce large transmembrane potentials in the vicinity of their somata. These output signals can be measured as a voltage difference by placing a conductor, such as the bare tip of an insulated wire, in close proximity to a neuron9. If there are many active (spiking) neurons in the vicinity of the tip, the electrode records from all of them (Fig. 9.2) [52]. Multisite electrodes (a wire tetrode, for example) can estimate the position of the recorded neurons by triangulation. Distance of the visible electrode tips from a single pyramidal cell (triangles) is indicated by arrows. The spike amplitude of neurons (>60 μV) within the gray cylinder (50 μm radius), containing ~100 neurons, is large enough for separation by currently available clustering methods. Although the extracellularly recorded spike amplitude decreases rapidly with distance, neurons within a radius of 140 μm, containing ~1,000 neurons in the rat cortex can be detected. Because neurons of the same class generate identical action potentials (all first violins sound the same), the only way to identify a given neuron from extracellularly recorded spikes is to move the electrode tip closer to its body (<20 μm in cortex) than to any other neuron. To record from another neuron with certainty, yet another electrode is needed.

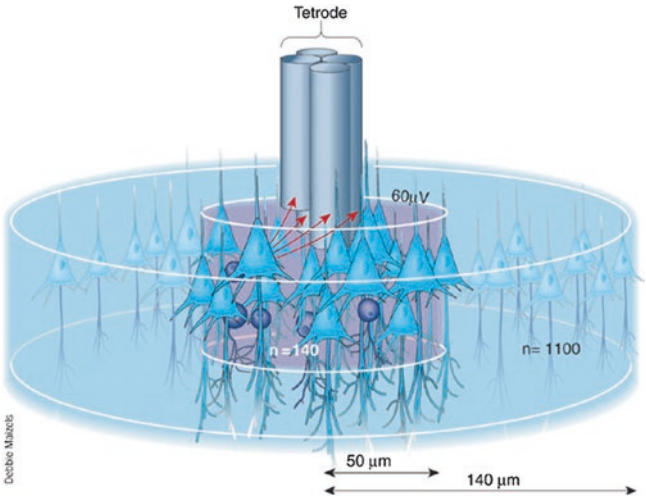

Fig. 9.2 Unit isolation quality varies as a function of distance from the electrode (Reproduced with permission from Ref. [52]. Copyright 2004 Nature Publishing Group)

Single and multiple microelectrodes have been used to record action potentials from olfactory system in mammals and the functional equivalent in insects [32, 53–57]. Although these techniques offer excellent temporal resolution, they report little information about how populations of these cells interact to contribute to concentration independent perception of odorants. Arrays containing large numbers of microelectrodes potentially could overcome the limitations of imaging and single microelectrode electrophysiological techniques by allowing the simultaneous recording of action potentials from many neurons. Taking advantages of multiple microelectrode implant technology, in vivo recordings continue for up to 10 months is even possible [31]. The efficacy for multielectrode electrophysiological investigation of neuronal populations is well established in both sensory and motor systems.

9.3 Design of the In Vivo Bioelectronic Nose

9.3.1 Preparation of Microelectrode

The three major categories of implantable microelectrodes are microwire, silicon-based [58], and flexible microelectrode arrays. Microwire electrodes are largely made of stainless steel or tungsten and they can be used to estimate the position of individual recorded neurons by triangulation. Silicon-based microelectrode arrays include two specific models: the Michigan and Utah arrays [59]. Michigan arrays allow a higher density of sensors for implantation as well as a higher spatial resolution than microwire electrodes. They also allow signals to be obtained along the length of the shank, rather than just at the ends of the shanks. In contrast to Michigan arrays, Utah arrays are 3D, consisting of 100 conductive silicon needles. However, in a Utah array signals are only received from the tips of each electrode, which limits the amount of information that can be obtained at one time. Furthermore, Utah arrays are manufactured with set dimensions and parameters while the Michigan array allows for more design freedom. Flexible arrays, made with polyimide, parylene, or benzocyclobutene, provide an advantage over rigid microelectrode arrays because they provide a closer mechanical match, as the Young's modulus of silicon is much larger than that of brain tissue, contributing to shear-induced inflammation [60].

Over the last decades, many laboratories around the world have started to rely on microelectrode arrays formed by fine microwires, organized in different geometrical configurations, to chronically record the extracellular activity of populations of individual neurons in both anesthetized and behaving animals [61]. Indeed, microwire array electrodes play an important role in multisite, multiple single-unit in vivo recording experiments [31, 62]. Duke University Center for Neuroengineering has specialized in producing a large variety of microwire array configurations that can now be utilized in a large variety of species (e.g., mice, rats, monkeys, and intraoperative human recording). Their efforts have

been directed at producing arrays that can be utilized in experimental protocols demanding simultaneous recording from large samples of single neurons (e.g., 50–500), distributed across multiple cortical and subcortical brain sites in fully awake and behaving animals over long periods of time (months–years). Various methods employed to manage and organize microwire into arrays can be classified as either layered or discretely wired.

Although microwire electrode array can be purchased from commercial vendors and are reliable for many experimental, economic considerations, and flexibility of the experimental design make it worthwhile to develop fabrication methods in-house [63]. Generally, we use 16-channel home-made arrays using the discrete-wired method which consist of: (1) 2 parallel rows of eight formvar-coated nichrome microwires (65 μm, AM system, WA; #762000) each, (2) a printed circuit board (PCB) connected to the microwires, and (3) a miniature 20-pin plastic connector connected to another side of the PCB (Fig. 9.3a). In the arrays, the distance between microwires in a row varied from 100 to 200 μm, and the distance between the rows varied from 400 to 500 μm. The impedance of each microwire is about 100 kΩ at 1 kHz (Fig. 9.3b) [64]. The magnitude spectra of home-made electrodes showed similar performance compared with commercial electrodes. The PCB was coated with epoxy for insulation. Several key steps in the fabrication of a good electrode include: (1) arranging microwires into the desired configuration, (2) maintaining the corresponding sequence of the microwires, (3) soldering the microwires to the pad of print circuit board.

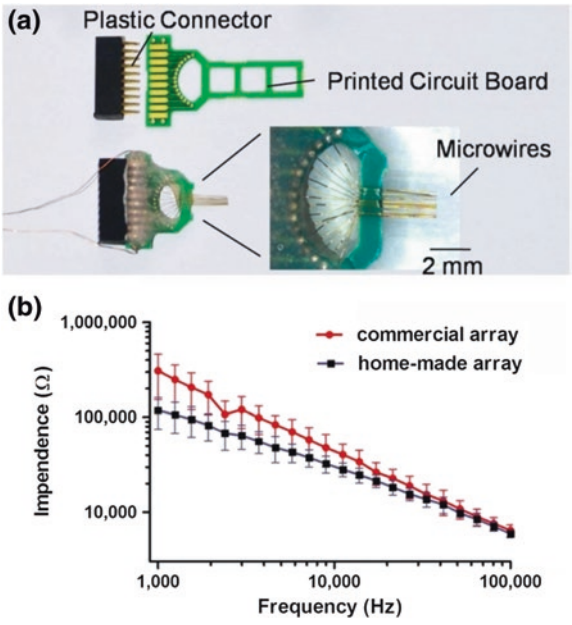

Fig. 9.3 **a** 16-channel home-made microwire array electrodes. **b** Impedance spectra of home-made and commercial electrodes were recorded. (Reproduced with permission from Ref. [64]. Copyright 2014 Science China Press and Springer)

9.3.2 Animal Training and Surgery Protocol

In order to implant the microelectrode into the OB of rat, craniotomy is necessary. Adult male Sprague Dawley rats (200–300 g) were often used. Rats were maintained in isolated cages for 2–3 days before surgery to adapt to experimental setting. They had free access to water but were on a food restriction schedule to keep them initiative on tracking odorants. During surgery, rats were anesthetized with an intraperitoneal injection of chloral hydrate (4 ml/kg) throughout the procedure. After shaving head, they will be held in a standard stereotaxic apparatus with ear bars. To access the dorsal surface of the skull, first a midsagittal incision was made on skin, and then removed the soft tissue and periosteum. Following washing away blood on the bone surface with saline, a craniotomy was performed at the stereotaxic coordinates of electrode implantation site (~8 mm anterior to bregma and ~1.5 mm lateral) and reference site (~1 mm anterior to bregma and ~5 mm lateral) in the dorsal side of the OB (Fig. 9.4a, b). After craniotomy had been done, a slit would be made in the pia mater overlying the OB. Then electrode could touch down the surface of dorsal OB to mitral/tufted layer which is located at the depth of 300–400 μm [55] using a hydraulic pressure microelectrode propeller (Narishige Group, Japan). After that, the craniotomy was sealed with a layer of medical glue. When the glue solidified, dental cement was applied to fix the electrode. An example of a conscious rat implanted with electrode is shown in Fig. 9.4c.

In some studies, behavioral testing was performed employing a go, no-go olfactory discrimination task in which all behavioral events and data collection were controlled and monitored by computer. For example, rats were water-deprived overnight prior to each recording session and, therefore, were strongly motivated to perform for fluid reward [65, 66]. On each trial, the rat poked its nose into an odor port to trigger odor presentation and then had 3 s after withdrawal from the port to respond by entering a nearby fluid well for reinforcement (go response). After a response was made, delivery of fluid was delayed by a variable period of

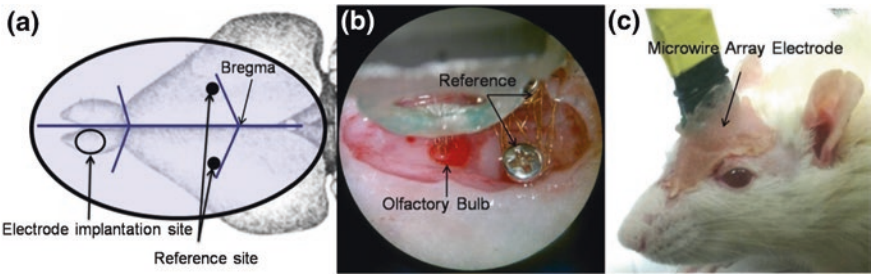

Fig. 9.4 **a** The location of the electrode array and reference electrode. **b** Implantation of a 16-channel microwire array electrode in the dorsal aspect of OB. **c** Microwire array electrode was implanted in OB of conscious rat (Reproduced with permission from Ref. [64]. Copyright 2014 Science China Press and Springer)

approximately 300–800 ms, providing a brief period in which neural activity could be examined independent of reinforcement. In the two-odor task, one odor signaled that a go response would produce approximately 0.05 ml of a palatable 10 % sucrose solution, whereas the other odor signaled that a go response would produce approximately 0.05 ml of a distasteful 0.03 M quinine solution. In the four-odor task, two distinct odors were associated with sucrose and two distinct odors were associated with quinine. In fact, as in any in vivo experiments, the learning and training effects on the same animal cannot be underestimated.

9.3.3 In Vivo Bioelectronic Nose

For decades, the creation of interface between brain and machine, like "brain-machine interface," "brain-computer interface": has received a lot of attention. Neurophysiologists have coupled devices to the nervous system of animals to record its electrical activity, and thereby infer its function, or to modify its function by stimulating it electrically. We chronically implanted the microelectrode in the dorsal aspect of rat OB to obtain neural activity. OB is the first processing center for olfactory information in brain. From the application point of view, we regard rat OB as "odor sensitive device." Distinct fields such as neurophysiology, genetic, and cellular biology have made considerable progress in understanding how olfactory system perceives, discriminates, and recognizes odorant molecules, which provide research basis for us. Combined brain–machine interface technology and mammals' olfaction ability, we present an in vivo bioelectronic nose system (Fig. 9.5). Simultaneous recordings could be obtained by attaching the connector of microelectrode to preamplifier with headstage cable connected to OmniPlex Data Acquisition System (Plexon, Inc., Dallas, TX). Neuronal signals from microelectrode were sampled at 40 kHz, amplified by 1500× gain, and filtered from 0.5 Hz to 8 kHz. Raw data were saved for offline analysis.

9.4 Realization of the In Vivo Bioelectronic Nose

9.4.1 Recording of Electrophysiological Signals

OB generates a tremendous amount of neural activity. These signals fall into two major classes: spikes and local field potential (LFP) [68]. Spikes (200–2000 Hz) reflect the action potentials of individual neurons and thus acquired primarily through microelectrodes implanted by invasive techniques. Spikes can be measured for a particular neuron or a group of neurons as shown in Fig. 9.6. The signal is a measure of the average rate, correlation, and temporal pattern of the neuronal firing. The nervous system presents information on the firing rate of each neuron. Therefore, odor information can be measured through changes in the average

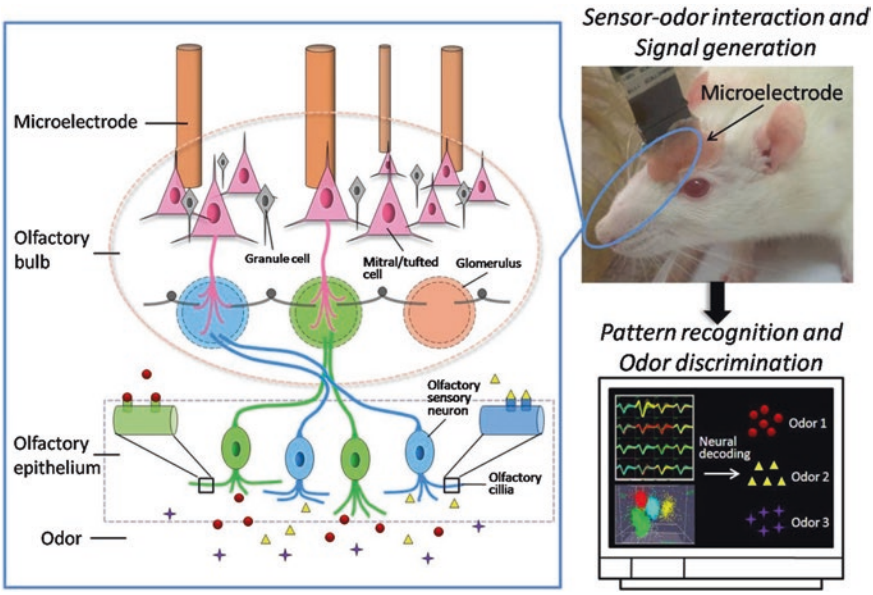

Fig. 9.5 Schematic of in vivo bioelectronic nose system (Reproduced with permission from Ref. [67]. Copyright 2014 Elsevier B.V.)

firing rate of neurons located in OB. In in vivo bioelectronic nose, the microelectrode tips contact with certain M/Ts and simultaneously recorded extracellular action potentials from a small population of M/Ts. The recorded M/Ts express and sustain an array of chemical gas transducers that can respond to odorant molecules. In the absence of odors, M/T cells displayed different degrees of spontaneous activity. Following odor onset, the firing rate would change. For example, in Fig. 9.6c, the mean spontaneous firing rate of recorded M/T cell is about 0.5 Hz, while the mean firing rate occurred (during 2.0 s) after odor onset is about 8.2 Hz. Rasters (left of graph) and peristimulus time histograms (PSTHs, mean firing rates/bin, bin = 0.5 s, right of graph) show spike firing rates during stimulation of isoamyl acetate (IAA). Eight trials are displayed. The dashed line indicates the odor onset time In addition, odor-evoked responses were reliable across multiple trials [67].

LFPs (1–100 Hz) are measures of combined synaptic, neuronal, and axonal activities of groups of neurons, which reflect the activity of neural network within a volume of tissue. Signals recorded from LFP are split into several bands: θ (1–12 Hz) oscillation tracks the respiration cycle and is also called respiratory oscillation, with frequency above 4 Hz usually defined as sniffing. β (15–30 Hz) and γ (40–100 Hz) oscillation are associated with olfactory coding and both play important roles in odor learning and discrimination [69]. LFP power increased evidently during odor stimulation and every odorant-evoked odor-specific oscillation. For each odorant, θ, β, and γ signals from raw LFP (1–100 Hz) were extracted.

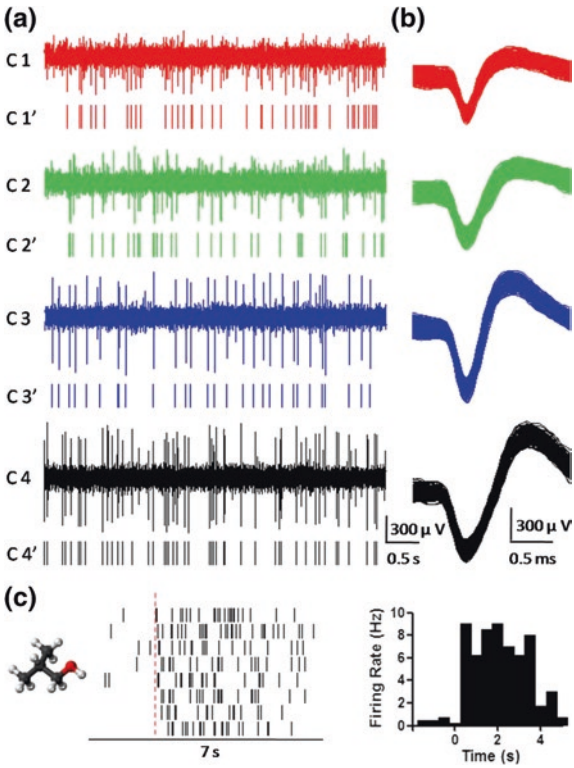

Fig. 9.6 Discriminated unitary activity from four channels of microelectrode. **a** The top panels of each *graph* shows raw spontaneous signals of four units. The *bottom panels* show their raster plots. **b** Four recorded M/Ts could be reliably discriminated with spike waveforms. **c** Odor-evoked unitary activities in a behaving rat from a single M/T cell (Reproduced with permission from Ref. [67]. Copyright 2014 Elsevier B.V.)

Spontaneous LFPs were fairly flat. Following odorant onset, the frequency of θ oscillation increased, and high-amplitude β and γ bursts were clearly observed. However, the β bursts seemed to have a more regular and consistent oscillation pattern than γ bursts. From top to bottom in each plot of Fig. 9.7, spectrogram of raw LFP (1–100 Hz) signal, filtered signals in the θ (1–12 Hz), β (15–30 Hz), and γ (40–100 Hz) band are shown. β and γ oscillations are from two trials of odorant stimulation. The color scale represents signal power. The dashed lines indicate the odorant onset time. Spontaneous LFPs are generally fairly flat.

9.4.2 Efficacy Analysis of In Vivo Bioelectronic Nose

In order to determine the efficacy of the developed bioelectronic nose system as a platform for odorant detection, we, respectively, explored its working life, specificity and sensitivity.

To examine the stability of developed bioelectronic nose system for continuous recording, we analyzed the responses of M/T cells from the same recording

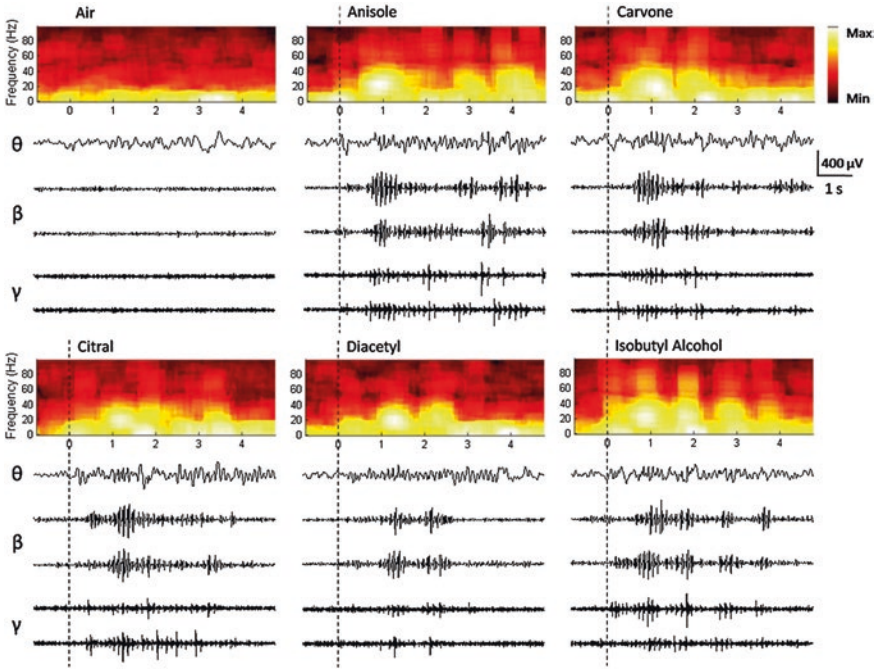

Fig. 9.7 Spontaneous LFP without odorant stimuli and LFPs evoked by monomolecular stimuli in a conscious rat

channel over multiple days. Figure 9.8 shows spontaneous (control group) and odor-evoked (10^{-1} M carvone) neuronal activity recorded at different days. The upper two panels show the raw data of spike discharge. The lower panels show raster plots of different trials recorded at a single day (5 trials). Each sweep of the raster lasts 2 s. The bottom panels represent peristimulus time histograms (PSTHs, spike counts/bin, bin = 0.1 s). The dashed bars indicate the time of occurrence of first 200 ms bin that significantly differed from baseline during

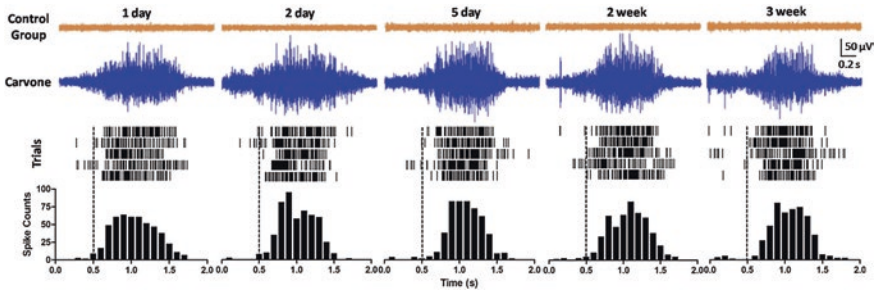

Fig. 9.8 Multiunit responses of M/T cells from the same channel over multiple days (Reproduced with permission from Ref. [70]. Copyright 2013 Elsevier B.V.)

odorant application. Raster plots for single trials and peristimulus time histograms (PSTHs) were created to compare the odorant response over time. The shapes of PSTHs were found to be similar over a period of 3 weeks. So the bioelectronic nose system has well stability and long working life.

To explore the specificity of developed bioelectronic nose system, we examined the responses of M/T cells to different odorants. Spike discharge and raster plots were created for carvone, citral, isobutyl alcohol (IBA), diacetyl, anisole, and IAA. For example, at concentration of 10^{-1} mol/L, the multiunit recorded from the same channel (Fig. 9.4) responded to all six odorants while the response patterns were not uniform based on spike discharge patterns and PSTHs. Further, we found a large proportion of examined units also responded to carvone ($C_{10}H_{14}O$), citral ($C_{10}H_{16}O$), IBA ($C_4H_{10}O$), and diacetyl ($C_4H_6O_2$) with robust discharge, indicating that M/T cells sensitive to these four odorants were distributed widely across the dorsal aspect of OB. During the four odorants stimulation, the mean firing rates of multiunit discharge were about 50 Hz. In Fig. 9.9, spike discharges (top panels

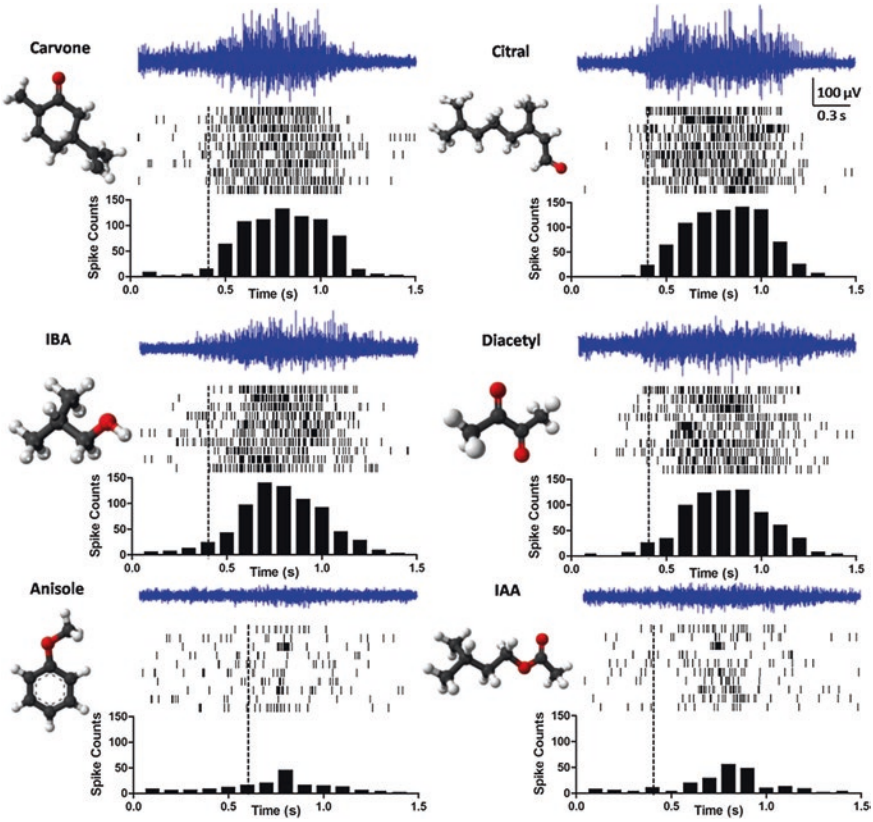

Fig. 9.9 Multiunit responses of M/T cells to six odorants from the same channel (Reproduced with permission from Ref. [70]. Copyright 2013 Elsevier B.V.)

of each graph), raster plots (lower panels of each graph), and PSTHs (bottom panels of each graph, bin = 0.1 s) of M/T cells during stimulation of carvone, citral, (IBA), diacetyl, anisole, and IAA. The dashed bars indicate the time of occurrence of first 200 ms bin that significantly differed from baseline during odorant application. However, there was no strong response to anisole (C_7H_8O) and IAA ($C_7H_{14}O$). The mean firing rates were below 20 Hz. We preliminarily found the odorants with same carbon atoms evoked similar discharge type. The results indicate that activity of multiple M/T cells provided sufficient information to discriminate between odorants. Thus, the bioelectronic nose system has high specificity for odorant detection.

To explore the concentration detection limit of developed bioelectronic nose system, we decreased the odorant concentrations to extremely low level. In this experiment, we applied different concentrations from 10^{-15} to 10^{-1} mol/L to have a better control of odorant response. Figure 9.10 shows the spike discharge from the same recording site evoked by different concentration of carvone. Both odor-evoked (a–k) and spontaneous (l) extracellular potentials repeatedly recorded

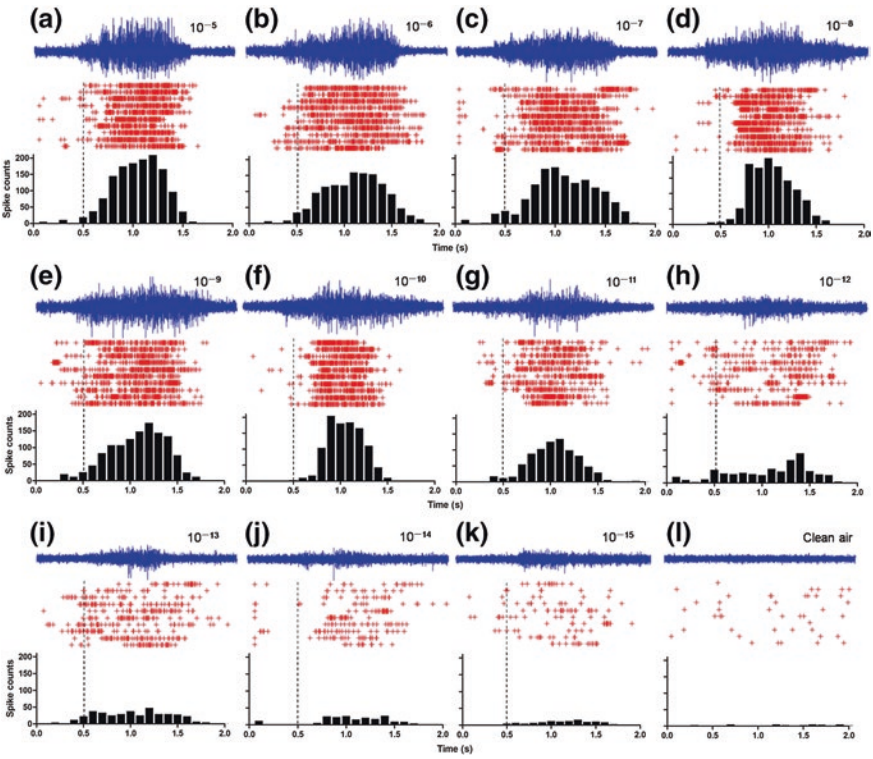

Fig. 9.10 Concentration detection sensitivity limitation analysis of bioelectronics nose system Both odor-evoked **a–k** and spontaneous **l** extracellular potentials repeatedly recorded from the same electrode. (Reproduced with permission from Ref. [64]. Copyright 2014 Science China Press and Springer)

from the same electrode. The concentration of carvone used in this experiment ranges from 10^{-15} to 10^{-5} mol/L. The upper panels show the raw data of firing spikes. The lower panels show dot raster plots of different trials (10 cycles). Each sweep of the raster lasts 2 s. The bottom panels show peristimulus time histograms (PSTHs, spikes/bin, bin = 0.1 s) for odorant stimulation. The dashed bars indicate the time of robust response onset to stimulation. With decreasing the concentration of carvone, the mean firing rate gradually decreased and the peak distribution of spikes shifted toward lower amplitude. The neuronal activities evoked by different concentrations of carvone were recorded from the same electrode. It was indeed the case that higher odorant concentration always activate more intensive response pattern. The response pattern changed slowly at concentration between 10^{-5} and 10^{-9} mol/L. However, once the concentration decreased below 10^{-10} mol/L, we found noticeable abrupt changes. Odorant concentration plays a critical role in determining the absolute detection threshold of an odorant. Quantitative changes lead to concentration-dependent response patterns. Although this phenomenon may depend a lot on the collection of odorants and recording site, we found the developed system has detection limit to carvone odorant as low as 10^{-15} mol/L. The results suggest that the bioelectronic nose system may contribute to the screening and detection of trace odorants, such as exhaled-breath detection.

9.4.3 Pattern Recognition for Odor Detection and Discrimination

Bioelectronic nose system has the same physiological model as traditional electronic nose dose. It is predicated that exiting algorithm for pattern recognition used in electronic nose also works in bioelectronics nose. According to plenty of experiments, the prediction is reasonable. Pattern recognition methods in electronic nose, to analyze odors or mixture of different compounds qualitatively, can be transplanted in bioelectronic nose system effectively.

The problem of pattern recognition analysis is closely linked to that of multivariate data analysis. Multivariate data analysis, as demonstrated in Fig. 9.11 generally involves statistical and biologically motivated methodologies. Common conventional statistical methods include multiple linear regression (MLR), partial least squares (PLS); principal components analysis (PCA), cluster analysis (CA) including nearest neighbor (NN); discriminant function analysis (DFA) such as linear discriminant analysis (LDA), principle component regression (PCR). Biologically motivated nonparametric intelligent algorithms based on physiological feature of human brain are also described, such as artificial neural networks (ANN) including self-organizing map (SOM), multilayer perceptron (MLP), probability neural network (PNN), radial basis function (RBF), Learning Vector Quantization (LVQ); fuzzy learning methods includes, fuzzy inference system (FIS), fuzzy neural network (FNN), adaptive resonance theory (ART); genetic algorithms (GA), neuro-fuzzy systems (NFS), and [71, 72]. Together these algorithms provide the reader with a comprehensive review of pattern analysis techniques for bioelectronic nose system.

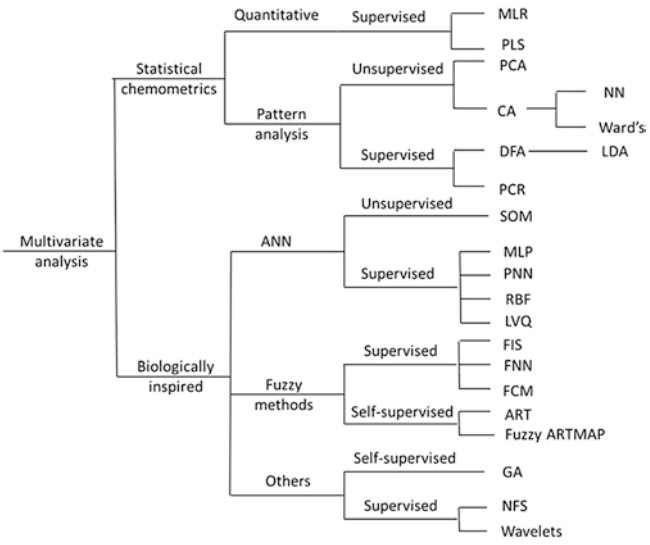

Fig. 9.11 Classification scheme of the multivariate pattern analysis

The nature of a (pattern recognition) PARC engine is usually classified in terms of being parametric or nonparametric, and supervised or unsupervised.

Parametric: A parametric technique is commonly referred to a statistical approach. Statistical methods are based on the assumptions that the data follow a normal distribution with a constant mean and variance. Thus the spread of the sensor data can be described by a probability density function (PDF). These techniques aim to find the underlying mathematically formulated relationship between system inputs, odor descriptor vectors and its outputs, classes.

Nonparametric: Nonparametric methods do not assume any specific PDF for the sensor data and thus apply more generally, such as widely used artificial neural networks (ANNS).

Supervised: In a supervised learning PARC method, a set of known odors are systematically introduced to the system. The parameters of classifier are adjusted while known odors are classified according to known classes in training data set, which is called the first stage of calibration, learning, or training. Then the second stage is to test the system containing learnt relationship and predicted class membership by an unknown odor as input to be identified. Testing a method using unclassified response vectors is well established and is often referred to as cross-validation.

Unsupervised: For unsupervised learning, PARC methods learn to separate the different classes from the response vectors routinely, discriminating between unknown odor vectors without being presented with the corresponding descriptors. These methods are closer to the way that the human olfactory system works using intuitive associations with no, or little, prior knowledge.

As summarized in Fig. 9.11, there are various multivariate data processing techniques, or PARC algorithms, which have been employed in the field of electronic nose and are explored in this chapter by describing three typical algorithms in details, PCA, KNN, and ANN.

(1) Feature extraction

Feature extraction is the key step for preprocessing odor response data. Differences in descriptor vector of multiple odors are supposed to be as distinguished as possible. The in vivo bioelectronic nose system takes advantage of olfactory system in mammals, thus descriptors of odor responses are chosen based on physiological changes in OB(OB). According to some primary findings in our experiments, firing rate and amplitude of extracellular action potential increases or decreases after odor exposure, which corresponding to the theory that neurons in OB are excitatory or exhibitory to odors. Besides, the power of action potential particularly in low frequency band increases obviously so it is another appropriate variable in descriptor vector. A neuron in OB responds to several odors with similar structure, and an odorant component are bonded by different olfactory receptor proteins which means several neurons in OB responds to an odor. That is why PARC is applied after efficient feature extraction.

(2) Principle Component Analysis (PCA)

Since neurons show highly overlapping sensitivity, the feature matrix X is expected to contain highly collinear variables. This characteristic means that the matrix X will have some dominating variables which carry most information. The aim of this method is to allow a visual approach to the problem in a reduced representative space defined by principal components. So linear combination relationships are calculated by the original feature vectors and the information in these original variables is carried in less new variables called principal components eventually. To apply this method, neural response features described in last paragraph were grouped into a response matrix X. Then we calculate eigenvalue of matrix X, which is equal to variance of new principle component. The larger eigenvalue, the larger difference in new component is. Finally, several new components with largest variance are chosen as descriptors of odor responses and all the redundancy variables are removed. The second step of data analysis, PCA is a typical data dimension reduction algorithm. Descriptor vectors in high dimension described by less dimensional feature vectors is critical in a multivariate problem.

(3) K-Nearest Neighbor (KNN)

K-nearest neighbor is a popular clustering algorithm. The classification rules are generated based on the training examples without any additional parameters. To classify a test sample, the K-nearest neighbors in the training data are found using Euclidean distance and labels the test sample with a class name by applying a majority rule among K neighbors. Accordingly, the rule applied in this study is: assume that there are m classes with a d-dimensional feature vector $X = (x_0, x_1, \ldots, x_{d-1})$ associated with each class and there are N training examples. Each jth training example can be written as $T_j\ (x_{j0}, x_{j1}, \ldots, x_{j(d-1)}) = Y$, where Y is the class label. For simplicity, each parameter is set as $m = 2$, $d = 2$, and $N = 4$, which means that there are two classes denoted by A and B, and each class has just two

attributes and four training examples in the database. The first step is to compute the distance between the query instance and each of the training samples. The distance between the query and the *i*th training sample is denoted by D_i and mathematically computed as:

$$D_i = \sqrt{(x_{i0} - u_0)^2 + (x_{i1} - u_1)^2} \tag{9.1}$$

The distance of the query sample from each training example obtained are $D1 = 4$, $D2 = 5$, $D3 = 9$, and $D4 = 2$. If K is 3, three training examples $T1$, $T2$, and $T4$ are chosen as those having the minimum distances from the query instance. In three chosen examples, two belong to class B; so the decision is that the query example belongs to class B. The class of the test sample can be determined through KNN mathematically as:

$$c = \arg_i \max \left\{ \frac{K_i}{K} \right\} i = 1, \ldots, m \tag{9.2}$$

where c is the label of the class assigned to the test sample, K_i is the number of nearest neighbors belonging to class I, and K is the total number of nearest neighbors. In another word, query example belongs to the class whose number is maxim within K-nearest examples, and the training vectors are classified in advance into m classes (Fig. 9.12).

(4) Artificial Neural Network (ANN)

As showed in Fig. 9.13, this learning algorithm that resembles the human brain process consists of a large number of processing elements also known as neurons. Interconnected processing function in parallel is associated with each neuron, and is used to map the inputs to the outputs of that neuron. Neurons are randomly assigned to one another by weights, which are adjusted by means of iterative or "learning" process. Learning means training the network by a set of examples whose output class is already known, and adjusting weights by error back-propagation, until minimum difference between actual and ideal output is obtained. The

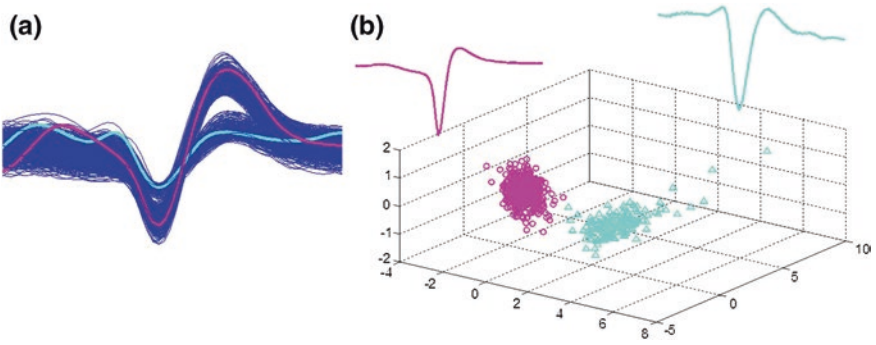

Fig. 9.12 **a** Spikes recorded by one channel of microelectrode simultaneously; **b** The result of clustering spikes in (**a**) by three steps: feature extraction, PCA, and k-means

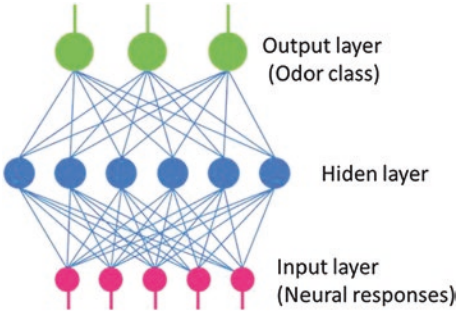

Fig. 9.13 ANN architecture, the first layer is called as neuron sensor layer; the second, hidden layer which might consist of several layers; the last, output concentration layer. Signals are transferred from input layer to output layer

ultimate combination of weights and functions is then saved as a "neural network." The ANN is a supervised method so plenty of known data is necessary to correctly train the system. The output will be unpredictable if the number of available data is not large enough. Unlike other PARC methods, a neural network is a dynamic and self-adapting system that can modify its response to external forces using previous experience, because it closely mimics mammalian neuron processing patterns responding to odors stimuli. A well-trained ANN achieves to predict which class unknown sample belongs to.

Multiple descriptors are left to identify odors more accurately even after feature extraction processing. Then ANN is the ideal algorithm to distinguish odors by training and modifying neural networks based on large amount of training data. ANNs are also an attractive approach to modeling parts of the biological nervous system, such as neural connections in OB, although ongoing research shows that this modeling is much too simple compared to the real neural circuits. Assuming that output transferred from layer to layer of built modeling is approximately predicted for any odor input, oppositely, the connection of each neuron might answer some unsolved biological questions about neural signal transmitting circuits in OB.

Signal processing in in vivo bioelectronic nose system includes four steps: feature extraction, data dimension deduction, clustering analysis, and intelligent identification. Extracting appropriate features influences the later recognition procedures significantly. The more features, the more accurate classification of odors is, but the more complicated the recognition algorithm is. Hence, the second step of principle component analysis is necessary to focus major information of odor response features in less dimensional vectors. Finally, if responses of an odor distribute together in principle components space and are regarded as from a class by clustering analysis, we train neural network by these data set. An unknown odor is predicted to a known class by recognition analysis as mentioned above and the in vivo bioelectronic olfactory system distinguishes odors accurately and efficiently.

9.4.4 *Smell Detection and Discrimination of In Vivo Bioelectronic Nose*

In mammalian olfactory system, each OSN expresses one odorant receptor but can recognize many ligand odorant molecules. In other words, OSNs are semiselective over a broad range of odors, and each type exhibits unique response pattern to different classes of odorant molecules, therefore giving rise to an odor-specific output response pattern to a given odor [73]. The M/T cells receive synaptic input from OSNs and thus generate odor-specific temporal patterns of neural discharge. In in vivo bioelectronic nose system, the recorded M/T cells were regarded as chemical gas transducers. Single M/T cells were classified based on waveform shape (Fig. 9.14a). In the absence of odors, M/T cells displayed different degrees of spontaneous activity (Fig. 9.14b). Following odor onset, the firing rate would change. Individual odors could evoke a variety of responses in different M/T cells.

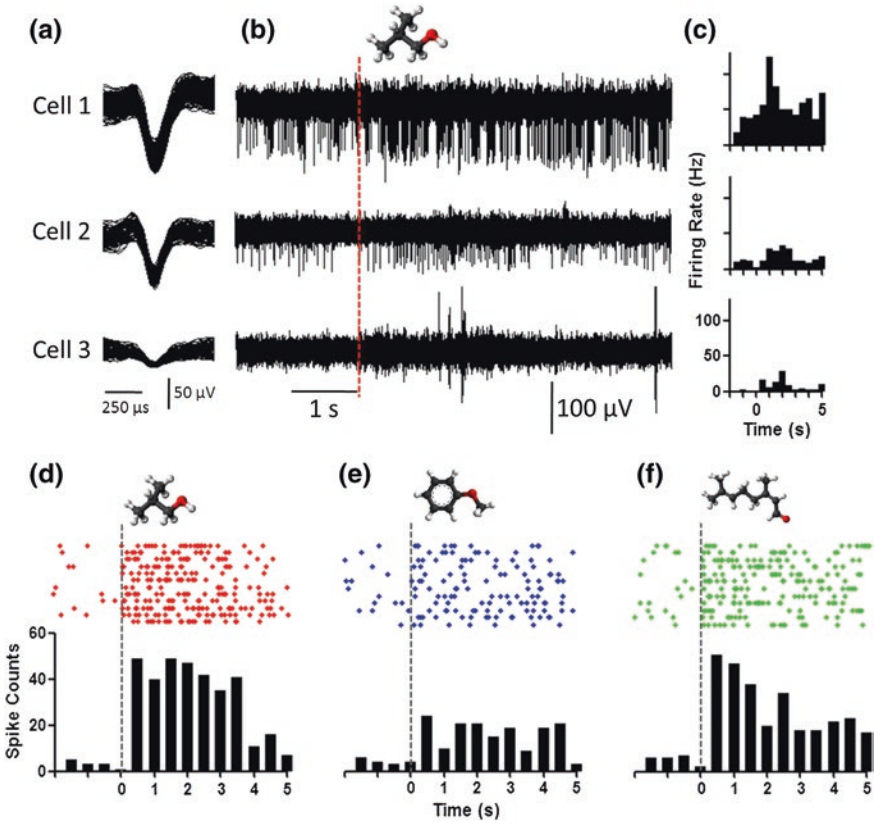

Fig. 9.14 Odorant-evoked unitary activities in a behaving rat from three channels of one microelectrode

For example, IBA causes robust excitation in some M/T cells and weak excitation in others (Fig. 9.14b, c). Thus, a given odor evokes a heterogeneous set of firing events among M/T cells.

In addition, for a single cell, different odors were able to evoke excitatory or inhibitory responses. As show in Fig. 9.14d–f, different odors (isoamyl acetate, anisole and citral) cause excitatory responses with different intensities and durations. Previous studies have reported that odorant-evoked responses can be classified as excitatory, inhibitory, and unresponsive, in some cases responding with mixture of excitation and inhibition [35, 74]. In the majority of M/T cells, we found odorant-evoked responses consisted of relatively simple odor-specific patterns dominated by either excitation or inhibition. The results demonstrated that the in vivo bioelectronic nose system has high specificity for odor detection. We focused on M/T cells with relatively simple patterns for additional analyses to evaluate odor discrimination in further study.

The response of single mitral/tufted cell expressed odor-specific spike firing pattern, such that a small number of simultaneously recorded cells carried sufficient information to discriminate odors. In fact, the responses of individual M/T cells to different odors could be remarkably similar, and different M/T cells could show similar types of responses to the same odors [75]. Further, we took advantage of this feature of M/T cells to express the original variables (multiple M/T cells activities simultaneously recorded) in lower new variables using PCA. PCA is a powerful, linear, unsupervised, and nonparametric pattern recognition technique that has been used to reduce the dimensionality of the pattern space leading to a better visualization of data clustering [76, 77].

Let $f_n(k)$ be the mean firing rate of M/T cell #n for an odor stimuli k. We defined odorant-evoked neural response (i.e., response feature) by $x_n(k) = f_n(k) - f_n(k_0)$, where $f_n(k_0)$ is the mean spontaneous firing rate of neuron before odor k delivery. The magnitude of $x_n(k)$ was calculated by subtracting the mean firing rate of spikes before odor onset from the mean firing rate of spikes that occurred (during 3.0 s) after odor onset.

If we use, for example, 10 M/T cells for odor detection (one detection can be represented as a point in a 10-dimensional space), some of them probably respond in a similar (but not identical) way. This means that the number of dimensions in the data set can be reduced without any loss of information. PCA consists of expressing the response vectors in terms of linear combinations of orthogonal vectors along a new set of coordinate axes, and is sometimes referred to vector decomposition and display multivariate data in two or three dimensions. A plot of a PCA shows what degree the different M/T cells contribute to the principal components. M/T cells with similar contributions will be close together. Figure 9.15 shows the PCA results of anisole and IAA data projected onto their first three principal components. The size of original data matrix was 17 detections (i.e., 9 trials + 8 trials of each odor, respectively) × 6 features (i.e., 6 recorded M/T cells). The first three principal components accounted for 92.0 % of the variance in the data set (PC1, PC2, PC3 accounted for 77.2, 9.1, 5.7 % of the variance, respectively). The PCA results show good recognition boundaries for the two odors, and

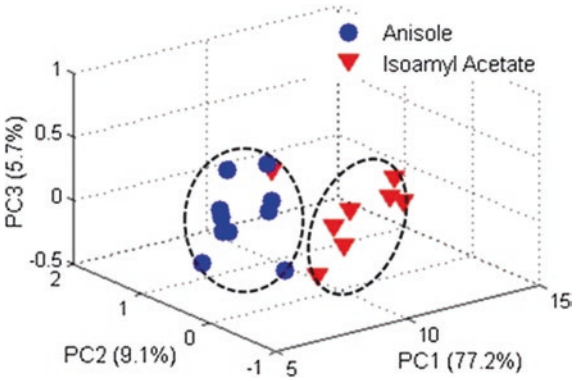

Fig. 9.15 PCA plot for the discrimination of two odors (isoamyl acetate, anisole)

only a few slight overlaps occurred at the edges. A high discrimination accuracy percentage could therefore be expected. These results indicate that in vivo bioelectronic nose system perform well in odor discrimination.

9.4.5 Application of the In Vivo Bioelectronic Nose

The odors we encounter in daily life are composed of many different odorant molecules. In practical applications, the detected odors are always natural odors that are complex mixtures of monomolecular components [78]. Although the capability of in vivo bioelectronic nose for monomolecular odors detection has been examined in our previous studies [70], little is studied about natural odor detection. Natural odors with monomolecular odors at concentrations (10^{-3} M) that smell alike and as strong as the natural odors were compared. Two different related odor pairs was used: (1) isomayl acetate and banana; (2) citral and orange. Actually, isomayl acetate ($C_7H_{14}O$) has a strong odor similar to banana [79], while citral ($C_{10}H_{16}O$) has a strong lemon odor.

Figure 9.16a shows an example of seven M/Ts recorded simultaneously from a behaving rat in response to two related odor pairs. The response of each M/T cell expressed odor-specific spike firing pattern as described above. However, the responses of individual M/Ts to different odors could be remarkably similar (e.g., cell 6 responses to isomayl acetate and banana), and different M/Ts could show similar types of responses to the same odors (e.g., cell 1 and cell 4 response to citral). That is, we regard seven M/Ts as semiselective sensor arrays for odor detection (one detection can be represented as a point in a seven-dimensional space), some of them probably respond in a similar but not identical way. This means that the number of dimensions in the data set can be reduced without any loss of information using principal component analysis (PCA). As shown in Fig. 9.16a, seven eigenvalues (odor-evoked responses of cell 1–7) were extract and analyzed by PCA. Figure 9.16c shows the PCA results of two related odor pairs data projected

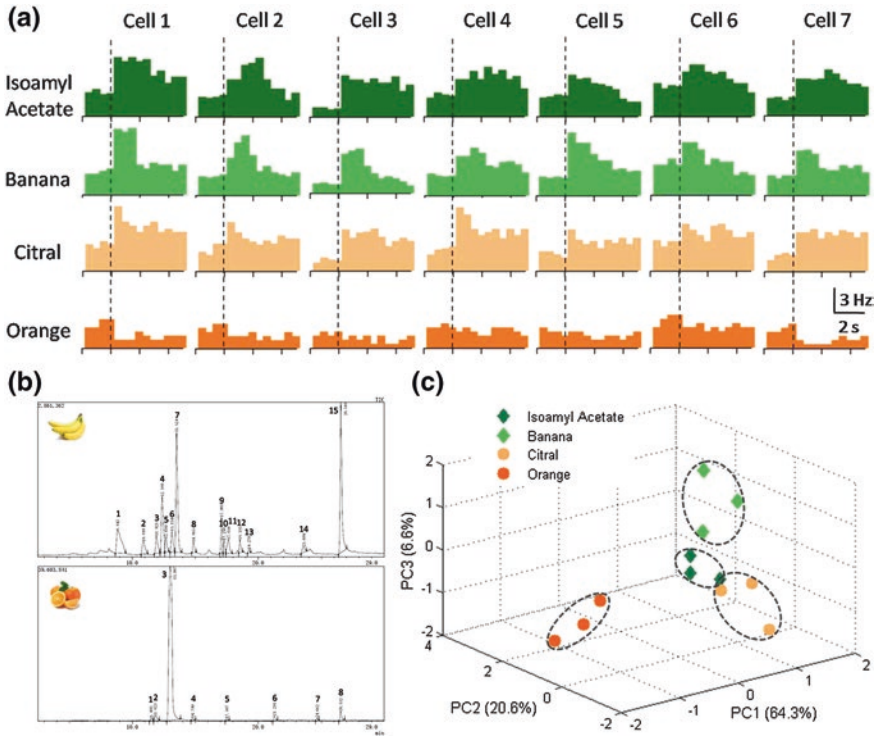

Fig. 9.16 **a** PSTHs of seven M/Ts recorded simultaneously from a behaving rat during stimulation of *isoamyl acetate*, *banana*, *orange* and *citral*. **b** The GC/MS chromatogram of volatiles released by banana (*top of graph*). **c** PCA plot for the classification of natural and its related monomolecular odor (Reproduced with permission from Ref. [67]. Copyright 2014 Elsevier B.V.)

onto their first three principal components. The first three principal components accounted for 91.5 % of the variance in the data set (PC1, PC2, PC3 accounted for 64.3, 20.6, 6.6 % of the variance, respectively). There is some slight overlapping between isomayl acetate and banana; however, citral and orange could be classified completely. Responses of monomolecular odors were representative of the simplest responses compared with complex mixtures, while the monomolecular component in natural odor can bind more different olfactory receptors. In the latter case, natural odor-evoked responses represent the nonlinear summation of multiple monomolecular odor-evoked responses [80]. As GC/MS chromatogram show in Fig. 9.4b, the monomolecular odor does not appear in the volatiles released by natural stimuli. Although the related odor pairs smell alike, they are differ in compounds and we human can discriminate them [1]. In this in vivo bioelectronic nose, natural and its related monomolecular odor was well discriminated using PCA, demonstrating that a small population of M/Ts ($n > 3$) carried sufficient information to discriminate odors.

In order to further determine the feasibility of in vivo bioelectronic nose for natural odor detection, we applied four stimuli (strawberry, pineapple, orange, banana) in this experiment. The odor-evoked response of each cell was calculated and used as eigenvalues. The size of original data matrix was 13 detections (i.e., 4 trials + 3 trials + 3 trials + 3 trials of each odor, respectively) × 7 features (i.e., 7 recorded M/Ts). The data were merged and a PCA plot was created (Fig. 9.17). The first three principal components accounted for 92.8 % of the variance in the data set (PC1, PC2, PC3 accounted for 67.7, 14.4, 10.7 % of the variance, respectively). The four odors could be well classified and a high discrimination accuracy percentage could therefore be expected. However, the methods to extract eigenvalues and preprocess raw data still need to be improved. Only then can we obtain better odor classification results. Although further data analysis and pattern recognition algorithm were not used, the in vivo bioelectronic nose presents emerging and promising potential in natural odor detection and discrimination.

The concept of in vivo bioelectronic nose system is recently proposed, and the applications of bioelectronic nose have not appeared in the literature. Our studies continue to be at the research level [81]. We also have examined the bioelectronic nose for food inspection through detecting the odorant generated from food. We simultaneously recorded the activity of five M/T cells from rats in response to stimuli spoiled for different time. The mean firing rates before and after odor stimulation were calculated and the firing rate change ratios were shown in polar plots. The discharge pattern of each cell changed due to the change of composition and concentration of volatile compounds released from food products. Thus, the collective firing rate change ratios of five M/T cells form response patterns

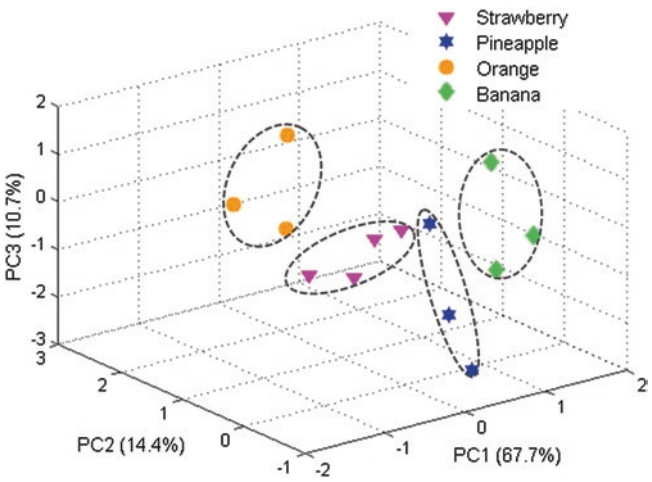

Fig. 9.17 PCA plot for the classification of four natural odors (*strawberry, pineapple, orange, banana*) (Reproduced with permission from Ref. [67]. Copyright 2014 Elsevier B.V.)

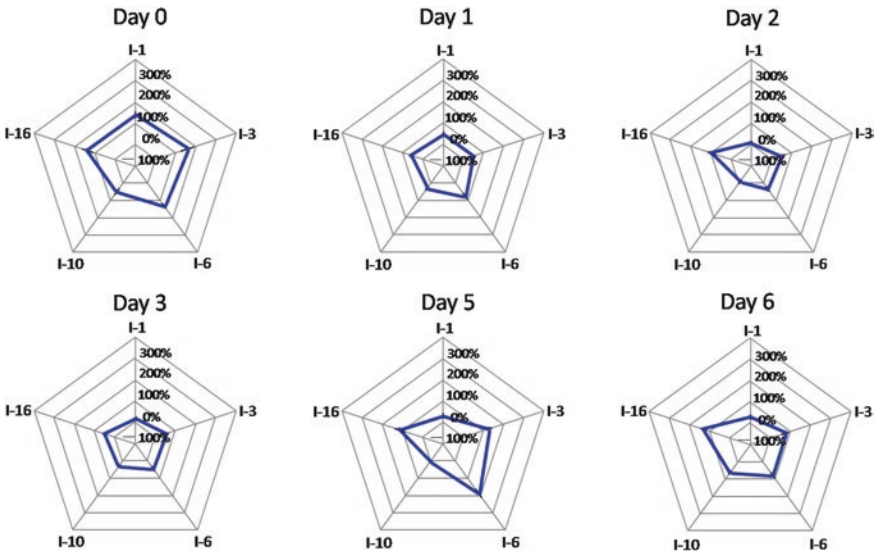

Fig. 9.18 Odor-evoked polar plots of average response of five M/T cells to cooked rice at six different storage days

according to the odor stimulation. There exists significant difference in polar plots shape evoked by fresh (day 0) and spoiled stimuli (Fig. 9.18). The response pattern changes with the length of storage.

To a certain extent, the activity of M/T cells in OB evoked by odorants could reflect the food freshness. Although further data analysis and pattern recognition algorithm were not used, the in vivo bioelectronic nose presents emerging and promising potential in determining the degree of food spoilage. In principle, both the electronic and the mammalian olfactory system operate by sensing simultaneously a high number of components giving rise to a specific response pattern. However, there are two basic differences between the mammal and the electronic nose that should be kept in mind. The electronic nose has both large differences in sensitivity and selectivity from the mammal nose. The sensors of an electronic nose respond to both odorous and odorless volatile compounds. Compared with electronic nose, we found in vivo bioelectronic nose system also has greater potential in the food industry, environmental monitoring, and medical diagnosis [82, 83].

9.5 Summary

Taking advantage of multiple microelectrode implant technology, we developed an in vivo bioelectronic nose system for odorant detection. Simultaneous recording from a number of M/T cells in conscious rats could be obtained by chronically

implanting the 16-channel microwire array electrode into the dorsal aspect of OB. The results show high-quality neuronal activity from a rat could be continuously recorded for at least 3 weeks. Based on spike discharge patterns, activity of M/T cells provided sufficient information to discriminate between odorants. Additionally, we found odorant concentrations affect the response patterns, and the rats could detect the carvone as low as 10^{-15} mol/L. For the in vivo bioelectronic nose system has characteristic of high sensitivity, continuous recording, specificity, it presents a promising platform for specific trace odorant detection in real application.

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Chapter 10
Taste Sensors with Gustatory Cells

Chunsheng Wu, Liping Du and Liang Hu

10.1 Introduction

With the fast advancements of cell-based biosensors in the past two decades, extensive amount of works have been performed in the development of gustatory cell-based taste sensors for chemical sensing that constitute an important class of cell-based biosensors [1–4]. Development of gustatory cell-based taste sensors has attracted more and more interest and become a popular research topic mainly due to their promising prospects and potential applications in many fields such as biomedicine, drug and food industry, and environmental protection. Biological, medical, or chemical processes involving complex chemical signal detection usually require very high detection spaces and performances. This is often a huge challenge for conventional sensitive materials (e.g., lipid membranes) [5–7]. Functional components originated from biological taste systems may provide a novel source of sensitive elements for the development of gustatory cell-based taste sensors. Furthermore, the advancements in the research of taste transduction mechanisms can also make a significant impact on the development of biomimetic gustatory cell-based taste sensors for chemical sensing. By imitating the biological mechanisms of taste systems, many kinds of gustatory cell-based taste sensors have been developed by the coupling of functional gustatory cells with various secondary sensors.

Gustatory cell-based taste sensors utilize the unique capability of gustatory cells to recognize a target molecule and to convert the recognized signals into cellular responses that can be detected by the transducers. Due to the unconventional

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using of gustatory cells as sensitive elements, gustatory cell-based taste sensors show distinct advantages such as fast response, high sensitivity, and excellent specificity. Recently, a variety of functional gustatory cells have been increasingly adopted in developing various gustatory cell-based taste sensors. The functional gustatory cells can be classified into two main categories: (1) primary gustatory cells isolated from the taste buds of animals, which contain native gustatory receptors responsive to chemical signals presented by various tastants [8–13], as well as (2) cell lines that heterologously expressed with functional gustatory receptors and usually co-expressed with essential molecules(e.g., α-gustducin) for intracellular taste signal transduction, which contribute to the transduction of chemical signals recognized by gustatory receptors to the cellular responses [14, 15]. It is worth to note that another type of cell-based biosensors have also been developed for investigating the mechanisms of taste bud cell-to-cell communications, which can detect local ATP or 5-HT secretion from single gustatory receptor cell by precise manipulation of biosensor cells closely to target cells [16–19]. Cell-based biosensors provide a novel and valuable tool for the research of taste transduction mechanisms.

Since the introduction of cell-based biosensors, intensive research efforts have been devoted in developing more reliable and biocompatible to facilitate the cells coupling to transducer surface. Gustatory cell-based taste sensors also show intensive demand on biocompatible sensor surface in order to obtain reliable and reproducible cellular responses. At present, majority of the works on sensor surface modification are based on the physical approach such as physisorption of biocompatible materials (e.g., poly-l-ornithine and laminin (PLOL)) [20, 21]. This could substantially improve the viability of gustatory cells cultured on sensor surface as well as enhance the cells coupling to transducers. In order to interpret the detected cellular response signals and describe the signal transduction mechanisms, some models are also proposed or modified based on cell–chip interface model. Some data processing methods and algorithms have also been employed for better understanding and presenting the mechanisms of gustatory cell-based taste sensors (e.g., principal component analysis (PCA)).

Despite the growing interests and potential applications of gustatory cell-based taste sensors, there are so far limited number of literatures specifically focused on the most recent progress in this field. It is thus necessary to make a comprehensive summary over various gustatory cell-based taste sensors. In the present chapter, we focus on gustatory cell-based taste sensors that combine functional gustatory cells with various transducers for the detection of different tastants as well as for the research of taste signal transduction. We also address issues such as preparation of functional gustatory cells, coupling of cells with transducers, and data processing as well as cell–chip interface models. In addition, cell-based biosensors applied in the research of taste signal transduction and gustatory cell-to-cell communications will also be discussed.

10.2 Fundamental of Gustatory Cell-Based Taste Sensors

10.2.1 Gustatory Cells for Chemical Sensing

Biological taste system is a kind of chemical senses that can detect the chemical substances presented by tastants, which provide very important information about the nature and quality of food. Figure 10.1 shows the structure and organization of taste buds [22]. Taste buds are the special organisms for taste sensation, which are distributed in tiny papillae on the lingual epithelium. One tiny apillae usually contains one to hundreds of taste buds. Taste buds are dominated with 50–100 gustatory cells that are around 50 μm in length and 5 μm in width. Gustatory cells can be classified into four categories based on their ultrastructural feature, which are type I (dark), type II (light), type III (intermediate), and type IV gustatory cells [23]. Gustatory cells with small microvilli at their apex are usually gustatory receptor cells (TRCs), which are taste sensation elements for five basic taste qualities including sweet, bitter, sour, salty, and umami [24, 25]. Small microvilli contain the specific gustatory receptors and are sensitive to various tastants dissolved in saliva. These microvilli are collectively exposed to external saliva via taste pores. It is reported that various gustatory receptors are expressed only in a different subsets of TRCs, which makes different TRCs show different functions and properties. This expression pattern may contribute to the encoding of taste information [24, 26]. TRCs bear the properties of neurons, which are a kind of specialized epithelial cells. TRCs are able to convert the taste signals into cellular responses through intracellular signal transduction pathway. The cellular responses include the generation of cellular membrane potential changes and the releasing of special neurotransmitter [27].

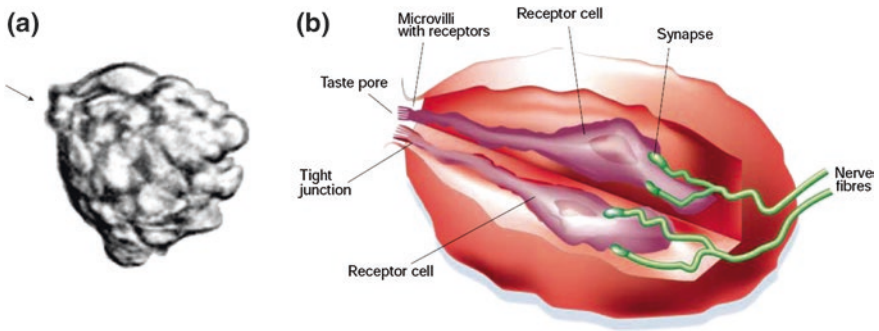

Fig. 10.1 Structure and organization of rat taste buds. **a** The isolated viable taste bud (30 μm in length) with taste pore (*arrow*); **b** schematic diagram of a taste bud in cut-open view shows two receptor cells with apical microvilli and basolateral synapses (Reproduced with permission from Ref. [22]. Copyright 2001 Nature Publishing Group)

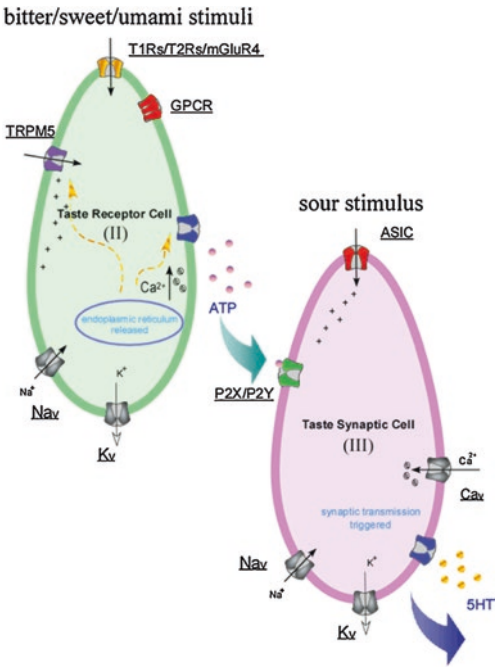

Fig. 10.2 The scheme of intracellular taste signal transduction and gustatory cell-to-cell communications via neurotransmitters (Reproduced with permission from Ref. [10]. Copyright 2011 Elsevier)

Figure 10.2 shows the intracellular taste signal transduction pathway in type II and type III cells as well as the cell-to-cell communications between them [10]. In taste buds, type II cells are responsible for the detection of sweet, bitter, and umami. Type II cells lack the classic synaptic connection with afferent nerves, which transduce the taste signals by the releasing of adenosine triphosphate (ATP) [28]. It has been demonstrated that ATP is secreted from type II gustatory receptor cells (TRCs) through specific hemichannels in response to electrical stimulation [16, 17] and chemical stimulation [18, 19]. In type II cells, taste signals are transduced via a special intracellular signal transduction pathway. Gustatory receptors can bind to the specific tastant molecules, which located on the membrane of TRCs. Gustatory receptors belong to the family of G protein-coupled receptors (GPCRs). The specific binding of gustatory receptors with their target taste molecules can activate the specific G proteins, which will initiate a cascade of intracellular enzymatic reactions. For the following, the activation of specific G proteins will result in the activation of PLC-β2, which will lead to the generation of inositol triphosphate (IP3). IP3 is able to bind IP3R3 and lead to the release of Ca^{2+} from intracellular Ca^{2+} stores. The released Ca^{2+} can activate TRPM5 and finally resulting in the secretion of ATP. The secretion of ATP from Type II cells plays crucial roles in taste bud cell-to-cell communications, which constitute a very important pathway for the taste signal transmission from taste buds to the central nervous system [28]. The ATP secretion can be detected by the P2X2 and P2X3 receptors located in the membrane of the afferent nerve endings [16, 18].

Moreover, the secreted ATP is able to stimulate type III TRCs to release another neurotransmitter, serotonin (5-HT) [19]. The taste signals are transmitted via the afferent fibers, including the chorda tympani, glossopharyngeal nerve, and vagus, to the brainstem, where the taste signals are further transmitted to the gustatory cortex via the specific neuronal projection. Type III cells form classic synaptic connection with the taste afferent nerve fibers, which can generate membrane potential changes in response to sour stimuli and transmit these taste signals to the taste afferent nerve via synaptic connection [29]. Type I and type IV cells show no distinct sensing functions and are considered as a kind of supporting cells and basal cells, respectively [30]. The basal cells usually distribute near the bottom of the taste buds, which can finally differentiate into the other types of gustatory cells.

TRCs can respond to various taste stimuli with extreme high sensitivity, specificity. In addition, TRCs inherit the features of neurons, which make the cellular responses fast and measurable by various potentiometric sensors. All these unique powerful capability make TRCs ideal candidate to be used as sensitive elements for the development of gustatory cell-based taste sensors.

10.2.2 Transducers for Extracellular Recording of Gustatory Cells

With the fast advancements of microfabrication technology in past two decades, a wide range of extracellular recording sensors or chips can be manufactured, which provide novel approaches for the development of new generation of cell-based biosensors. This also opened up an exciting realm for the research and application of gustatory cell-based taste sensors [31]. At present, various potentiometric sensors have been applied in the cell-based biosensors for extracellular recording, including microelectrode array (MEA) [32, 33] and field effect transistor (FET) [34–36]. Both MEA and FET have been used for the monitoring of extracellular potential changes, which can realize the long-term cellular measurement in a non-invasive way. However, the limited and discrete recording spots of MEA and FET have hampered their applications in the development of gustatory cell-based taste sensors. The main challenge is the control of target cell position on sensor surface to satisfy the need for extracellular recording. For example, it is necessary to culture target cells exactly on the gate-electrode of individual FET or the tip of individual microelectrode of MEA in order to obtain successful extracellular recording of cellular responses.

On the other hand, light addressable potentiometric sensor (LAPS), which is another commonly used extracellular recording chip, have been widely adopted in the development of gustatory cell-based taste sensors due to its added advantages compared with FET and MEA [21, 37]. LAPS can select the desire measurement spot by a movable focused light beam illuminated on the sensor surface, making

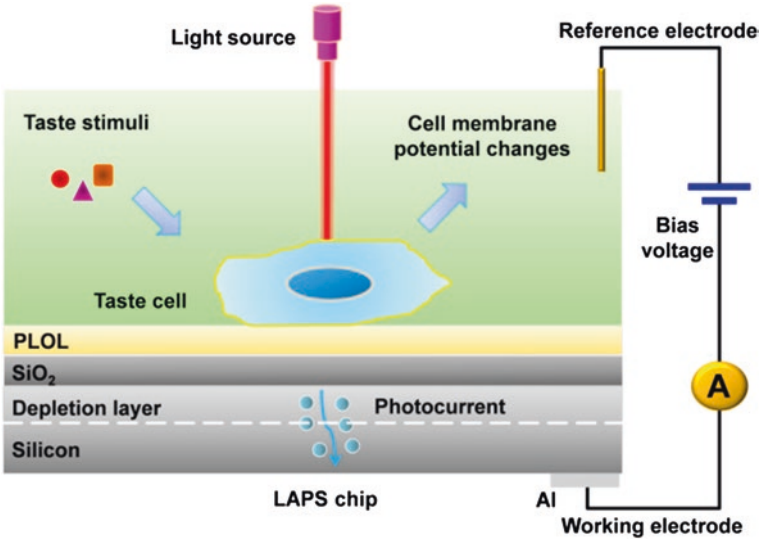

Fig. 10.3 Schematic diagram shows the detection mechanisms of gustatory cell-based taste sensors using LAPS chips as secondary sensors for extracellular recording of gustatory receptor cells in response to taste stimuli

it possible to overcome the intrinsic geometry limitations of MEA and FET. In addition, LAPS also have the advantages of simple structure, low cost, and easy integration. These features have make LAPS favorable for the development of gustatory cell-based taste sensors for the monitoring of gustatory cell membrane potential changes in response to various taste stimuli. Figure 10.3 shows the basic mechanisms of LAPS chips used for extracellular recording of gustatory receptor cells in response to taste stimuli. LAPS chip is a kind of surface potential sensitive device, which has the structure of electrolyte insulator [SiO_2] semiconductor [Si] (EIS). When the focused light with a certain wavelength illuminates on one spot of the LAPS chip, the corresponding region of semiconductor will absorb the light energy, which will result in the generation of electron–hole pairs within the illuminated region. In this case, the semiconductor does not generate the photocurrent due to the rapid recombination of electron and hole. The surface potential changes are not able to be measured by the monitoring of the photocurrent. At this time, if a bias voltage is applied to the LAPS chip to make it in depletion, the semiconductor will generate the photocurrent that can be measured by the peripheral circuits. In addition, the photocurrent is related to the width of the depletion layer, which is in turn related to the bias voltage. The local surface potential changes can be considered as an additional voltage added to the bias voltage, which can subsequently result in the changes in photocurrent. This makes it possible to measure the surface potential changes by monitoring the changes in photocurrent, which can be readout by the peripheral circuits. When the TRCs are cultured on the sensor surface, the cell membrane potentials are coupled to the sensor surface potentials.

Once the TRCs are stimulated by the specific taste stimuli, the cells will respond to the stimuli by giving the corresponding cell membrane potential changes. This will finally lead to the generation of photocurrent fluctuation within the semiconductor. Therefore, the responses of TRCs to taste stimuli can be measured by monitoring the photocurrent changes. In addition, due to the light addressability of LAPS, it is possible to select the desire cells for measurement by illuminated the focused light on the target TRCs that coupled to the sensor surface. By this method, the chemical information of taste stimuli can be converted into measurable electrical signals.

In addition to potentiometric sensors, electrochemical sensors have also been applied in the development of gustatory cell-based taste sensors. Electrochemical sensors were initially developed for the detection of biomolecules and have been widely applied in the medical and clinical diagnostics, which can realize highly sensitive, specific, and real-time measurements for the interactions between biomolecules [38–43]. Furthermore, electrochemical sensors have decisive advantages on miniaturization and small sample size, making them suitable for the development of devices for in field applications. Due to all these advantages, electrochemical sensors have shown emerging potential in cell-based biosensors. For example, electrical cell-substrate impedance sensing (ECIS) sensors have become very promising and attractive devices for the development of cell-based biosensors that can be applied in many fields such as toxicity evaluation and drug industry [44–46]. In principle, electrochemical sensors for cell-based biosensors are based on the impedance changes resulting from cell morphological changes induced by various cellular stimulating substances. Cell morphological changes could be the results of cell adhesion, spreading, and motility on the sensor surface, which could in turn change the electrode impedance. This makes it possible to detect the cellular responses by measuring the changes in electrode impedance. By culturing the gustatory cells on the surface of electrochemical sensors, impedance measurements can monitor the cellular morphological changes in response to various taste stimuli. Based on this mechanism, electrochemical sensors have been successfully used for the development of gustatory cell-based taste sensors, which allow for the specific detection of sweet and bitter substances in a quantitative manner [14, 15]. Electrochemical sensors are becoming promising devices for the research and development of gustatory cell-based taste sensors, which broaden the area of gustatory cell-based taste sensors and contribute to the miniaturization and improvements on detection efficient.

10.2.3 Modeling of the Gustatory Cell Electrophysiological Recording

Taste sensation is one of the most complex chemosensations for recognizing and discriminating chemicals in environment. TRCs hold the features of neurons that

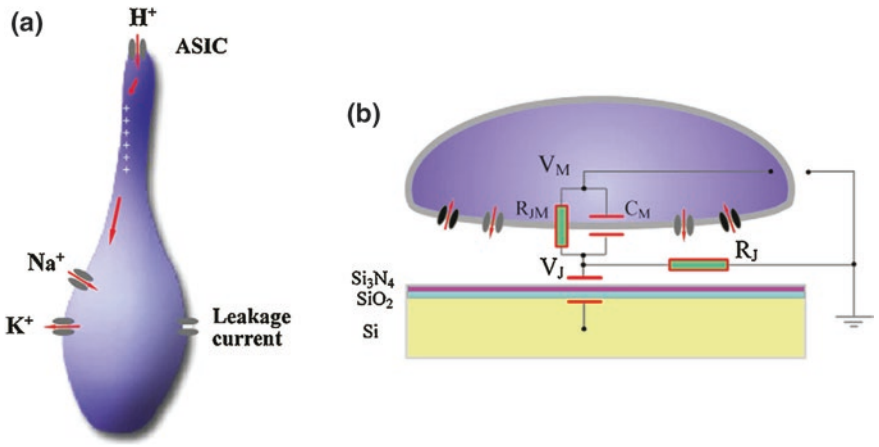

Fig. 10.4 **a** A Hodgkin–Huxley type model to simulate mechanisms of sour taste transduction. **b** The equivalent circuit of cell-LAPS electric coupling. V_M is the intracellular potential. V_J is the extracellular potential. C_M is the cellular membrane capacity. R_{JM} is the cell membrane resistance. R_J is the sealing resistance between cell and chip (Reproduced with permission from Ref. [12]. Copyright 2009 Elsevier)

can not only convert the chemical signals into the electrical signal of membrane potential changes, but also can realize the primarily processing of the sensed taste signals. It has been demonstrated that TRCs can generate the membrane potential changes when stimulated by various taste stimuli such as sour, sweet, bitter, and salty tastants. In addition, the firing patterns of TRCs are related to the taste modality encoding. Much effort has been devoted in exploring the underlying mechanisms of taste signal transduction and much progress has been achieved. Electrophysiological recording of TRCs has been proved to be an efficient approach for the research of taste transduction mechanisms. However, the detailed mechanisms of TRCs responding to taste stimuli is still far from completely understood. Therefore, when TRCs are employed as sensitive elements for the development of gustatory cell-based taste sensors, the modeling and simulation of TRCs in response to taste stimuli based on the electrophysiological recording are required to interpret the recording signals and describe the cell–sensor interface. This plays an important role both in the basic research and practical applications of gustatory cell-based taste sensors, which could help to deploy the biological transduction mechanisms of taste sensation and solve the key questions in taste sensing encoding mechanisms.

In theory, some of the cell–sensor coupling models have been employed to explain the signal pathway of extracellular recording. Cell–electrode electric coupling is usually adopted for the modeling of cell-based biosensors for extracellular potential recordings. This is also suitable for the case of gustatory cell-based taste sensors that are developed on the basis extracellular electrophysiological recordings. Figure 10.4b shows the equivalent circuit of cell-LAPS electric coupling

[12]. In this coupling model, C_M represents the cellular membrane capacity. V_M and V_J are intracellular and extracellular potentials, respectively. R_J is the sealing resistance that represents the leakage current between cells and microelectrodes. When TRCs are stimulated by the taste stimuli, receptors or ion channels located in cell membranes will change their status and result in the generation of transmembrane current. This will lead to the cell membrane potential changes. Due to the cells are attached on the sensor surface, the cell membrane potential can couple to the sensor and change the electric field. This will consequently lead to the reestablishment of charge at the interface between electrode and electrolyte. By this signal transduction pathway, the cell membrane potential changes are transferred to the electrical sensor signals, which can then be readout by the peripheral circuits for signal amplification and further signal processing.

Hodgkin–Huxley type models are commonly used to quantify the transmembrane currents originated from ion channels and recorded from whole cell patch clamp. These kinds of models have been applied to create models of gustatory cell-based taste sensors for acid detection developed on the basis of LAPS. The main components of this model include voltage-gated sodium currents (I_{Na}), delayed rectifier outward potassium currents (I_K), nonspecific leakage current (I_l), and currents through acid-sensing ion channels (I_{ASIC}), as illustrated in Fig. 10.4a. When the cell is attached on the LAPS surface, a thin film of electrolyte will form between the cell and LAPS chip surface. Due to the separation of silicon and cytoplasm by insulation layers (e.g., SiO_2, Si_3N_4, and lipid membrane), this model was defined as Sandwich cable model [47]. Based on the model, the calculated simulation results are in good agreement with the experimental results for gustatory cell-based taste sensors for acid detection.

10.3 Design of the Gustatory Cell-Based Biosensors

10.3.1 Gustatory Cell Preparation and Characterization

In order to use gustatory cells as sensitive elements for the development of gustatory cell-based taste sensors, it is necessary to achieve sufficient functional gustatory cells that can not only respond to specific taste stimuli, but also convert the taste signals into measurable cellular responses. At present, the main sources for achieving functional gustatory cells can be classified into two main categories: (1) primary TRCs directly isolated from taste buds of animals, and (2) bioengineered cell lines that expressed with specific gustatory receptors (usually co-expressed with special molecular components for intracellular taste signal transduction).

Sprague Dawley (SD) rats are the most commonly used animals for the preparation of primary gustatory cells for the purpose of developing gustatory cell-based taste sensors. Gustatory cells are isolated from the epithelium of the dissected rat tongue according to a well-documented procedure [48]. Elastase/collagenase enzyme mixture solution is usually employed for the separation of

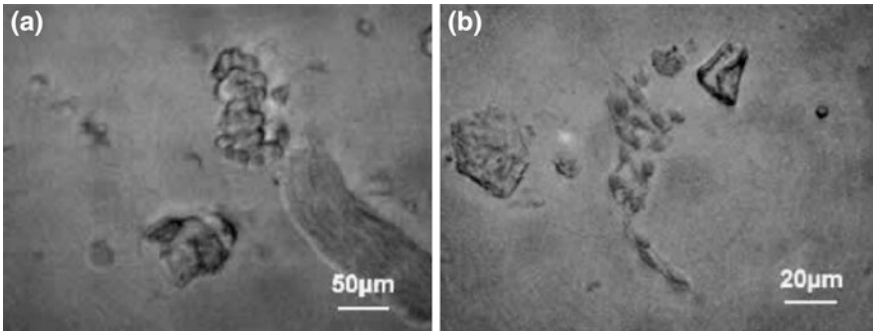

Fig. 10.5 **a** Primary taste bud and **b** gustatory receptor cells isolated from rats cultured on the surface of LAPS chip pretreated with PLOL for improving the cell attachment (Reproduced with permission from Ref. [13]. Copyright 2008 Elsevier)

lingual epithelium from the underlying connective tissue of tongue. Taste bud cells are dissociated from epithelium using a petridish, which contain various types of gustatory cells including TRCs that can respond to specific taste stimuli. Then the gustatory cells can be transferred to cell culture medium and cultured on the sensor surface, which are often pretreated with a thin layer of biomaterials to improve the biocompatibility of sensor surface and allow the formation of enhanced attachment between cells and sensor surface. The most commonly used biomaterials include the PLOL mixture (100 μg/ml poly-l-ornithine and 8 μg/ml laminin mixed by the rate of 1:1), and Cell-TAK. The gustatory cells are usually needed to be maintained on the sensor surface at 37 °C under standard conditions of humidified air with 5 % CO_2. The time for gustatory cells to form well coupling with sensor surface are varied from 2 to 7 days, which are mainly depended on the properties of sensor surface as well as the cell culture conditions. Figure 10.5 shows the results of primary taste bud and gustatory receptor cells cultured on the surface of LAPS chip [13].

To characterize the cells cultured on the sensor surface, biological methods are usually applied to make sure that the specific type of gustatory cells are functionally available on the sensor surface. Immunofluorescent staining can be adopted for the purpose of determining if the desired types of gustatory cells are cultured on the sensor surface using special fluorescent-labeled antibody that can recognize the specific gustatory receptors [10, 15]. On the other hand, calcium imaging can be used for the functional assays of gustatory cells for the detection of specific taste stimuli, which uses calcium indicators to show the calcium status of a living cell allowing investigation of the cell activity. The main advantage of primary gustatory cells is the easily preparation from animals. However, it is not easy to control the gustatory cell types that contain the desired gustatory receptors, which makes the repeatability of the gustatory cell-based taste sensors very low. In addition, the amount of primary gustatory cells is limited by the number of living animals.

In order to overcome the drawbacks of primary gustatory cells, bioengineered cell lines expressed with defined types of gustatory receptors have been used for the development of gustatory cell-based taste sensors. The human enteroendocrine NCI-H716 cells expressed with sweet gustatory receptors (GPCRs, gust TIR2 plus T1R3) have been applied in the development of a sweet gustatory cell-based biosensor, which can detect different concentrations of sucrose solutions [15]. The human enteroendocrine STC-1 cells, expressing G protein-coupled receptors and bitter receptors (type 2 members) have been used as sensing devices for the construction of gustatory cell-based taste sensors for bitter detection [14]. In principle, bioengineered gustatory cell lines can be used for unlimited times once the stable cell line that express the desire gustatory receptors is achieved. Moreover, the cell lines could provide more stable cellular responses, which can contribute greatly to the repeatability of gustatory cell-based taste sensors. But the screening process of stable cells that can continuously express gustatory receptors is usually time-consuming and the expression efficiency of gustatory receptors in a heterologous cell system is often low. These disadvantages have hampered their practical applications and gradually become the bottle-neck of bioengineered gustatory cell-based taste sensors. In the future, the main research focus of this field could be how to achieve functional gustatory cells in a more convenient and efficient way to meet the need for the development of gustatory cell-based taste sensors.

10.3.2 Sensor Design and Fabrication

In gustatory cell-based taste sensors, sensors play important role in the detection of cellular responses of gustatory cells to various taste stimuli. Different gustatory cells have different requirements on the sensor design and fabrication. For example, potentiometric sensors are suitable for the electric excitable cells, while electrochemical sensors are more sensitive to the cell morphological changes. It is crucial to choose appropriate sensors according to the responsive properties of gustatory cells.

As for primary gustatory cells that are electric excitable cells, LAPS chip is the most often used sensor for the extracellular recordings of action potentials originated from gustatory cells under different status. LAPS chip can monitor signal gustatory cell responses by illuminating the focused light on the desire single gustatory cell. LAPS chip has pretty simple structure (usually with EIS structure) and the special design is usually not necessary to meet the requirement of gustatory cell measurement. LAPS chip can be fabricated by conventional microfabrication process on the basis of silicon wafer [13, 21]. A layer of SiO_2 with thickness about tens of nm is thermal oxidized on the silicon surface to serve as the insulation layer. Sometimes, a layer of Si_3N_4 about tens of nm is further deposited upon the SiO_2 layer to achieve more stable insulation layer. In addition, the sensor sensitivity can be enhanced by reducing the thickness of silicon layer. A layer of Al is evaporated on the back side of silicon wafer to create an ohmic contact for the

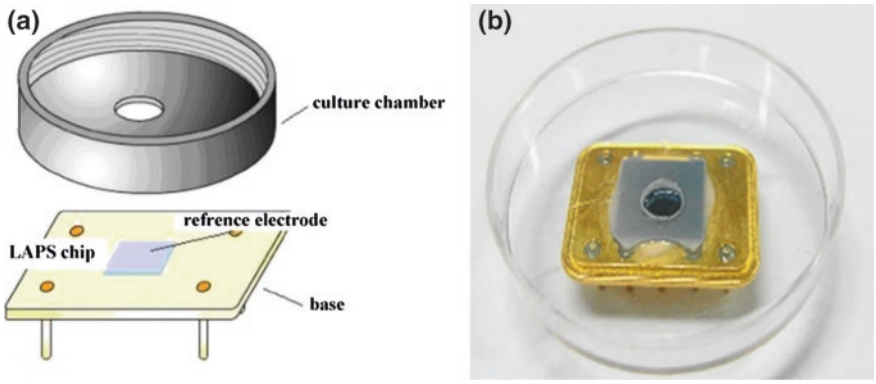

Fig. 10.6 **a** Schematic diagram shows the package of LAPS chip. (Reproduced with permission from Ref. [13]. Copyright 2008 Elsevier) **b** LAPS chip packaged with a cell culture chamber (Reproduced with permission from Ref. [9]. Copyright 2009 Elsevier)

output of sensor signals. When the chip is fabricated, it is necessary to be packaged with a cell culture chamber that is usually made by poly(dimethylsiloxane) (PDMS). The chamber should have no toxic effect or other influences on gustatory cells cultured on the sensor surface. Figure 10.6 shows the sketch of LAPS chip package and a packaged LAPS chip with a cell culture chamber [9, 13].

Moreover, a reference electrode that can reach the inside of the cell culture chamber is necessary for the detection of action potentials from gustatory cells as well as for the application of bias voltages to the LAPS chip. LAPS chip have shown promising potentials in the development of gustatory cell-based taste sensors, especially for the extracellular recording of single gustatory cell.

For the cell lines expressed with gustatory receptors, which are nonexcitable cells, electrochemical sensors are employed for the detection of cellular responses based on the impedance changes of the interface between gustatory cells with sensor surface. A commercially available electrochemical sensor named carbon screen-printed electrode (CSPE) has been used for the measurement of gustatory cell lines expressed with sweet receptors or bitter receptors in response to various sweet or bitter substances (Fig. 10.7a) [14, 15]. This electrochemical sensor is usually used in combination with a flow cell, which is also commercially available and can provide suitable environments for cell culture on sensor surface as well as for the introduction of various taste substances via solution (Fig. 10.7b). Electrochemical sensors have been proved to be an efficient tool for the nonexcitable gustatory cell measurements.

10.3.3 Measurement Setup and Data Processing

For the gustatory cell-based taste sensors, there are two types of sensors are used, which are potentiometric sensors and electrochemical sensors, respectively.

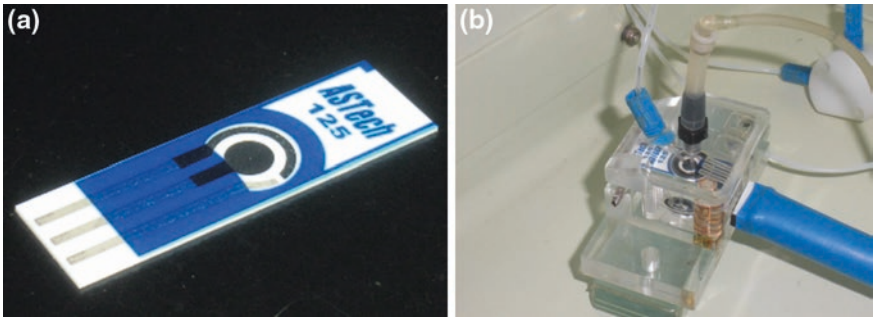

Fig. 10.7 **a** Photo of a carbon screen-printed electrode (CSPE). **b** Flow cell for CSPE (Reproduced with permission from Ref. [15]. Copyright 2012 Elsevier)

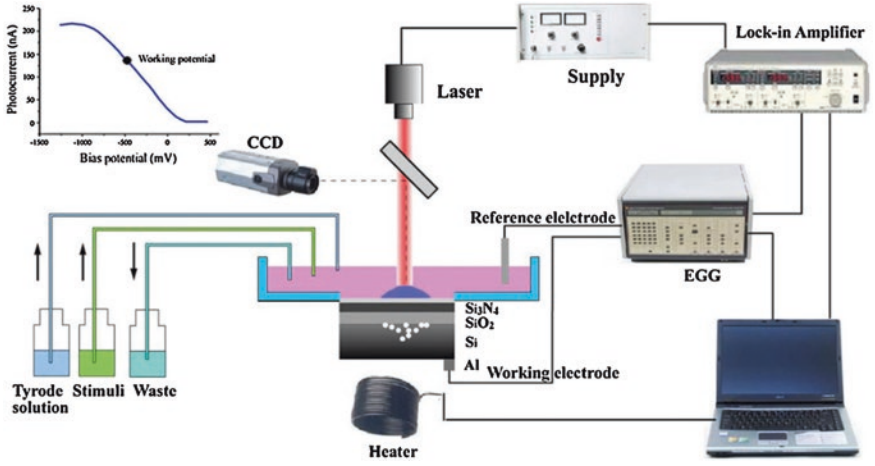

Fig. 10.8 The LAPS measurement setup developed for extracellular recordings of single gustatory receptor cell in response to various taste stimuli (Reproduced with permission from Ref. [9]. Copyright 2009 Elsevier)

Because the most commonly used sensors in gustatory cell-based taste sensors are LAPS chips and CPSE sensors. The measurement setup for both LAPS chips and CPSE sensors will be briefly introduced here. Due to the complex of cellular responses of gustatory cells to taste stimuli, various data processing method have been used in order to show more clearly the cellular responses. Here, we will also introduce some methods for processing the data originated from measurement of gustatory cell-based taste sensors.

Figure 10.8 is the schematic diagram of LAPS measurement setup for extracellular recordings of single gustatory receptor cell in response to various taste stimuli [9, 12]. The main functions of LAPS setup include the recording of LAPS chip signals, amplification of the recorded signals, and provide the bias voltage

for LAPS and supply for the modulated light source. In order to select the desired gustatory cells for measurement, the LAPS chip with cultured gustatory cells is fixed under a microscope, by which the light can be focused and illuminated on the desire single gustatory cell. The He–Ne semiconductor laser was one of the most often used light sources, which can generate the modulated light not only with a certain wavelength but also with very small power. The diameter of the focused light is usually less than 10 μm allowing for the single cell measurement. The potentiostat and lock-in amplifier are usually used to provide bias voltage for LAPS chip and to amplify the recorded signals, respectively. The data collection card and the special software for data storage and analysis are also needed to be included in the LAPS setup. Due to the frequency of action potential are usually high, the sampling rate of the setup should be higher enough in order to achieve good signals without distortion. In addition, the whole setup is packaged in a mental shielding box in order to keep the constant temperature and humidity during measurement (37 °C for cell measurements) as well as to minimize the influences of environmental factor on measurements.

Peak value extraction method was utilized to analyze the LAPS extracellular recording data from the gustatory cell-based taste sensors [9]. By this method, the firing spikes elicited by taste stimuli can be obtained. Basically, the recording signals when no taste stimulus was applied were considered as the reference signals, whose mean value was used to quantify the baseline noise level. Then a threshold was set to discriminate the firing spikes from noise, which is usually 2–3 times of the baseline noise level depending on the experimental conditions and recording data. The spikes that the amplitude is higher than the threshold can be extracted and used for further data analysis. There are three main analytical parameters used to analyze the temporal firing signals recorded from gustatory cells, which are mean firing rate, peristimulus time histograms (PSTHs), and interspike interval histograms (ISIHs). Mean firing rate is the total number of spikes elicited by taste stimulus in a second. PSTHs are the histogram of the times when cells fire, which can visualize the firing rate and timing of gustatory receptor cell in response to a stimulus. ISIHs are the distribution of the intervals between spikes, which can unravel the patterns of spike activities. In order to discriminate different taste stimuli, PCA is usually employed to process the firing spikes recorded by LAPS chip from gustatory cells in response to various taste substances. For example, PCA was used to discriminate different bitter substances by analyze the recording data from taste-based biosensor in response to three different bitter stimuli, which include $MgSO_4$, denatonium, and D-(-)-salicin [11].

The measurement setup developed for gustatory cell-based taste sensors on the basis of electrochemical sensors usually include four parts: the electrochemical sensors cultured with gustatory cells, fluidic system for cell culture and stimulation, signal detection and amplification equipment, and data collection and signal processing unit [14, 15]. In the measurement setup for CSPE, flow-injection analysis (FIA) combined with three peristaltic pumps was employed for controlling the different solutions to be introduced to the detection chamber. The potentiostat (EG&G 273A) was used to measure the interface impedance changes

between gustatory cells and electrochemical sensor surface. High-performance dual phase analog lock-in amplifier 5210 was utilized to amplify the sensor signals. The whole setup was shielded in a mental box to reduce the influences of electromagnetic interference on measurement. As for the data processing, a signal-to-noise ratio (SNR) method based on stochastic resonance was adopted in order to improve the SNR level of detected cellular signals by applying an additional noise to a nonlinear system, which has been proved to play important roles in data processing, especially for the weak signal detection. This method was first introduced by Benzi in 1981 to explain the dynamic mechanisms of Earth's ice age, which described the small periodic perturbations could be greatly amplified by a large environmental fluctuations [49]. It has been widely used in many fields such as biological modeling, neural systems, and electrical circuits [50].

10.4 Application of Gustatory Cell-Based Biosensors

10.4.1 Research on Taste Signal Transduction Mechanisms

Biological taste systems have extreme high performance to recognize and discriminate environmental chemical signals presented by taste substances, which can provide valuable information on nature and quality of food. In the recent decade, considerable amount of effort has been devoted into the research of taste signal transduction mechanisms. Much progress has been achieved, which provide deep insights into the mechanisms underlying the taste sensation. However, the taste signal transduction mechanism is still not completely understood due to the complications of taste sensation as well as the lack of powerful and convenient tools for the research of taste transduction. Patch clamp have been widely used in the research of taste transduction mechanisms, which is the golden standard of cellular electrophysiology. But it has been proved to be expensive, invasive, and complicated to use, which hamper its applications in cellular physiology. The development of cell-based biosensors provides a promising alternative approach for the research of taste transduction, which can realize noninvasive, long-term, and low cost measurements on gustatory cells. Various sensors have been applied in the development of cell-based biosensors including the FET and MEA sensors. But both of them required to culture the desire cells on the discrete active sites of sensor surface (e.g., the gate-electrode of individual FET and the tip of individual microelectrode), which makes cell culture really difficult due to the random distribution of cells on sensor surface. Therefore, LAPS is favorable for the development of gustatory cell-based taste sensors for the research of taste transduction due to some obvious advantages of LAPS compared with FET and MEA including the simply structure and no cell position requirements.

Gustatory cell-based taste sensors developed on the basis of LAPS chip have been used to investigate the electrophysiology property of living TRCs in response to the five basic taste substances including NaCl, HCl, $MgSO_4$, sucrose, and

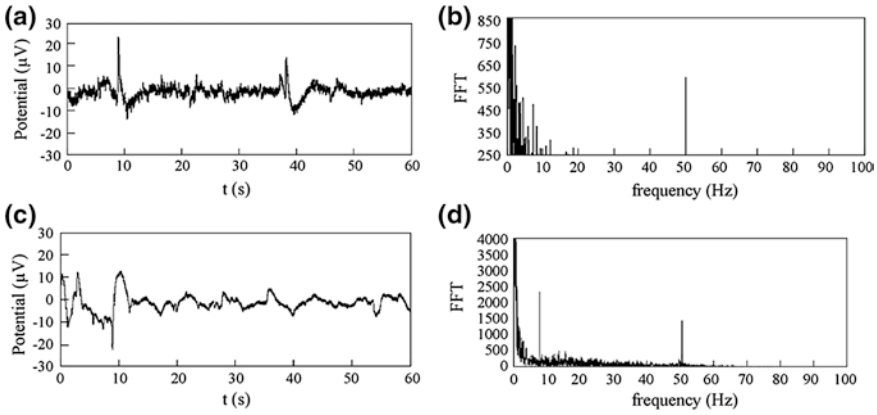

Fig. 10.9 LAPS extracellular recordings of gustatory receptor cells in response to **a** culture medium and **c** tastants mixture. (**b**) and (**d**) are results of signal processing with FFT from (**a**) and (**c**), respectively (Reproduced with permission from Ref. [13]. Copyright 2008 Elsevier)

glumate [13]. Taste buds as well as TRCs isolated from Sprague Dawley rats were cultured on the LAPS surface, which was confirmed by the immunofluorescence imaging. LAPS chip is able to record the extracellular potential changes of taste buds and TRCs cultured on its surface. As shown in Fig. 10.9, the results indicate that this gustatory cell-based biosensor can respond to taste mixture containing five basic taste substances mentioned above. In addition, a specific frequency component of 7–10 Hz was found to be related to the cellular responses to taste stimuli, which may reflect the sensitive property that conveys the taste information like taste intensity or taste modality. This study proves that gustatory cell-based taste sensors are feasible to be applied in the research of taste sensation mechanisms.

In another study, similar gustatory cell-based taste sensors were further used for the research of cell-to-cell communications between different types of cells to explore taste sensation and analyze taste-firing responses [9]. Rat TRCs were culture on the surface of LAPS chip to serve as target cells for the investigation of cellular responses under various taste stimuli as well as neurotransmitter. LAPS chip can efficiently record the temporal firing responses of TRCs under different stimulations (Fig. 10.10). The results obtained from the gustatory cell-based taste sensors are in good agreement with that of from patch clamp and molecular biology experiment. The results show that the firing rate of TRCs is dependent on the concentration of stimulus. The PCA analysis on the temporal firing responses indicates that different types of TRCs show distinguishable cellular responses. In addition, the exogenous ATP, which play important role in taste bud cell-to-cell communications, was used to stimulate TRCs to mimic the effects of transmitter ATP released from type II cells onto type III cells. The results show that both enhancive and inhibitory effects of ATP on spontaneous firing of TRCs can be successfully observed, which were evaluated by means of PSTHs and ISIH. This

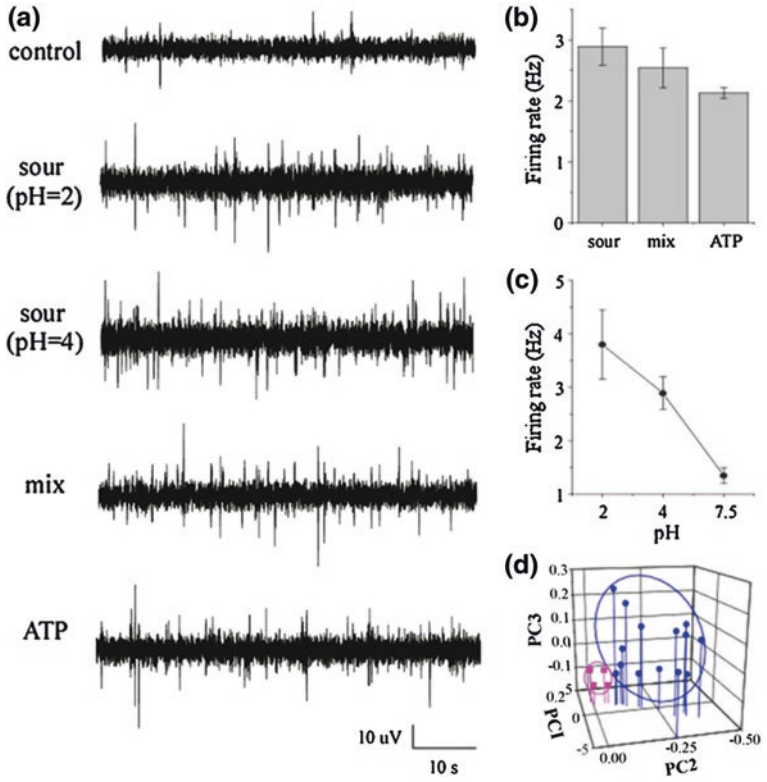

Fig. 10.10 Responses of gustatory cell-based taste sensors in response to various taste stimuli monitored by the extracellular recording of LAPS chip. **a** LAPS recorded firing spikes of gustatory cells upon different taste stimuli, which include sour (HCl), mix (bitter ($MgSO_4$), sweet (sucrose), umami (monosodium glutamate) tastants, and exogenous ATP. **b** Firing rate of gustatory cells upon different stimuli. **c** The relationship of firing rate with pH value of sour tastants. **d** PCA analysis of the temporal firing responses to sour (*blue*) and ATP (*red*) (Reproduced with permission from Ref. [9]. Copyright 2009 Elsevier)

suggests that gustatory cell-based taste sensors can not only be used for specific taste sensation and transduction mechanism, but also for the research of gustatory cell-to-cell communications.

10.4.2 Acid Detection

A subset of taste bud cells is acid-sensing TRCs that is sensitive to acidic substances and is able to convert the acidic chemical signals into cell membrane potential changes. Acid-sensing TRCs contain specific membrane ionic channels that can interact with protons, which are major sources of acid stimuli. Various

ionic channels have been demonstrated contributed to the transduction of acidic chemical signals, which include ASIC2a/2b (acid-sensing ionic channels) [51] permeable to Na^+ and H^+, PKDL (polycystic kidney disease-like) channels [52] permeable to Ca^{2+} and H^+, and HCN (hyperpolarization-activated cyclic nucleotide-gated cation channels) [53]. The acidic ionic channels can be activated by H^+ and generate the transmembrane current, which can depolarize the gustatory cells and finally lead to the generation of action potentials. The unique feature of acidic sensitive TRCs makes them ideal candidate for the development of gustatory cell-based taste sensors for the detection of acidic chemical signals. Acid sensitive TRCs have the properties of neurons, which can realize the acid chemical signal transduction by the generation of specific action potentials. The firing pattern of action potentials can be recorded and analyzed to indicate the relationship between the cellular responses and acidic stimuli.

Based on neuron–silicon interface, gustatory cell-based taste sensors that are sensitive to acidic stimuli have been developed on the platform of LAPS setup that is similar as mentioned above [12]. Acid sensitive TRCs originated from rat were cultured on the surface of LAPS chip. The cellular responses originated from single acidic sensitive TRC were monitored by extracellular recording of LAPS before and after acidic stimuli (HCl) were introduced. The recorded signals were further analyzed and extracted out in both time domain and frequency domain to reveal the characteristic features of recorded firing spikes of TRCs and to distinguish the responses of TRCs to acidic stimuli. The results indicate that LAPS can efficiently record the cellular responses and all the responsible cells can be categorized into two types: with high signal-to-noise firing spikes in time domain (type I); with low signal-to-noise response (type II). In order to decipher the LAPS recorded signals and achieve more insights into the mechanisms of acid sensation, a Hodgkin–Huxley (H–H) type model of mammalian gustatory receptor cells was constructed based on patch–clamp experimental recordings to simulate the action potentials of TRCs in response to acidic stimuli. In this model, three corresponding ionic components are included, which are voltage-gated Na^+ currents, outward delayed rectifier K^+ currents, and transduction current upon sour stimulus. An extension to neuron–silicon interface is also included to help explain the characteristic patterns of extracellular signals obtained by gustatory cell-based taste sensors. The results demonstrated that the temporal firing and characteristic features of acid sensitive TRCs can be successfully recorded by LAPS chip, the simulation results are in good agreement with the experimental results (Fig. 10.11). This gustatory cell-based biosensor has great potential to be used a novel and valuable tool for the detection of acidic substance as well as for the research of acid sensation mechanisms.

10.4.3 Sweet and Bitter Detection

In taste buds, cells can be classified into four types according to their morphological features. Among them, type II cells are responsible for sensing and

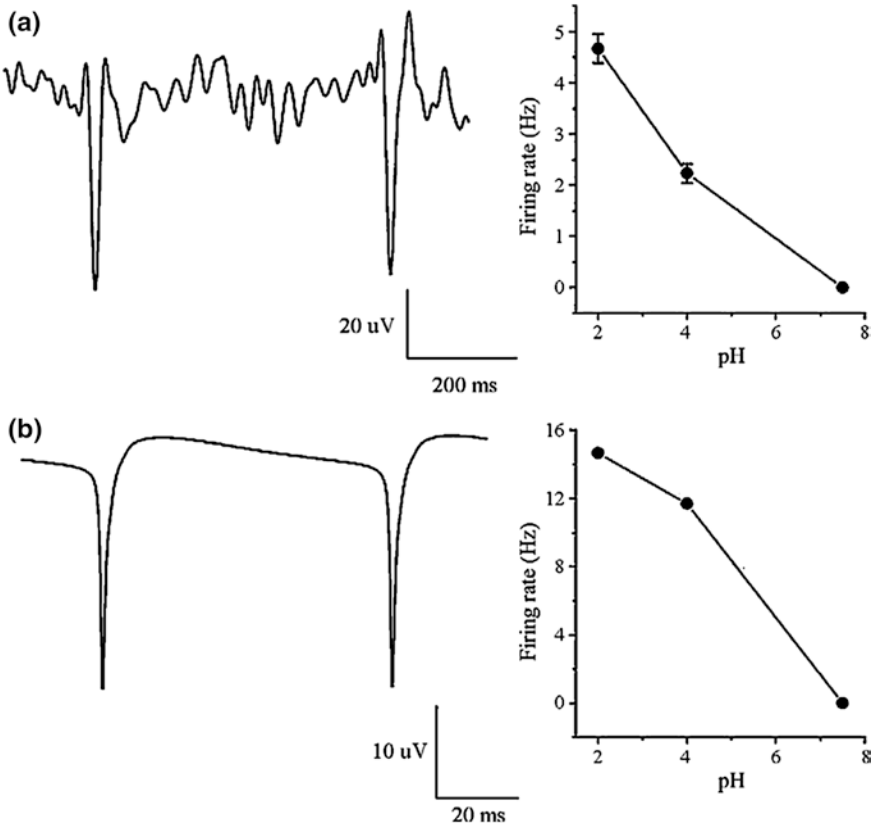

Fig. 10.11 **a** Typical extracellular signals recorded by LAPS chip from gustatory cells in response to sour stimuli (*left*). The firing rate of gustatory cells upon different sour stimuli with various pH values. **b** The simulation results of the extracellular potential (*left*) and the firing rate (*right*) (Reproduced with permission from Ref. [12]. Copyright 2009 Elsevier)

transduction of sweet, bitter, and umami signals. Type II cells transmit the taste signals to other taste bud cells via ATP releasing instead of membrane potential signals due to the lack of classic synaptic connection with taste afferent nerves. Gustatory receptors for sweet and bitter detection are seven transmembrane G protein-coupled receptors (GPCRs). Sweet compounds are detected by the combinations of T1R family members, which form T1R2/T1R3 heteromers on the cell membrane [54–57]. On the other hand, bitter compounds are detected by T2R family members, which consist of 25 members in humans and 34 members in the mouse [58–61]. With limited types of bitter receptors, mammalian taste systems can detect thousands of different bitter compounds. The unique powerful capability of TRCs for sweet and bitter detection have been employed for the development of gustatory cell-based taste sensors for the detection of specific sweet and bitter compounds.

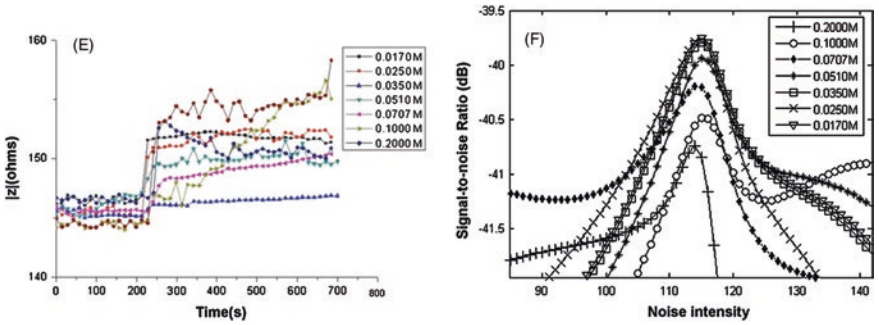

Fig. 10.12 Responses of NCI-H716 cell-based sensor to sucrose in 7 concentrations (*left*) and the corresponding BSR processed responses (Reproduced with permission from Ref. [14]. Copyright 2010 Elsevier)

A sweet gustatory cell-based biosensor has been developed on the platform of electrochemical impedance measurement for the detection of four basic tastants and sucrose solutions in seven concentrations [15]. Sweet gustatory receptors, T1R1/T1R3, together with α-gustducin, were expressed on the human colorectal carcinoma NCI-H716 cell lines. The bioengineered cells were cultured on the surface of carbon screen-printed electrode (CSPE), which was the pretreated with PLOL to enhance the cell attachment. The electrochemical impedance spectrum measurement was carried out to monitor the responses of this biosensor to four basic tastants and sucrose solutions. The results indicate that NCI-H716 cell-based biosensor can specifically responded to sucrose more intensively than other for basic tastants (Fig. 10.12). In addition, four basic tastants and different concentrations of sucrose can be distinguished. On the contrary, the negative control (COLO-205 cell lines without gustatory receptor expression) shows no response to the tastants tested. In addition, based on the same electrochemical measurement platform, gustatory cell-based taste sensors have also been developed for the detection of bitter compounds, in which human enteroendocrine STC-1 cells expressed with bitter receptors were used as sensitive elements [14]. The experimental results show that this biosensor can respond to the specific bitter substances, while the negative control cells lack the similar responses. The gustatory cell-based biosensor is proved to be a valuable and promising method for sweet and bitter compound detection as well as for the research of sweet and bitter signal transduction mechanisms.

LAPS chip has also been applied in the development of gustatory cell-based taste sensors for the detection of different bitter compounds [11]. Primary TRCs isolated from the taste buds of rats were cultured on LAPS surface and used as sensitive elements, which can respond to different bitter stimuli with extreme high sensitivity and specificity. LAPS chip was used as a secondary transducer to monitor the single cell responses due to its light addressable capability. In addition, LAPS chip can realize the noninvasive measurement in real-time for a long-term. The extracellular recordings of TRCs by LAPS proved that this gustatory

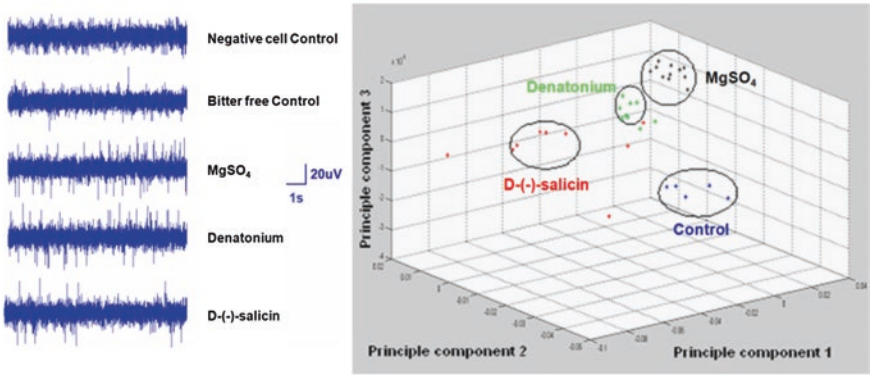

Fig. 10.13 Typical extracellular recordings by LAPS chip from gustatory cells in response to different bitter stimuli (*left*). PCA analysis of LAPS recordings was carried out to discriminate different bitter stimuli (*right*) (Reproduced with permission from Ref. [11]. Copyright 2012 World Scientific Publishing)

cell-based biosensor can respond to different bitter stimuli including $MgSO_4$, denatonium, and D-(-)-salicin (Fig. 10.13). Different responses can be efficiently discriminated by PCA analysis on the LAPS recording data. This gustatory cell-based biosensor shows promising prospects and potential applications in bitter compound detection, which may provide a valuable tool for functional assays of bitter receptors to study the interactions between bitter molecules and bitter receptors. This may also contribute to the bitter signal transduction as well as to the prediction of any compound's bitterness.

10.4.4 Taste Neurotransmitter Detection

TRCs can detect the taste signals and transmit the detected taste signals to other taste bud cells via the cell-to-cell communications between taste bud cells. It have been demonstrated that ATP and serotonin (5-HT) play an important role in taste signal processing and transmission. ATP has been demonstrated to be a pivotal extracellular neurotransmitter in various tissue and organs, which is conventionally considered as a universal energy source for all cells [62–64]. ATP is also proved to be the key afferent neurotransmitter in taste buds, which is essential for taste signal transmission of type II gustatory cells that lack the classic synaptic connections with afferent taste nerve [28]. ATP is secreted from type II gustatory cells through specific hemichannels in response to electrical stimulation [16, 17] and chemical stimulation [18, 19]. On the other hand, 5-HT is released by type III gustatory cells when stimulated by sour tastants or ATP released by type II gustatory cells, which is essential in taste information transmission from TRCs to taste afferent nerve [65, 66]. Type III cells are the only type of cells that form synaptic connection with the

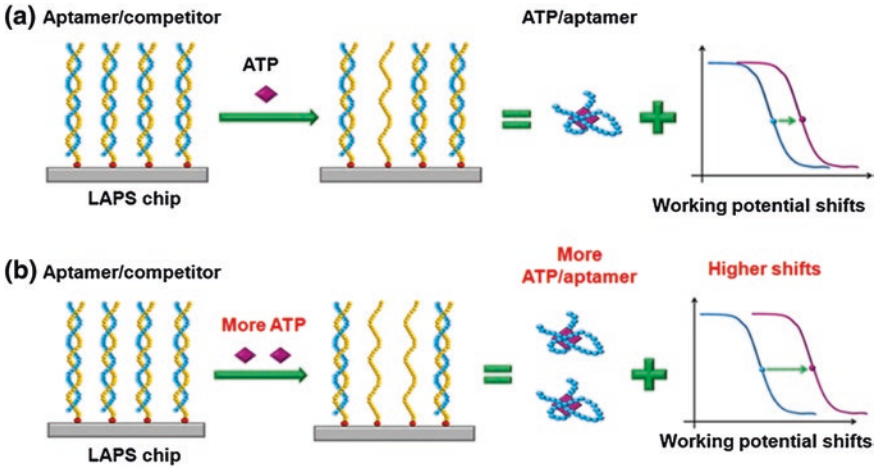

Fig. 10.14 Schematic diagram shows the detection mechanisms of ATP-sensitive aptamer-based biosensor for the detection of local ATP secretion from single gustatory cells. Working potential shifts of LAPS in response to **a** a certain ATP concentration and **b** higher ATP concentration (Reproduced with permission from Ref. [8]. Copyright 2012 Springer)

taste afferent nerve that can finally transmit the taste signals from taste buds to the brain. Due to the important functions of ATP and 5-HT in taste buds for taste signal transmission and processing, various cell biosensors have been developed for the detection of neurotransmitter secretion for TRCs to explore the detail mechanisms of ATP and 5-HT in gustatory cell-to-cell communications, especially for the measurement of ATP and 5-HT releasing from single TRC.

The bulk concentration of ATP secretion from taste buds can be detected by stand luciferin–luciferase assay [28]. But this method is limited due to the differences between bulk ATP concentration in taste buds and local ATP concentration in the vicinity of the cell surface. To address this problem, ATP-sensitive cell-based biosensors have been developed and used for the detection of local ATP secretion from single TRC [16–19]. However, some intrinsic difficulties have hampered their practical applications. For example, the preparation of ATP-sensitive cells is tedious and time-consuming. This method requires the precise manipulation of biosensor cells very close to the desire target cells for local ATP measurement.

ATP-sensitive aptamer has been applied in the development of simple and convenient method for ATP detection due to its decisive advantages such as high stability, high performance, and low cost [67]. The combination of ATP-sensitive aptamer and LAPS chip has been proposed for the development of ATP-sensitive biosensors for single gustatory cell extracellular monitoring [8]. ATP-sensitive DNA aptamer was utilized as sensitive element for ATP detection, while its DNA competitor (with full complementary sequence to ATP aptamer) was covalently immobilized on the LAPS surface to serve as signal transduction element (Fig. 10.14). Benefit from the light addressable capability of LAPS, this biosensor

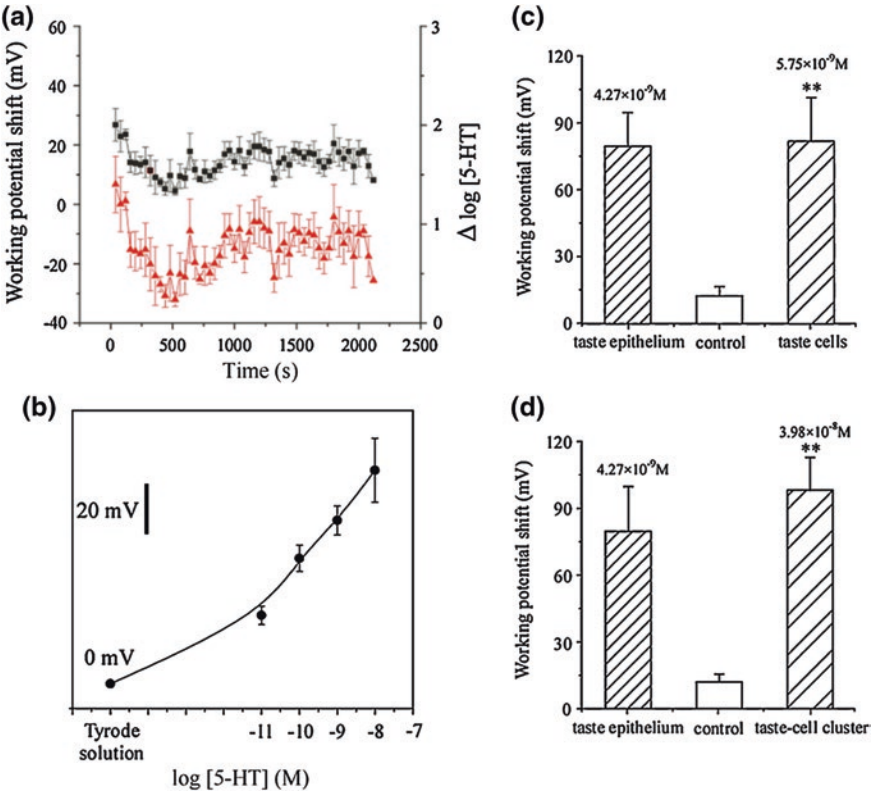

Fig. 10.15 **a** Stability of 5-HT sensitive LAPS chip and **b** responses to different concentrations of 5-HT. The detection of 5-HT released from gustatory cells and epithelium upon **c** sour and **d** mix tastant (Reproduced with permission from Ref. [10]. Copyright 2011 Elsevier)

is able to measure the local ATP secretion from single cell by illuminating the focused light on the desire single gustatory cell. The local ATP concentration was detected by recording the working potentials shifts of LAPS. The results show this biosensor can effectively detect the local ATP secretion from single TRC isolated from rats in response to tastant mixture. This biosensor can be used as a novel tool for ATP detection secreted from gustatory cells as well as other types of ATP secreting individual cells. This biosensor also provides a novel and valuable tool for the research of cell-to-cell communications.

The standard method for 5-HT detection is HPLC (high-performance liquid chromatography), which has been applied in the detection of 5-HT secreted from taste organs [68]. This method has good selectivity and high sensitivity. But the HPLC instruments are usually complicated, inconvenient, and expensive. Cell-based biosensor are also an efficient method for the detection of 5-HT released from single gustatory cell, in which HEK 293 cells transfected with 5-HT

receptors are used as sensitive elements [16, 19, 66]. However, the successful 5-HT detection requires the biosensor cells to be cultured adjacent to the targeted gustatory cell cluster, which makes this method difficult and limit its practical applications.

A 5-HT sensitive biosensor has been developed for the detection of 5-HT released from single gustatory cell as well as from taste epithelium [10]. A poly(vinyl chloride) membrane was deposited on the LAPS surface and used as sensitive elements for 5-HT detection, which consists of special ion-exchanger and solvent mediator [69]. The detection limit of this sensor was as low as 3.3×10^{-13} M. The sensitivity is 19.1 mV per concentration decade. This biosensor have been successfully applied in the detection of 5-HT released from gustatory cells (Fig. 10.15). The measurement results are consistent with those from other methods. Immunofluorescent imaging verified the functional existence of type II and III gustatory cells on LAPS surface, which were labeled with the corresponding specific antibodies. This 5-HT sensitive biosensor shows good performance for 5-HT detection and could be a potential and promising method for 5-HT detection as well as for the research of the mechanisms of taste signal transduction and gustatory cell-to-cell communications.

10.5 Summary

The increase of using functional cells, especially the gustatory cells, becomes the recent trend of constructing cell-based biosensors for chemical sensing due to the unique features of gustatory cells in chemical signals transduction. The unique capability of converting the taste signals into cellular responses make gustatory cells more and more attractive to be used as sensitive elements for the detection of specific taste substances. Gustatory cells have been employed in the development of different types of gustatory cell-based taste sensors by combining with various secondary sensors. This chapter first introduced the basic theory of cell-based biosensors in three aspects, which are biology and electrophysiology of the gustatory cells, extracellular recording of gustatory cellular responses, and modeling of the gustatory receptor cell electrophysiological recording. Then the design principle of gustatory cell-based taste sensors was discussed in the following three aspects including gustatory receptor cells preparation, sensor design and fabrication, and sensor setup and data processing. Finally, the most recent progress in the applications of gustatory cell-based taste sensors were summarized, which include taste signal transduction mechanisms, acid sensation, sweet and bitter detection, and cell biosensors for taste neurotransmitter detection.

Gustatory cell-based taste sensors have shown great potential for chemical sensing, providing novel and valuable method for the detection of specific taste compounds as well as for the research of taste signal transduction mechanisms. The ultimate goal of the gustatory cell-based taste sensors is to offer high sensitivity and specificity essential for the detection of specific chemical substances in

a complex background. Advancements in bio- and nanotechnologies, micro fabrication techniques, and new information technology, are mainly responsible for the improvements in gustatory cell-based taste sensors. In particular, the studies on taste transduction mechanisms can not only provide deep insight into the underlying mechanisms of taste sensation, but also advance the development of gustatory cell-based taste sensors. The progress in taste transduction could contribute to the solving of some key problems for the development of gustatory cell-based taste sensors, such as how to achieve sufficient functional gustatory cells in a more cost-effective and convenient way, how to improve the sensitivity and specificity in a biomimetic approach. Contributions from nanotechnologies and microfabrication process could allow for gustatory cell-based taste sensors to be further developed into sensor arrays and biochips containing different types of gustatory cells, which can perform rapid, multiplexed, and high throughput analysis and characterized with miniature, integration, and intelligence.

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Chapter 11
Gustatoty Epithelium-Based Taste Sensors

Diming Zhang and Qingjun Liu

11.1 Introduction

For sensors mimicking biological taste sensory, many achievements have been made in liquid detection with special-sensitive lipid/polymer membrane [1–6]. Notably, a series of sensors were developed by Toko's group to evaluate beer, tea, and food by discriminating several basic tastants [7, 8]. However, the sensitivity and selectivity of detection using these electronic tongues were lower than those of biological taste sensation, which mainly lies in the biological receptor structures and information coding mechanisms. Thus with advancements in tissue culture protocols, tissue-based biosensors were developed to mimic biological taste sense for analyzing the functional information of taste substances by treating living units as sensing elements [9–11]. Recently, Ozdener and Rawson proposed a method for primary culture of mammalian gustatory epithelium, which provides a useful model for molecular studies of the proliferation, differentiation, and physiological function of mammalian gustatory receptor cells [12]. The cultured tissue can keep taste sensitivity and electrophysiological activity, which can be recorded and analyzed in pattern recognitions. Although the cultured gustatory tissue loss the three-dimensional structure of the intact taste bud, the study opens a great starting for potential application of gustatory tissue in biosensor for taste detections.

Gustatory epithelium is sensory epithelial tissue containing specialized cells within taste buds, which plays a role in biological taste receptions for a wide range

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of both simple and structurally complex molecules [12]. Over last couples of years, the applications of molecular biology, electrophysiology, and genetic were carried out to understand and mimic biological taste sense on gustatory epithelium [13–15]. Now, it is known that taste buds constitute basic gustatory receptor units on surface of gustatory epithelium. They were often associated with fungiform papillae on the anterior two-thirds of the tongue, and circumvallate and foliate papillae were on the posterior third of the tongue. During biological taste sensory, the taste response was initially coded in taste buds on gustatory epithelium by action potentials. The first stage of coding was specific interaction of taste substances with their receptors and ion channels in taste buds, where a complex series of events follows taste stimulation, when the generations of potentials were culminated in the receptor cells [16–18]. Different from single gustatory cells, taste buds were the natural gustatory cell networks, comprising all types of gustatory receptor cells. Therefore, the whole intact gustatory epithelium, preserving natural state of the taste buds, will be one kind of good candidates for the sensitive elements of the taste sensors.

In this chapter, we introduce gustatory epithelium-based biosensors which can detect five basic taste qualities including sour, sweet, bitter, salt, and umami. Extracellular potentials of gustatory receptor cells can be recorded with hybrid structure of microelectrodes and gustatory epithelium, and analyzed by spatiotemporal signal process. With these engineering efforts, the gustatory epithelium-based biosensors can be used to recognize five basic taste qualities, discriminate analyte belong to same taste, and detection for taste substance concentrations.

11.2 Theories of Gustatory Epithelium-Based Biosensors

11.2.1 Structure and Electrophysiology of Gustatory Epithelium

Gustatory epithelium-based biosensors employed electrophysiological signals of gustatory epithelium as responses to taste stimuli [14, 19]. For all five basic tastes, a taste bud of the epithelium initially coded the responses of gustatory receptor cells to tastants by action potentials. Molecular studies implied that individual taste qualities were encoded by different gustatory receptor cells and transduced by different ways, which can be discriminated at the receptor cell level. To be more specific, evidence has demonstrated that Na^+ ion was the source of biological salt perception. The ions entered the receptor cells directly via epithelial-type Na-channels (ENaCs), yielding the depolarization of gustatory receptor cells (TRCs) [20, 21]. While in sour perception, the proton (H^+) can block, permeate or activate ion channels that allowed other positive ions to enter the cells and resulted in the depolarization and neurotransmitter release [13, 22]. Bitter stimuli acted through G-protein-coupled T2R receptors and second messengers which caused the release of calcium ions leading to depolarization and neurotransmitter release [23]. Sweet tastants bond to dimeric receptors (T1R2 and T1R3) that were coupled

to G-proteins, which activated the second messengers that closed potassium channels indirectly [24, 25]. Umami stimuli bond to G-protein-coupled dimeric receptors (T1R1 and T1R3) and activated second messengers, which led to the release of packets of neurotransmitters [26–28]. That is to say, sweet, bitter, or umami tastants were all detected by different G-protein-coupled receptors (GPCRs) and transduced by a common signaling pathway, while sour and salty tastes were mediated by different ion channels [29, 30]. These different spatiotemporal properties provided a possibility to discriminate taste quality by electrophysiological response signals of TRCs on gustatory epithelium to taste stimuli.

11.2.2 3D Tissue Electrophysiological Recording

The electrophysiological recording for tissue culture in vitro was an important method for the study of disease mechanisms and drug efficacy. Compared to animal models and cadaveric tissues, tissue cultured in vitro can provide a systematic, repetitive, and quantitative electrophysiological recording with state best mimicking physiological functions in vivo [31]. The most commonly used tissue culture for in vitro electrophysiological study was the monolayer of cells. Although the electrophysiological recording for two-dimensional (2D) tissue has made significant contributions to biological researches, it has certain intrinsic limitations. Thus, several groups have proposed the development of three-dimensional (3D) in vitro tissue cultures for electrophysiological recordings [32–34]. Compared to 2D tissue for electrophysiological recording, the 3D tissue provides electrophysiological activities that more closely mimics the microenvironment observed in native tissues. This feature was critical for fabrication of excellent biosensors using gustatory epithelium for taste detection.

11.2.3 Electrophysiological Recording on Microelectrode Arrays

Microelectrode arrays (MEAs), in a noninvasive and long-term manner, can monitor electrophysiological activities of gustatory cells from multiple network sites with high spatial and temporal resolution, and thus are a promising detecting method to cellular electrophysiology researches. In recent years, a number of studies have reported that cells and tissues were cultured on surface of MEAs in vitro for various applications in pharmacology, toxicology, developmental biology, and basic research [35–38]. With advances in micro-electromechanical systems (MEMS), remarkable progress has been reported at most bioelectronic levels, but the electrode–tissue contacts remain one of the major obstacles. In recent years, MEAs fabrication techniques have been tried to obtain consistent recording signals from small groups of neurons without losing microstimulation capabilities.

For instance, Robinson et al. developed a nanowire electrode arrays to quantify the strength of individual synaptic connections and also scalable enough to simultaneously measure and control a large number of mammalian neurons with single-cell resolution [39]. For gustatory epithelium-based biosensor, natural epithelium can be employed as a biological sensitive element to keep biological structure and properties of taste buds, while maintaining advantages of in vitro experiment such as controllable experiment conditions.

11.3 Design of Gustatory Epithelium-Based Biosensors

11.3.1 Microelectrode Array Preparation

MEAs were composed of an array of electrodes and fabricated by MEMS technology. The electrodes often can be fixed from 1–100 μm in diameter with a several hundred micrometer center to center spacing, which can avoid the electric interference between the neighboring electrodes effectively. Since development of the first MEAs, the quality of information from extracellular recordings technological was mainly improved by technical efforts to increase the density and the number of the electrodes that can be constructed and addressed over a single MEAs [40, 41]. Now, in vitro MEAs may contain over 10,000 electrodes. Nevertheless, the recording qualities of these devices, reflected by the electrical coupling coefficient between single neuron and the device, and the signal-to-noise ratio, remained poor. Typically, the amplitudes of field potentials ranged between 10 μV and 1 mV and a great deal of computational power was required to extract data and sorted out the recorded signals [41–43]. Thus, recent works for MEAs were focused on nanofabrication of electrodes into 3D model and chemical modification for electrode surfaces, in order to improve attachment of electrodes and tissue [44, 45]. Details have been more introduced in the previous chapter of "Olfactory epithelium biosensor."

11.3.2 Isolation and Fixation of Gustatory Epithelium

The gustatory epithelium-based biosensors usually used rat gustatory epithelium as biosensitive components [14, 19, 46]. The rat can be anesthetized by intraperitoneal injection of urethane. The tongue was excised proximal to the circumvallate papillae and immediately incubated in Ringer's solution and washed several times. The buffer was uniformly injected under the lingual epithelium of fungiform papillae of the dissected tongue with injector. The isolated epithelium was rinsed with Ringer's solution and observed by the scanning electron microscope (SEM) (Fig. 11.1). After rinsing, the solution was removed from the MEAs chamber and the tissue was fixed by a plastic ring-shaped frame covered with a tightly stretched

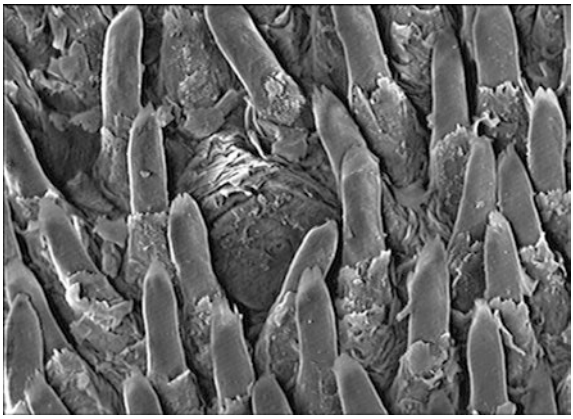

Fig. 11.1 Fungiform papillae of the gustatory epithelium observed by the scanning electron microscope (Reproduced with permission from Ref. [14]. Copyright 2013 Elsevier)

piece of mesh. Besides attachment of gustatory epithelium directly, the tissue containing taste buds can also be used to form a sandwich-type sensing membrane for taste detections. Qiao et al. isolated gustatory epithelium from SD rat by similar technique above and used the sodium alginate-starch gel as a fixing agent to fix on two microporous membranes [47]. By this method, glassy carbon electrodes can be used to monitor ligand–receptor interaction on fixing taste bud tissues and achieved detection for tastants.

11.3.3 Sensor System and Data Processing

Multichannel electrophysiological recording system can be used to measure action potentials of TRCs during taste stimuli [14, 19, 46]. The whole recording system was placed in a shielding box to avoid external disturbances. Noise of blank measurement should be kept under 10 μV. The softwares such as MC_RACK and MATLAB were often used to display and analyze signals, respectively. More specifically, electrophysiological data can be analyzed in software by spatial and temporal domain. In spatial domain, several parameters including the total number of firing spikes of multichannel signals, displaying the activated subsets of gustatory receptor cells and the corresponding responding regions can be calculated after every taste stimuli. When in temporal domain, the typical long-time signals in their basic characteristics of firing rate (the total number of signal spikes in a second), duration (interval between onset and end of one complete spike), and amplitude (voltage difference between the maximal positive and negative peak) can be analyzed accordingly. Fast Fourier transformation (FFT) spectra were calculated, and the power spectrum analysis of taste signals was used to calculate the distribution of frequency components. To discriminate the signals detected under different taste stimulations, the sorting algorithms such as 3D principal component analysis (PCA) also can be introduced into investigations about gustatory epithelium-based biosensors.

11.4 Application of Gustatory Epithelium-Based Biosensors

11.4.1 Recording of Taste Electrophysiological Signals

Combining the intact gustatory epithelium with MEAs, the tissue-MEAs hybrid biosensors can be fabricated to detect electrophysiological activities of the gustatory receptor cells (Fig. 11.2a). With several reports, the gustatory epithelium can be well stripped from tongue of rat and immobilized on the surface of MEAs device [14, 19]. The filiform papillae and fungiform papillae formed a dense meshwork on the epithelium surface (Fig. 11.2b). The natural taste buds were well preserved, and primary structures of receptor cells population were kept integrated. MEAs had the benefit of detecting signals of many cells synchronously, which was convenient to comparatively analyze recorded information in parallel. Taste buds had different populations of gustatory receptor cells which mediated different patterns of signal responses. Therefore, the multichannel signals in our experiment can properly reveal the coherent activities of different gustatory receptor cells in taste buds.

The most advantage of MEAs device was multichannel electrophysiological recording in parallel, which can provide multipoint information in time and space domain. The study was originally from 16-channel simultaneous recording for gustatory epithelium [14]. 16-channel was typical method to display electrophysiological signal from MEA chip. With technology advances in MEMS and sensor system, MEAs chip can be improved to 32, 64, and even 128 recording channels for gustatory epithelium. For instance, 64-channel recording can be used to measure electrophysiological activity of gustatory epithelium under stimuli of sweet

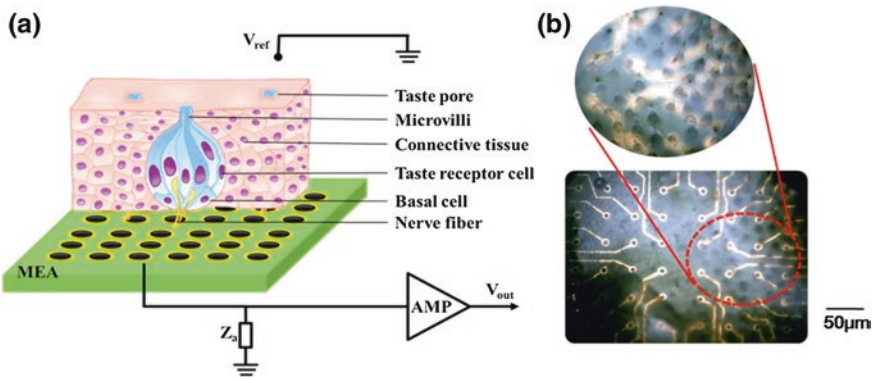

Fig. 11.2 Recording extracellular potentials of gustatory receptor cells in taste buds by microelectrodes. **a** Schematic diagram of the tissue biosensor (Reproduced with permission from Ref. [14]. Copyright 2013 Elsevier). **b** Observation of gustatory epithelium coupled to microelectrodes with a light microscope (Reproduced with permission from Ref. [50]. Copyright 2013 Elsevier)

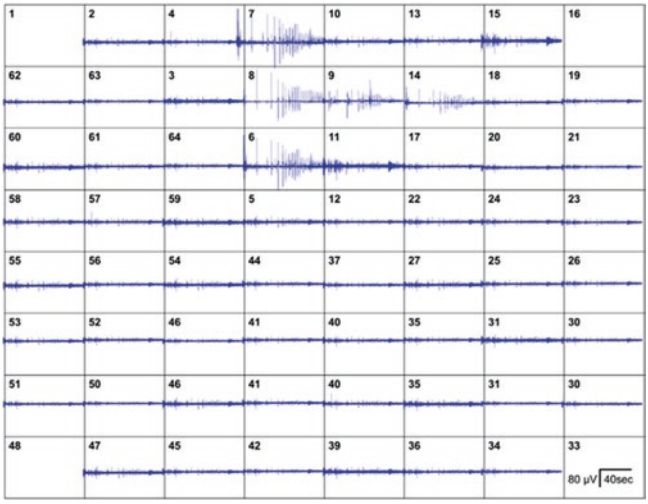

Fig. 11.3 64-channel electrophysiological recording for gustatory epithelium (Reproduced with permission from Ref. [51]. Copyright 2013 Elsevier)

taste (Fig. 11.3). Compared to 16-channel recording, it can provide higher resolution to observe electrophysiological activities of gustatory epithelium, allowing more information analyzed for taste discrimination. It can be seen that almost all the channels recorded the peak potentials and all the responses exhibited coherent patterns in spike firing, indicating the synchronized activities in cell networks of taste buds. However, researchers have found that the amplitude of the multichannel signals presented clear differences due to signals from different position and adhesion of the gustatory epithelium in MEAs chips. It was an important feature which can be analyzed for taste detection and recognition.

11.4.2 Analysis of Multichannel Taste Signals

Gustatory epithelium contained different TRCs specifically responded to different tastants. Thus, using multichannel signals recorded from different points of gustatory epithelium, the tissue electrophysiological behavior can be tracked for spatial segregation pattern of taste stimuli [14, 46]. Multichannel signal responses can be recorded and studied by analyzing the distribution of the activated receptor cells to different tastes stimulation. We explored the multichannel signals in different stimulations of NaCl (salt) at increasing concentrations [46]. It can be found that NaCl at different concentrations elicited electrophysiological responses of different channel of MEAs. As shown in Fig. 11.4a, salt stimuli at low concentration can only activate gustatory cells in several channels (marked in red), while salt stimuli at high concentration fired continuous potentials of gustatory

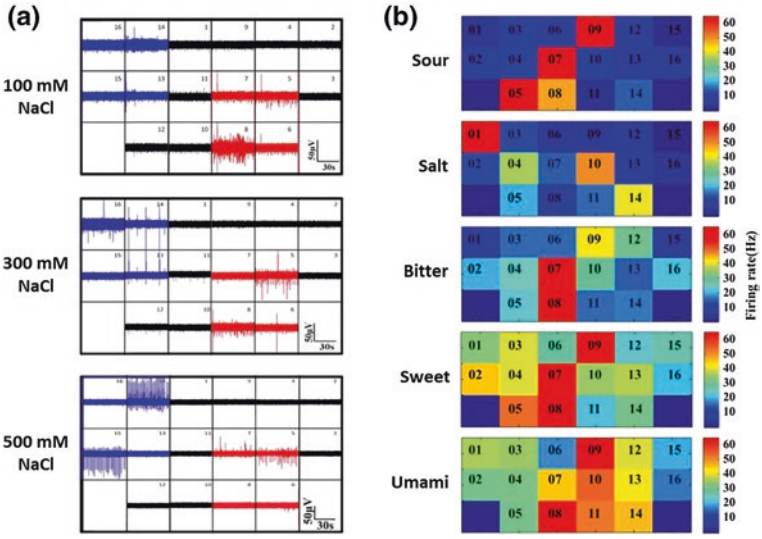

Fig. 11.4 Spatial segregation pattern of gustatory epithelium-based biosensor. **a** The multichannel signals in different NaCl concentrations (Reproduced with permission from Ref. [46]. Copyright 2013 Elsevier). **b** Normalized comparison diagram of the firing rates of multichannel responses in five basic tastes stimulations (Reproduced with permission from Ref. [14]. Copyright 2013 Elsevier)

cells covering in other channels (marked in blue). Therefore, from the multichannel results, two nonoverlapping populations of gustatory receptor cells in taste buds were demonstrated to be activated by salt stimuli. One responded to all concentrations, whereas another responded only to high concentrations. This showed potential application of the gustatory epithelium-based biosensors for investigation about distribution of gustatory cells in epithelium.

We further investigated spatial segregation patterns of gustatory epithelium-based biosensor for five basic tastes of sour, salt, bitter, sweet, and umami [14]. We used signal firing rates, the main characteristic of signal firing pattern, to evaluate the overall activation extent of multichannel signals in different tastes perception. The channel with high firing rate reflected high activation of gustatory receptor cells on its surface. The intact signals were extracted from multichannels with original arrangement of all stimulations, respectively, and calculated firing rate sequences of multichannel signals (Fig. 11.4b). The distributions of activated subsets of gustatory receptor cells after different taste stimulation can be observed to reveal the spatial segregation in gustatory perceptions in the epithelium. It was obvious that gustatory receptor cells with different spatial distributions were best activated to different tastes. The result of multichannel analysis indicated that a subset of gustatory receptor cells responded to a single tastant and that these cells were distributed across a wide area of the gustatory epithelium. Therefore, the multichannel analysis of the taste signals can reveal the spatial segregation of

gustatory receptor cells in epithelium. Actually, some studies in the primary taste cortex of mice have demonstrated topographic segregation in the functional architecture of the gustatory cortex [15, 48, 49]. As the taste information was always transmitted from gustatory epithelium to the brain, the gustotopic map in the brain was likely to come from and related to the spatial information in gustatory epithelium.

11.4.3 Taste Detection and Analysis

After the multichannel analysis of the signals in gustatory epithelium, typical temporal analysis can be used to extract effective information from original signals. Long-time responses of different channels can first be filtered and plotted to different taste stimuli. Our previous study has demonstrated that the electrophysiological signals from different channels of gustatory epithelium-based biosensor can be modulated by different stimulations of HCl, NaCl, quinine-HCl, glucose, and sodium glutamate [14]. These five substances represented five basic tastes of sour, salt, bitter, sweet, and umami, respectively. Experiments showed taste buds delivered significant action potentials in present of taste stimulations compared with the native activities (Fig. 11.5a). Significantly, obvious differences in characters of the action potentials such as amplitude and duration can be found with different taste stimuli. Thus, the statistics for several character parameters of action potentials

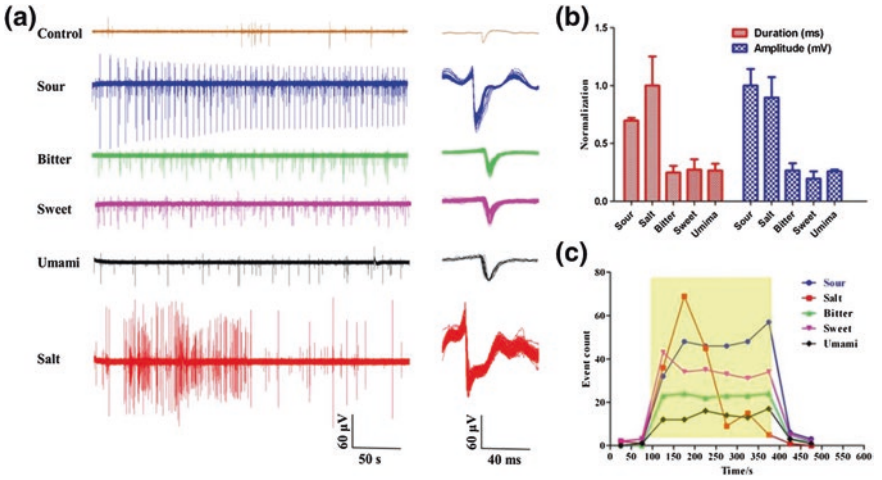

Fig. 11.5 Analysis of electrophysiological signals in long-time recording. **a** Changes electrophysiological signals of after the stimulation of basic taste qualities. **b** The mean amplitude and duration of recorded signals to five basic taste stimulations. **c** The firing rate comparison of potentials in five taste simulations (Reproduced with permission from Ref. [14]. Copyright 2013 Elsevier)

were introduced into recognition for different tastes. The statistic indicated that the amplitude and duration characteristics in sour and salt signals were much bigger than those in bitter, sweet, and umami responses (Fig. 11.5b). This result accorded with physiological study of biological taste sense that sour and salt were mediated by ion channels and bitter, sweet and umami were modulated by gustatory receptors. Moreover, the firing rate in unit time interval can also be used as a parameter to evaluate five basic tastes (Fig. 11.5c). The peak events increased immediately with the present of five stimuli, and the events presented obvious differences in the firing rate. With these results, we can find the gustatory epithelium-based biosensors had different electrophysiological responses to different taste stimuli. These differences can provide theoretical basis for taste recognition using the gustatory epithelium-based biosensors.

Besides taste recognition, temporal analysis was also used to evaluate concentrations of taste substances. We reported a gustatory epithelium-biosensor for umami detection using electrophysiological action potentials [50]. In the study, several different umami tastants such as L-glutamic acid (L-Glu), L-aspartic acid (L-Asp), L-monosodium glutamate (L-MSG), and L-monosodium aspartate (L-MSA) were used as umami stimuli for gustatory epithelium-based biosensor. Individual waveforms evoked by different concentrations and extracted from recording trains were plotted together with increasing concentrations (Fig. 11.6a). Visible changes in amplitude of action potentials can be observed with increasing of concentrations of all four umami tastants. Dose-dependent behavior can also be found in statistics for umami tastants at increasing concentrations (Fig. 11.6b). Thus, amplitude characters of individual waveforms were demonstrated as effective parameters to detect and evaluate tastants.

The above analysis in time domain was applied to individual signals in order to estimate their basic properties. In fact, a signal recording can be regarded as a population of several samples in a statistical sense and not estimated within a complete

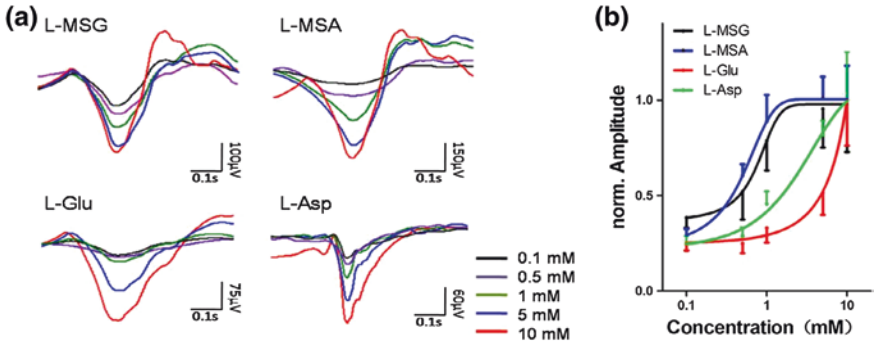

Fig. 11.6 Response of the gustatory epithelium-based biosensor to umami tastants at different concentrations. **a** Action potential recordings of the biosensor for different umami tastants. **b** Mathematical statistic curves on normalized amplitude for four umami tastants (Reproduced with permission from Ref. [50]. Copyright 2013 Elsevier)

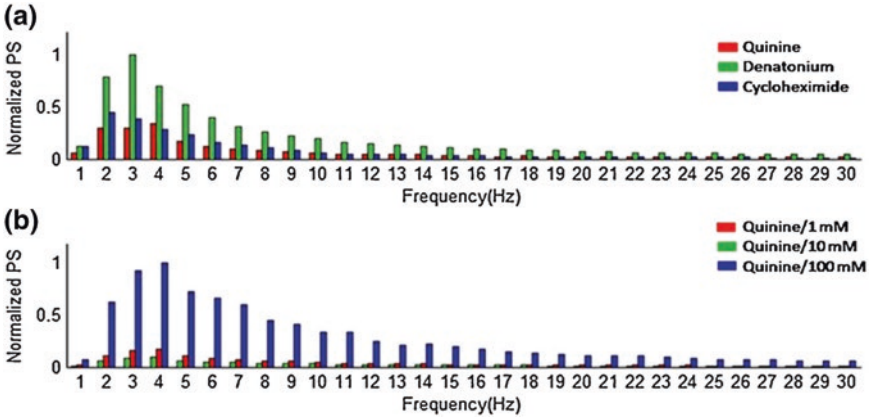

Fig. 11.7 Power spectrum analysis of the recorded signals for bitter sensing (Reproduced with permission from Ref. [19]. Copyright 2013 Elsevier)

signal recording in time domain. Thus, analysis in frequency domain can be employed to estimate holistic properties of a long-time recording. The power spectrum was a classical method in frequency domain which described how the energy of a long-time series signals. Thus, we also tried to use power spectrums of response signals to recognize and detect bitter tastants [19]. The power spectrums of response signals can be analyzed and plotted in a low band (Fig. 11.7a). For different bitterness, response signal to bitter tastant with higher bitter value showed larger energy in power spectrum than bitterness with low bitter value. It was consentient to the physiological study for bitter taste and well-satisfied biomimetic sensing demand for bitter taste. Moreover, for one kind of bitterness at increasing concentrations, magnitudes of power spectrums also increased with concentrations in a dose-dependent manner (Fig. 11.7b). The increasing power spectrum not only evidenced that electrophysiological activities of gustatory epithelium changed with concentrations of taste stimuli, but also provided a pathway to evaluate tastant concentrations.

11.4.4 The Gustatory Epithelium-Based Biosensors for Taste Discrimination

Through above analysis, gustatory epithelium-based biosensors can be demonstrated to detect taste qualities in high sensitivity and selectivity. In fact, researches have demonstrated that gustatory epithelium-based biosensors can discriminate five basic taste qualities including sour, sweet, bitter, salt, and umami, and can even evaluate different substances belong to same taste (Table 11.1). In the following, we will introduce several applications of gustatory epithelium-based biosensors for taste discrimination using different analyzing methods.

Table 11.1 Several applications of the gustatory epithelium-based biosensors for taste detections

	Taste	Substances	References
Gustatory epithelium-based biosensors	Five basic taste	Na^+/H^+/glucose/quinine/glutamic acid	[14]
	Salt	Na^+	[46]
	Bitter	Quinine/denatonium/cycloheximide	[19]
	Umami	Glutamic acid/aspartic acid	[50]
	Sweet	Natural sweetener/artificial sweetener	[51]

Principle component analysis (PCA) was one kind of typical pattern recognition methods used in the study of traditional electronic tongue. It can reduce size of data and extract key information to realize the tastes classification [1, 2]. In studies about gustatory epithelium-based biosensors, the signal features were also applied to discriminate the taste response signals by PCA in the time domain. Based on the signals detected in the condition of absence of basic tastes, the temporal characteristics were extracted in terms of maximum value, minimum value, amplitude, duration, rising time, decreasing time, and the firing rate of signals for PCA. In gustatory epithelium-based biosensor, we first used a 2D pattern clustering using amplitude and duration of waveform from recorded signals directly [51]. The pattern clustering can obviously distinguish artificial sweetness and natural sweetness into two main regions (Fig. 11.8a). It suggested the gustatory epithelium-based biosensors can discriminate different tastants through character clustering.

Furthermore, 3D pattern clustering can be used in gustatory epithelium-based biosensors for recognition of five basic tastes [14]. Using three principle components calculated from PCA, responses to five basic tastes can be clustered into five main regions in 3D space, respectively, indicating all five basic tastes sensed by gustatory epithelium biosensors showed characteristic regions related to the taste information (Fig. 11.8b). Notably, these five clusters, although different from each

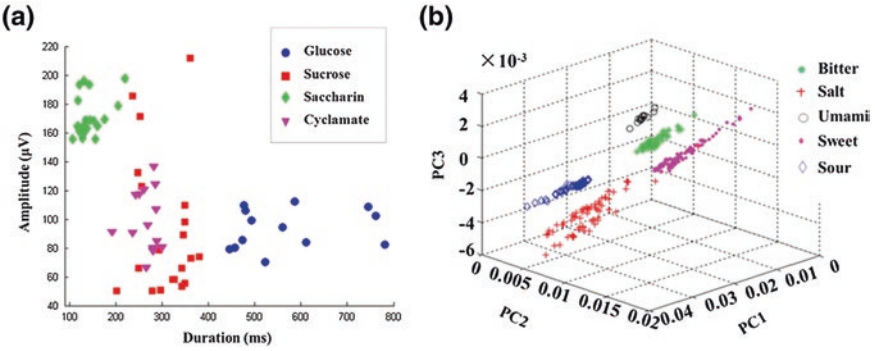

Fig. 11.8 The PCA analysis of action potentials. **a** Recognition pattern of the biosensor for several natural sweetness and artificial sweetness (Reproduced with permission from Ref. [51]. Copyright 2013 Elsevier). **b** Recognition pattern of the biosensor for five basic tastes (Reproduced with permission from Ref. [14]. Copyright 2013 Elsevier)

other, can be observed to gather into two groups. The sour, salt group and bitter, sweet, umami group was likely to come from the two perception pathways. Apart from this, the mechanism of basic tastes discrimination and recognition laid mainly in the activated subsets of gustatory receptor cells. As has been mentioned in multichannel analysis, different subsets of gustatory receptor cells were involved in basic tastes perception. Theses all suggested that the gustatory epithelium-based biosensor can be used to distinguish different tastants.

Moreover, the gustatory epithelium-based biosensors also provided a tool to investigate taste mechanism. As reported in physiological study, topographic segregation for five basic tastes in the functional architecture of the gustatory cortex was demonstrated by in vivo two-photon calcium imaging [15]. For instance, the salt point in gustatory cortex showed significant responses to NaCl, while no electrophysiological activities can be observed with sweet and bitter stimulations (Fig. 11.9). The similar specific responses can also be found in sweet, bitter, and umami stimulations. Accordingly, in the tongue, the five basic tastes are mediated by separate classes of gustatory receptor cells on gustatory epithelium, respectively. Although gustatory receptor cells for five basic tastes were demonstrated to distribute everywhere of gustatory epithelium, the principle of the distribution can be further explored with the gustatory epithelium-based biosensors.

In conclusion, gustatory epithelium-based biosensors can provide multichannel electrophysiological signals from different points of gustatory tissue with taste stimuli. Using several methods such as spatiotemporal analyzing and PCA, gustatory epithelium-based biosensors were demonstrated to achieve three targets: (i) recognition for five basic taste which can be sensed by human and animals, (ii) discrimination for taste substances belong to same taste, (iii) evaluation for concentrations of taste substances. In taste recognition, the gustatory epithelium-based biosensors can detect analytes based on their intrinsic tastes, rather than detection for chemical components. It was helpful to scan and discover new taste substance, such as powerful sweeteners, to replace taste substances which were loved by persons, but harmful for our health.

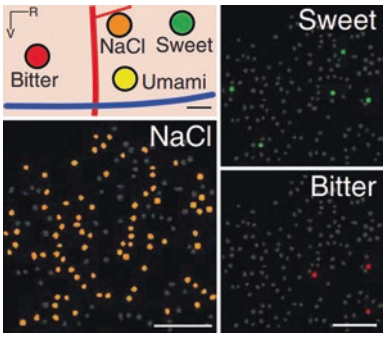

Fig. 11.9 The basic tastes represented in a spatial map in the primary taste cortex (Reproduced with permission from Ref. [15]. Copyright 2011, American Association for the Advancement of Science)

11.5 Summary

Combining biological gustatory tissue with microchip technology, gustatory epithelium-based biosensors were designed for taste detection and recognition by electrophysiological sensing measurements of gustatory tissue. Extracellular potentials of gustatory receptor cells in taste buds were recorded through multichannel MEAs recording systems. When stimulated by taste qualities, the recorded potentials presented different spatiotemporal properties to analyze and evaluate taste quality information. With function units well preserved and successfully recorded, the biosensor technology was a promising platform for bioelectronic tongue with respect to tastes detection in the intact taste buds.

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Chapter 12
Gustatory Receptor-Based Taste Sensors

Ling Zou, Chunsheng Wu and Liping Du

12.1 Introduction

A number of studies are dedicated to studying "electronic tongues" to imitate human taste, which can be applied at the food and beverage industries by using sensor arrays in previous decades [1–3]. Briefly, electronic tongues discriminated and quantified tastes by analyzing the output signals using pattern recognition techniques. Even though some advances have been achieved, electronic tongues are still not used in the quality control of foods and tastant screenings as well for their low selectivity and high cross-selectivity [4, 5]. To date, the commercial taste sensor systems are very rare. Therefore, the research about artificial taste system is indispensable in the future.

Receptor is a kind of important biological macromolecules which can bind signal substance out of cell and triggers a series of biochemical reactions inside the cell. The signal substance which specifically binds to the receptor is named as ligand. There are so many different kinds of receptors and they have many different ligands. The binding of ligand to receptor shows a high specificity, selectivity, and affinity. As a type of membrane receptor, gustatory receptors recognize specific tastants is the first stage of taste sense. Humans have five recognized taste qualities, so there are also five types of gustatory receptors [6, 7]. Different types of gustatory receptors expressed on gustatory cells may selectively recognize their specific ligands and provide corresponding taste signals. Gustatory receptor-based biosensor, which used gustatory receptor as sensing elements, have attracted more attention due to their ability of discriminating specific tastants from mixtures of food and beverage at very low concentrations with high selectivity in recent years

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[8–14]. Compared to electronic tongues, gustatory receptors were used as recognition elements for the development of novel artificial taste sensors that have great potential for the assessment of food quality, drug discovery, basic research on the human taste system, and so on. In this chapter, we address the most recent progress in this field, which include the functional gustatory receptor obtained and combined different transducers, examples of its use, and so on.

12.2 Theories of Gustatory Receptor-Based Taste Sensors

12.2.1 Biological Structure and Function of Gustatory Receptor

12.2.1.1 G-Protein-Coupled Receptors-Sweet, Umami and Bitter Receptors

Among the five gustatory receptorss, sweet, umami, and bitter receptors belong to G-protein-coupled receptors (GPCRs), salty and sour belong to ion channel receptor family. GPCRs located on the cell membrane, with thousands of members, are the largest and most diverse transmembrane receptor family in mammalian cells. Normally, GPCRs are composed of three main domains: seven helix TM domains (7TM), N terminal, and C terminal domains. Like all GPCRs, N terminal is the domain expressed outside the cell membrane, whereas C terminal is inside the cells. Sweet and umami are detected by T1R1, T1R2, and T1R3; they belong to class C heptahelical GPCRs. According to current studies, the T1R2 and T1R3 (T1R2 + 3) heterodimer act as sweet gustatory receptors which can detect sugars, synthetic sweeteners, and sweet tasting proteins; and T1R1 and T1R3 (T1R1 + 3) heterodimer function as umami gustatory receptors which respond to umami stimuli [15–17]. Class C GPCRs have a large amino-terminal domains (ATD) which contain a Venus flytrap (VFT) binding motifs and served as the active site for typical ligands [18].

On the contrary, bitter taste is detected by T2Rs, a large family of class A GPCRs, which with a short extracellular ATD and more than 30 members have been found in present [19, 20]. Owing to lack of extensive ATD, the active sites of bitter compounds are shaped by the 7TM. In contrast to sweet and umami taste, which allow recognition of a limited amount of nutritious content of food, bitter taste needs to identify the toxic compounds from tremendous compounds. Therefore, each T2Rs can recognize a good deal of bitter compounds to complement the small number of bitter receptors for the flexibility of the TM domains [19, 21, 22] (Fig. 12.1).

Although bitter gustatory receptors have different N terminal and are expressed in different Type II cells, from sweet and umami gustatory receptor, they share common signal transduction components [23, 24]. In brief, when taste compound bind to gustatory receptors, a heterotrimeric G-protein consisting of three subunits (α, β, and γ) is activated by taste GPCRs (Fig. 12.2a). Then, the three subunits

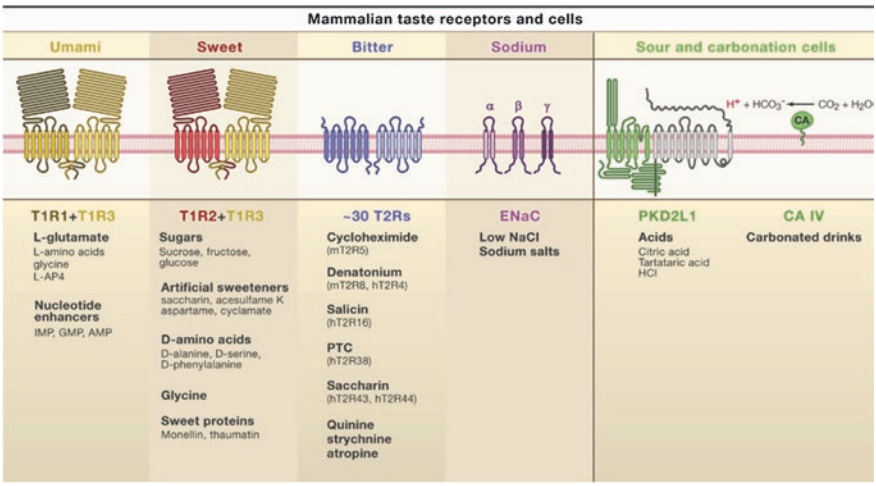

Fig. 12.1 Mammalian gustatory receptors, cells, and ligands [25]. (Reproduced with permission from Ref. [25]. Copyright 2009 Elsevier)

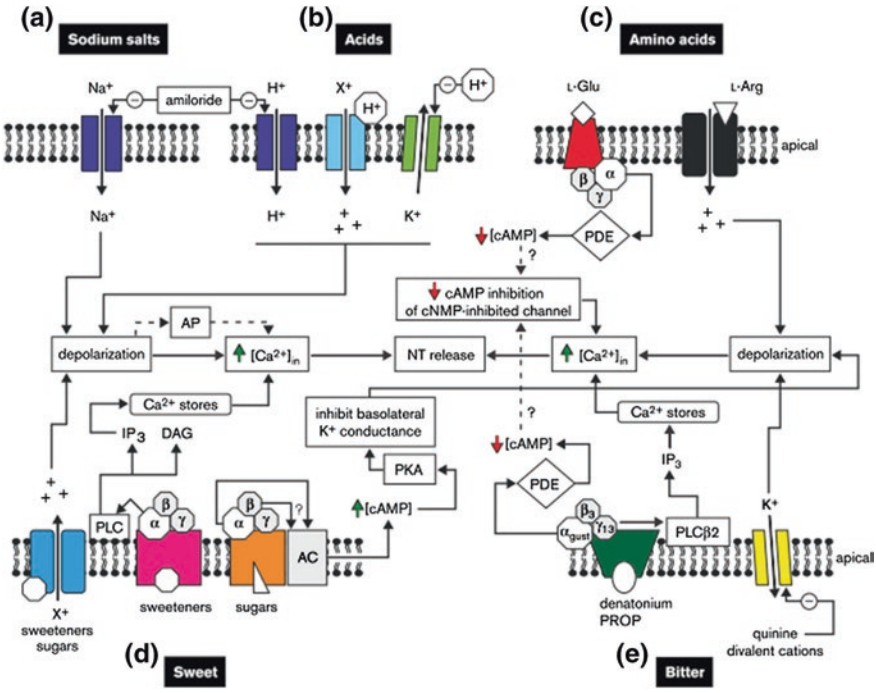

Fig. 12.2 Mechanisms of five taste qualities are transduced in gustatory cells [26]. (Reproduced with permission from Ref. [26]. Copyright 2000 Elsevier)

were separate from the receptors and β/γ dimer interaction with PLC-β2 to stimulate the second messenger inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 opens the IP3R3 channels to release Ca^{2+} from intracellular stores in the smooth endoplasmic reticulum. And the increase of intracellular Ca^{2+} activated taste-selective cation channel, TRPM5, and a gap junction hemichannel, which causes the release of ATP or other taste bud transmitter.

12.2.1.2 Ion Channel Receptors−Salt and Sour Tastes

Unlike the sweet, umami, and bitter gustatory receptors, sour and salt gustatory receptors are ion channel receptors. The polycystic kidney disease-like ion channel PKD2L1 and its associated partner PKD1L3 were proposed as candidate sour gustatory receptors which has been demonstrated by genetic-ablation experiments. The proximate stimulus for sour taste is intracellular acidification, but the PKD2L1 and PKD1L3 is more sensitive to extracellular pH. Several other candidate receptors for sour taste have been proposed in recent researches, like HCN1, HCN4 (common sense), and certain K channels located on cell membrane. No comprehensive theory accounts satisfactorily for the complete signal transduction pathway for carbonation and sour taste.

The amiloride-sensitive epithelial Na channel (ENaC) and the transient receptor potential cation subfamily V member 1 (TRPV1) channel were proposed as candidate salt gustatory receptorss [27, 28]. ENaC's plays a keyrole in salt taste was recently confirmed in ENaC gene knockout mice. ENaC is a heterotrimer which consists of three subunits: α, β, and γ in rat and mouse, whereas another δ subunit is expressed in humans [29, 30]. Current studies suggest that Na^{+} enters the gustatory cells through ENaC and depolarizing gustatory cells [6]. Electrophysiological evidence from the chorda tympani nerve (CT) showed that (TRPV1) channel is a major component of amiloride-insensitive salt taste transduction, but the behavioral results produced equivocal evidence to support. Consequently, amount of work have yet to be done to study the mechanisms for salt taste and the identity of salt gustatory receptor.

12.2.2 Production Techniques of Gustatory Receptors

Currently, the sources of gustatory receptors which are used as the sensing elements of various sensors mainly include two aspects: Using the primary gustatory receptor cells it expresses specific gustatory receptor using protein engineering method. The first method has been introduced in Chap. 10. In this chapter, we focus on the second method. The level of gustatory receptors expression in the gustatory cells is too low and it is difficult to extract from a great deal of cell protein. Therefore, the in vitro overrepresentation of gustatory receptors is indispensable to study the structure and function of receptor, as well as

gustatory receptor-based biosensors. Investigation into the mechanisms of cell surface expression of taste receptors is important in order to successfully establish the gustatory receptor-based biosensor. Both GCPRs and ion channel receptors have hydrophobic transmembrane regions, which make it difficult to heterologous expression. Generally, there mainly are three expression systems including prokaryotic expression system, eukaryon expression system, and cell-free protein synthesis system. Although the entire expression system can successfully express functional protein, the optimum expression conditions were different for various receptors. Now, we will discuss the three expression systems in detail.

Prokaryotic expression system (e.g., *E. coli*) has the advantages of low cost, homogeneity of the recombinant proteins, and short generation time [31]. Kim and co-workers expressed human bitter gustatory receptor T2R38 in BL21 (DE3) *E. coli* cells successfully [32]. Nevertheless, this system is not the best choice for functional expression of the gustatory receptor in vivo, since prokaryotes do not contain any endogenous membrane proteins. Prokaryote cells were dying in the formation of transmembrane regions of exogenous membrane protein. Therefore, this approach has already been employed for several soluble proteins, but much more rarely for membrane proteins, especially the gustatory receptors [33]. The protein expressed by the eukaryon expression system, which contains the yeast, insect, and mammalian cell expression system, has the most similar structure to natural protein, especially the mammalian cell expression system. Although the yeast expression system presents short generation times, is easy to manipulate, and inexpensive, the oligosaccharide structures that are essential to GPCR functionality is not precise due to the composition and quantity of N-glycans added by yeast are different [34]. Thus far, no gustatory receptor expressed by this system has been reported. The insect cells expression system is more appropriate to express gustatory receptors for the insect cells provide the posttranslational modifications of proteins [35]. However, the limitation of this system is that it often brings about heterogeneous populations of receptors. A *C. elegans*-based expression system to express functional human and rodent bitter gustatory receptors which combined the advantage of *Caenor-habditis elegans* (*C. elegans*) and mammalian GPCR signaling pathways was developed by Conte et al. [36]. And the *C. elegans*-based system realized the functional expression of the mammalian bitter gustatory receptors such as human T2R4, T2R16 and mouse ortholog T2R8. The main advantages of the mammalian cell expression system are that it can provide about the same environment to the gustatory receptor in the native tissues and has the ability of posttranslational modifications of the receptor production needed. Thus, mammalian cells as the most appropriate expression system for functional studies, which was most commonly used in gustatory receptor-based biosensor to date. Many kinds of mammalian cell lines like HEK293, CHO-K1, Hela, and so on have been used to express different gustatory receptors [9, 37, 38]. Heterologous expression of receptors using mammalian expression system has two ways: transient and stable expression. As the name suggests, gustatory receptor expression is transient, which usually lasted several days until the transfected cells died. Therefore, if high quantities of proteins are necessary in a repeatable way, establishment of a stable

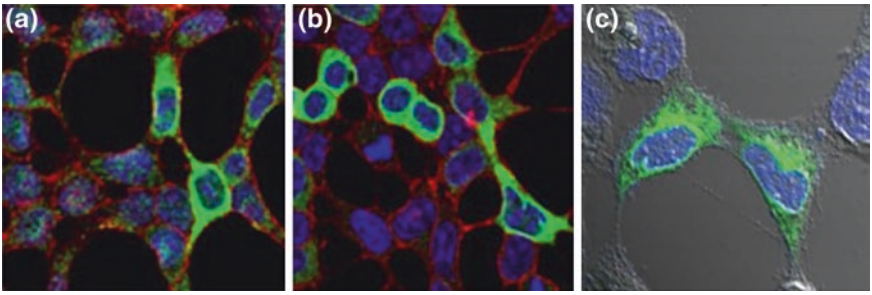

Fig. 12.3 Cell-surface localization of HEK293 cells were transiently transfected with plasmids expressing T1R1 (**a**), T1R3 (**b**) [41] (Reproduced with permission from Ref. [41]. Copyright 2011 Oxford University Press) and T2R4 (**c**) [9] (Reproduced with permission from Ref. [9]. Copyright 2013 Royal Society of Chemistry)

cell line which expresses specific receptor is highly recommended. Compared to transient expression, the stable cell line has high efficiency, easy operation, and good stability. However, transient expression is used frequently in gustatory receptor expression as it is usually difficult and time-consuming for generation of an amplified stable cell line overexpressing receptors [15, 20, 39]. As a promising technique, alternatives to classical cell-based receptor overexpression system, cell-free protein synthesis system has many advantages, such as short time-consumption, convenient operation, and widely applicable in protein expression. Briefly, this system provided a similar environment that natural receptor needed, by adding all the necessary elements for protein synthesis. Many receptors, like olfactory receptor, were successfully expressed at a high level using this system; but gustatory receptors have never used this system before [40]. We believed that this method would play a great role in gustatory receptors production in the future (Fig. 12.3).

12.2.3 Natural Structure Maintenance of Gustatory ReceptorS

How to maintain the natural structure of receptor protein is a crucial matter for gustatory receptor-based biosensor. Before the heterologous proteins are used in receptor-based biosensor, they need to be solubilized, purified, concentrated, and then reconstituted in a lipid environment. The purpose of solubilization is to extract the expressed protein from its initial environment. In this step, the hydrophobic receptor protein will move from a lipid environment to a detergent micelle environment and will be converted to a strong detergent and denaturant solubilized form from an aggregated form [42]. There are two major criteria for the selection of detergent used in protein solubilization. One is the compatibility of detergent to preserve the functionality of the receptor during solubilization and

purification steps. Generally, nonionic detergents which break lipid–lipid interactions and lipid–protein interactions could allow the membrane protein maintenance in the structural features [43]. Ionic detergents such as sodium dodecyl sulfate (SDS) could make protein denaturation, although they are extremely effective in the solubility process of membrane proteins. Zwitterionic detergents have all the properties of ionic and nonionic detergents. The other one is that the facility can subsequently be removed. Moreover, the factors that are likely to influence the solubilization step not only those two, but also include buffer composition, salt concentration, pH, and so on. The objective of protein purification is to remove the excess protein from all the proteins. Although the purification methods for membrane and water-soluble proteins are generally the same, the purification of membrane proteins is more difficult. All the gustatory receptors belong to the hydrophobic molecules, which tend to form aggregates and will increase the difficulties during purification. Among the purifying methods, chromatographic methods that are based on the intrinsic properties of protein could be selected in gustatory receptor purification. The specific recognition of a recombinant protein toward a chromatographic matrix can be realized by specific tags which added to the N or C terminus of the protein by using genetic engineering. The most common tags include his-tag, flag-tag, and GST-tag [9, 44–46]. The renaturation process of protein was still needed in numerous recombinant proteins. Membrane proteins can be solubilized in membrane-mimetic environments through a series of detergents used. However, the best environment for the functional reconstitution of membrane proteins is the original membrane that is not located on the detergent environment. Therefore, the reconstitution of membrane proteins should be performed in artificial membranes. Similarly, gustatory receptor should be solubilized by different detergents in the same way. Different receptors have different membrane compositions, so the membrane compositions must be tested for the given receptors. Nie and co-workers have successfully expressed the saccharide-binding domain of the T1R3 sweet receptor as a soluble and functional protein. In the study, they used BL21-CodonPlus(DE3)-RIL *E. coli* as the host cell to express the protein. The protein was purified by chitin affinity chromatography and then eluted with additional chitin column buffer [33]. In another study, the functional human bitter receptor hTAS2R38 was resuspended in PBS containing protease inhibitor [32].

12.3 Design of Gustatory Receptor-Based Taste Sensors

12.3.1 Secondary Transducer for Gustatory Receptor-Based Taste Sensors

In a gustatory receptor-based biosensor, the gustatory receptor is the primary transducer which can convert the physical signal into mechanical signal. The secondary transducers used in this area include quartz crystal microbalance (QCM)

and field-effect transistors (FETs) [8, 9, 13, 32]. QCM is one kind of mass sensitivity sensor based on the piezoelectric effect of quartz crystals, which is often used to detect interactions between biomolecules and gas detection [47, 48]. The mass change caused by the adsorption between specific molecules and substance immobilized on the sensor chip can be detected by the shift of the QCM resonant frequency. The mass change was directly proportional to the frequency shift. Frequency measurements are easily reached to a high precision; hence, it can measure mass densities as low as a level below 1 $\mu g/cm^2$. Besides to measure the frequency shift of QCM, the dissipation is often measured to help the result analysis. The dissipation is a parameter quantifying the damping which is related to the sample's viscoelastic properties. The dissipation is equal to the ratio of bandwidth, w, and frequency, f. The interaction of tastants and gustatory receptors would induce the shift of the QCM resonant frequency and can be recorded to analyse different tastants label free [9]. Figure 12.4a shows a typical schematic of a piezoelectric quartz crystal; QCM usually fabricated on a thin plate of quartz and the gold electrodes were fixed on each side of the plate.

The FET biosensor is composed of two parts: the receptor and the field-effect transistor. The receptor includes many biomolecules that are immobilized on the sensor. The FET controls the shape and conductivity of the channel by an electric field. By applying an additional ion-and/or charge-sensitive gate layer, the FET can detect any kind of electrical interaction at or nearby the interface of the electrolyte. The current in the FET's channel would be modulated by surface electron charges and potential changes in the gate layer. Therefore, the adsorption of

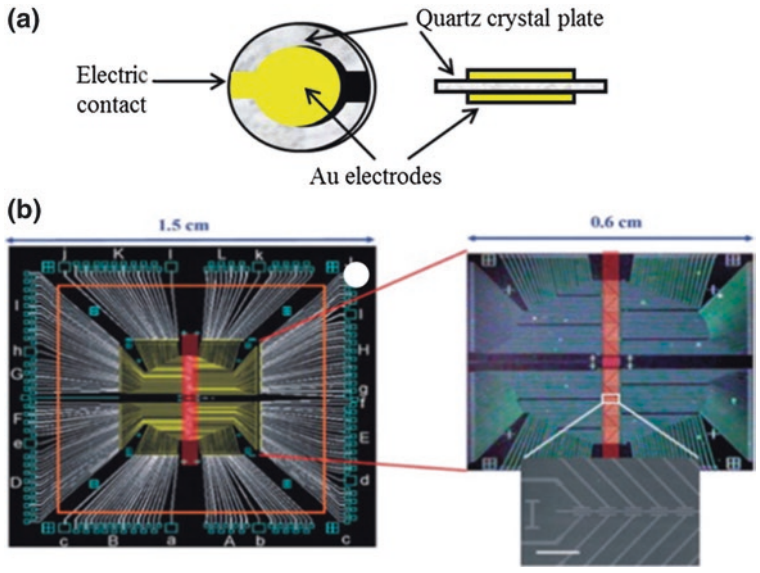

Fig. 12.4 Schematic of a piezoelectric quartz crystal and a SiNW-FET device [50, 51]. **a** (Reproduced with permission from Ref. [49]. Copyright 2014 Giovanna Marrazza). **b** (Reproduced with permission from Ref. [50]. Copyright 2011 Elsevier)

charged macromolecules (e.g., polyelectrolytes, proteins, and DNA) as well as potential changes (e.g., action potential of nerve cells, metabolic processes of bacteria or cells, and ligand–receptor interactions) can be detected by the FET [49]. FET arouses fascinating attention recently for it is easy to modify by many nanomaterials, as well as gustatory receptor biosensor. Figure 12.4b shows a SiNW-FET device.

12.3.2 Coupling Techniques of Gustatory ReceptorS

Highly efficient and convenient methods for the immobilization of membrane receptors to different receptor-based biosensor are indispensable. A large number of methods for sensor surface modification has been developed to meet the specific and effective requirements in functional membrane receptors applied [52]. Physical absorption, as the most commonly used method to immobilizate the membrane receptor, was attributed to the membrane receptors can be coupled directly to the sensor surface without any modification. However, this method lacks in good repeatability and stability of biosensor since the chemical and physical properties of adsorbed sensitive molecules is not easy to control [53, 54]. Therefore, a variety surface-based treatments have been developed to obtain the high stability and repeatability [8, 55]. For example, covalent anchoring provides better stability for analyte sensing in the liquid phase compared to physical absorption. As shown in Fig. 12.5a, Kim and his team have been using this method to couple the human bitter gustatory receptor T2R38 with FET device [8]. Although these methods can improve the thermal, mechanical, and stabilities of the surface, they are typically laborious, costly, and time-consuming. Furthermore, these physical and chemical methods cannot offer sufficient specificity for the efficient immobilization and purification of membrane receptors. Hence, the most satisfying methods of membrane receptors need to be not only of low cost and convenience, but also with sufficient specificity. Over the last decade, self-assembled monolayers (SAMs) have been introduced to surface modification in order to improve the efficiency and stability of the coupling between sensitive receptors and transducers [56]. SAMs that has the advantages of easy preparation, low cost, and versatility is formed by molecular with active chemical moieties spontaneously onto reactive solid surfaces. On the other hand, aptamers, which are nucleic acid ligands, have also been applied in biosensors as sensitive elements due to their high sensitivity and specificity to the specific molecular. Aptamers have been widely used as promising complementary molecules to antibodies due to the high affinity and good specificity [48, 57]. Wu and his co-workers use the method that combines SAMs and aptamers to provide a novel solution for the high efficiency coupling of sensitive T2R4 bitter receptors with QCM due to the combination of high stability of SAMs with aptamers [9]. As schematically illustrated in Fig. 12.5b, thiol-modified anti-His_6-tag aptamer forms a monolayer through the self-assembly process via Au-thiol binding on the gold surface of QCM. The

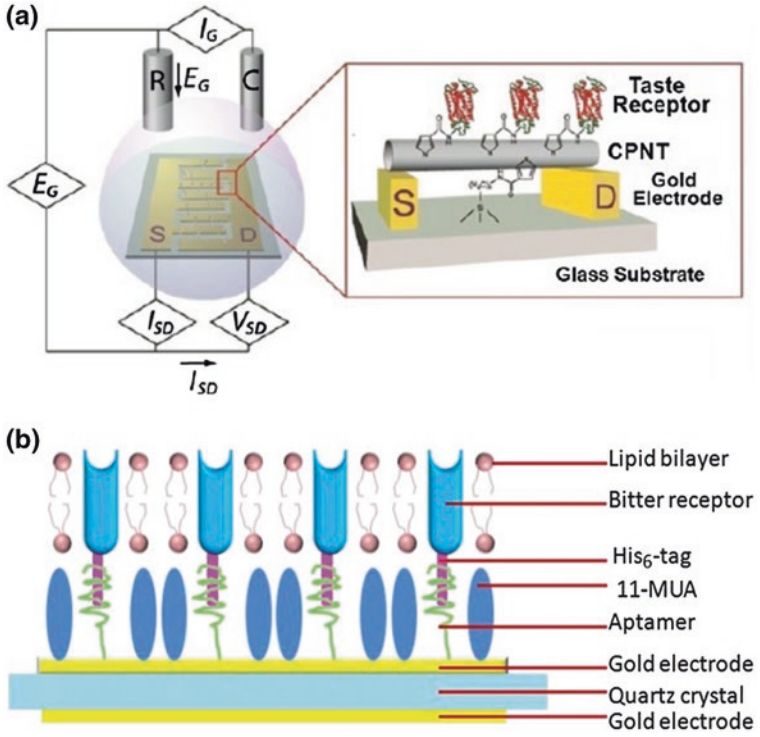

Fig. 12.5 Schematic diagram shows the surface structure of bitter receptor-based biosensors prepared by the covalent polymer coating (**a**) [8] (Reproduced with permission from Ref. [8]. Copyright 2013 American Chemical Society) and the self-assembled aptamer-based method (**b**) [9] (Reproduced with permission from Ref. [9]. Copyright 2013 Royal Society of Chemistry)

results demonstrated that self-assembled aptamer-based method significantly simplified the preparation of bitter receptor-based biosensors and highly improved the performance due to the higher efficiency of immobilization and purification on bitter receptors.

12.3.3 Sensor System and Data Processing

12.3.3.1 QCM for Gustatory Receptor-Based Receptor

To date, there are only few biosensors used to study the gustatory receptor, and even fewer in receptor-based biosensor. Most of the biosensors were cell-based and the cells used include primary gustatory receptor expression cells and gustatory receptor overrepresentation cell lines. Gustatory receptor-based biosensor will attract more attention as it has a higher specificity and stability than gustatory

receptor cell-based biosensor. In receptor-based biosensor, QCM is well suited for studying the interaction between the receptor and the tastants. For example, a biomimetic bitter receptor-based piezoelectric QCM sensor was used to detect specific bitter substances by Wu et al. [9]. In the study, human bitter receptor T2R4 has been extracted from HEK293 cells which successfully expressed that T2R4 receptor were immobilized on the QCM chip to establish a sensitive piezoelectric T2R4-based biosensor. The QCM system includes piezoelectric quartz crystal, resonance circuit, mixers, filters, and a computer which analyzes and processes data. The bitter receptor T2R4 immobilized on the sensor chip will induced the frequency change of QCM, which could be detected by a series data processing. The electrochemical methods like cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) have been employed to demonstrate the immobilization process of the receptor. Figure 12.6a and b shows the CV curves and the Nyquist plots of sensing electrode response at different stages in immobilization process. EIS is a good method of probing the interfacial properties of the electrode in different modification-layers. Figure 12.6a shows the cyclic voltammograms of the bare Au, Au/aptamer, Au/aptamer/11-MUA, and Au/aptamer/11-MUA/receptor. The CV scans of the bare Au electrode indicate a clean gold surface. After the immobilization of the aptamer, the peak-to-peak separation increased, and a fast decrease in the peak current was also observed. This change is due to the insulating property of the aptamer monolayer. Then, there are no CV redox waves that can be observed after blocking the electrodes with 11-MUA and receptors because of the lack of substance with electrochemical activity in the gold electrode, which provides a low and reproducible background current. Figure 12.6b shows the Nyquist plots of the bare Au, Au/aptamer, Au/aptamer/11-MUA, and Au/aptamer/11-MUA/receptor. Each plot consists of two portions: a semicircular part in a high-frequency region and a linear part in a low-frequency region, corresponding to the electron-transfer process and the diffusion process, respectively. The charge transfer resistance (Rct) was selected to demonstrate the interfacial

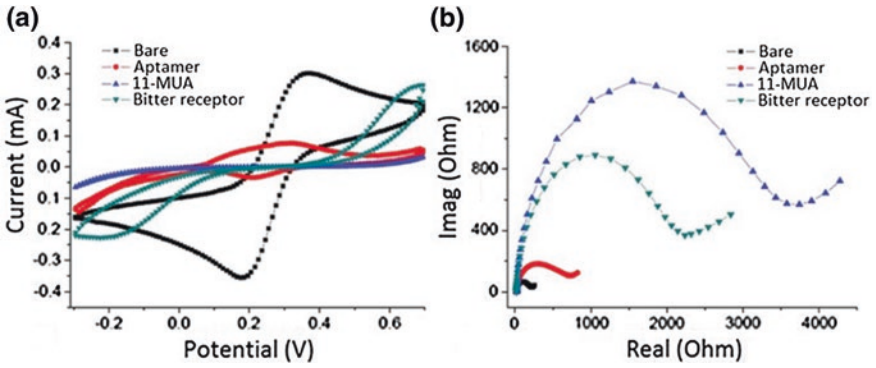

Fig. 12.6 T2R4 receptor immobilization on the gold surface of the QCM device with the self-assembled aptamer-based method characterized by **a** CV, and **b** EIS [9] (Reproduced with permission from Ref. [9]. Copyright 2013 Royal Society of Chemistry)

properties of the prepared receptor-based biosensor during stepwise immobilization procedures. The Nyquist plot of Au electrode showed a characteristic small semicircle domain indicating a diffusion-dominant process with a diffusion-limiting step (black curve). The diameter of the semicircle after the self-assembly of the aptamer on the electrode surface was obviously enlarged owing to the repulsion of the aptamer on the surface which blocks the electron-transfer. After the blocking of 11-MUA, the Rct was further increased for the blocking of nonspecific adsorption spots of some of the exposed gold areas. Finally, the immobilization of bitter receptor on the aptamer causes a considerable increase in the diameter. In addition, immunofluorescent staining was employed to verify the coupling efficiency and the density of bitter receptors on the sensor surface of the QCM device. Therefore, all the results demonstrated that the receptor T2R4 had successfully immobilized on the surface of the electrode.

In order to investigate the established receptor-based biosensor, further experimentation should be done. A variety of bitter substances including MgSO4, denatonium, D -(−)-salicin, and quinine have been utilized to test the performance of bitter receptor-based biosensors. Denatonium is the natural ligand of T2R4 receptor which has been demonstrated in previous the study [58]. The mass change on the sensor surface which was induced by the molecular interaction can be detected by monitoring the frequency shift of the QCM according to the Sauerbrey equation. As shown in Fig. 12.7, the T2R4 receptor-based biosensor shows specific responses to denatonium significantly, which is corresponding to the interaction between the receptor and its ligand. Two negative control biosensors that are without T2R4 receptor and with a olfactory receptor (ODR-10), show no significant response to the bitter substance, including denatonium. The much smaller responses of the negative biosensors to all the substance and the T2R4 receptor-based biosensor to other tastants may be caused by the nonspecific adsorption of bitter substances onto the sensor surface. The results obtained by the biosensors with ODR-10, with and without T2R4 receptor, indicates that the T2R4 receptor-based biosensor have a high sensitivity in bitter substance detection. The limit of

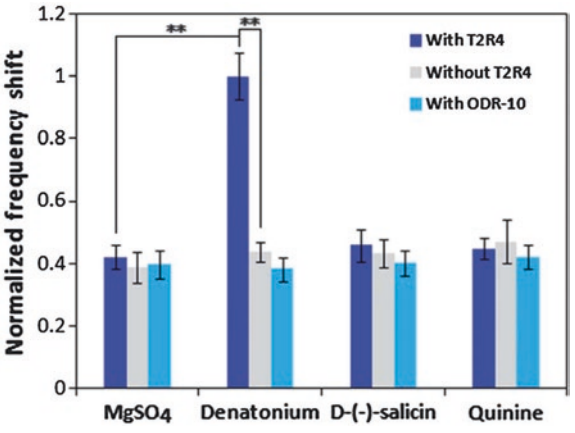

Fig. 12.7 Responses of biosensors with different sensitive elements (with T2R4, without T2R4, and with ODR-10) to various bitter stimuli. The responses of various bitter stimuli were normalized to that of denatonium [9] (Reproduced with permission from Ref. [9]. Copyright 2013 Royal Society of Chemistry)

detection (LOD) of the T2R4 receptor-based biosensor is 1.58×10^{-6} M, which was a theoretic value. It was calculated by the intersection point of the extrapolated linear region of the calibration curve with abscissa. The sensitivity (S) of T2R4 receptor-based biosensors was calculated by the following equation: $S = \Delta f / \Delta c$, where Δf represents the resonant frequency shifts of the QCM, and Δc means the concentration of denatonium.

12.3.3.2 FET for Gustatory Receptor-Based Receptor

As nanomaterial progresses, nanostructured materials, including carbon nanotubes (CNTs), nanowires, etc., have attracted much attention in various field such as micro- and nano-electronic devices, chemical, and biosensors [59–62]. The increasing number of FET sensors based on CNTs has been designed during recent years [63, 64]. Vertically aligned arrays of single-walled CNTs FET (swCNT-FETs) have been developed for bitter gustatory receptor-based biosensor by Kim and co-workers [32]. Conducting polymers (CP) have great potential to provide increased surface area, reduced costs, and improved flexibility of device [65]. Seen in this light, the application of CP in the field of biosensors is highly attractive to solve diverse application demands [66, 67]. For instance, there is growing interest in the use of swCNT-FETs and CP in biological sciences. Recently, swCNT-FETs and CPNT-FETs have good sensitivity and high specificity to detect particular analytes with different recognition elements, such as cells, aptamers, antibodies, and receptors [8, 68–70].

In this chapter, we will introduce the bitter gustatory receptor-based swCNT-FETs in detail. The basic steps to prepare the bioelectric tongue used in hT2R38 bitter gustatory receptor CNT-FETs are as follows: Frist, the substrate was dipped in octadecyltrichlorosilane (OTS) solutions to obtained a self-assembled monolayer (SAM) with functionalization nonpolar terminal groups; Second, the substrate was placed in the solution of swCNTs to obtain a single layer of swCNTs; Finally, the electrodes were fabricated by conventional photolithography [71]. Subsequently, the human bitter gustatory receptor protein which was produced by recombinant *E. coli* was spread on the swCNT-FET, and then it was dried for 4 h via vacuum drying technique. Figure 12.8 A showed the structure of the swCNT-FET-based hT2R38 biosensor. PROP and PTC as the ligand of hT2R38 receptor were selected to assess the performance of the swCNT-FET-based hT2R38 biosensor. The bitter gustatory receptor is GPCRs that usually contain ionizable cysteine residues which have active and inactive biophysical states. The interaction between the gustatory receptor and its ligand may influence the state of the cysteine residues and cause negative charges on the receptor [72, 73]. As a consequence, PROP and PTC binding to the receptor would change the conformation, which increased the contact resistance between the electrodes and swCNTs. Then, the conductance of the swCNT-FET decreased, and the specific bitter tastants can be detected by the change of conductance. The real-time response of a swCNT-FET-based hT2R38 biosensor to various tastants: phenylthiocarbamide (PTC) (bitter tastant), L–glutamate (umami tastant),

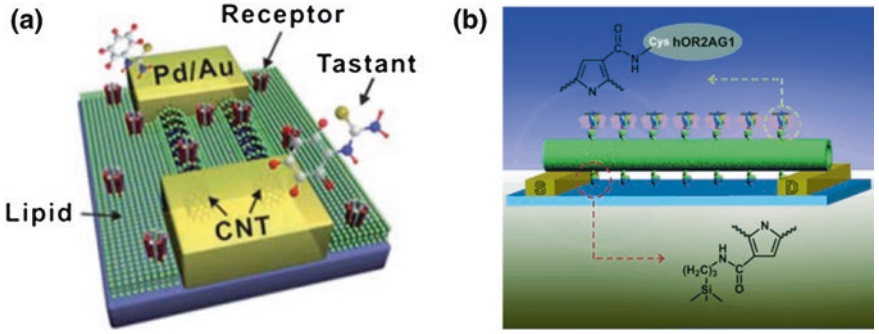

Fig. 12.8 **a** Lipid membranes with hTAS2R38 cover the CNT-FET device [32]. **b** Human olfactory receptor hOR2AG1 immobilize on the nanotube [65]. (Reproduced with permission from Ref. [65]. Copyright 2009 John Wiley and Sons)

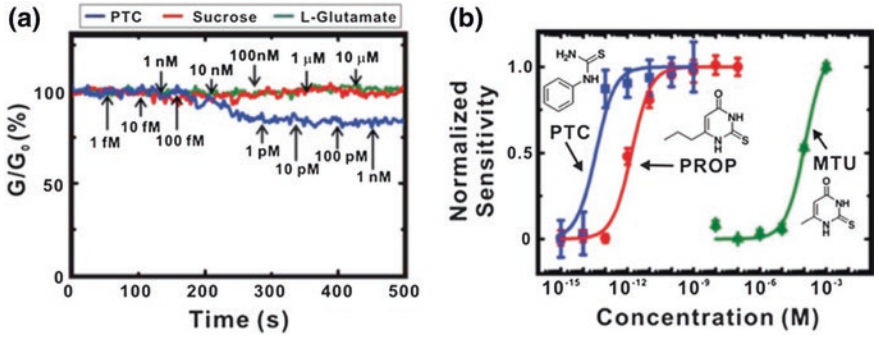

Fig. 12.9 **a** Real-time response of BST devices to PTC (target bitter tastant), L -glutamate (umami tastant), and sucrose (sweet tastant), and **b** Response curves of BST devices to PTC (target bitter tastant), PROP (target bitter tastant), and MTU (nontarget bitter tastant). Note that the BST devices responded to both PTC and PROP (target bitter tastants) although they have quite different chemical structures [32]

and sucrose (sweet tastant) was show in Fig. 12.9a. A model based on the Langmuir isotherm theory was introduced to analyze the response of biosensors. Cs max is the surface density of gustatory receptors on the biosensors; C represents the concentration of tastant molecules in the solution and the equilibrium constant is K [74]. The density Cs of tastant molecules bound to tastant receptor on the device can be calculated by the Eq. (12.1):

$$\mathrm{Cs} = \frac{\mathrm{Cs\ max}}{1/K + C} \tag{12.1}$$

The sensor signal |ΔG/G0| can approximately be considered as |ΔG/G0| ≈ kCs, because the conductance change ΔG compared to the original conductance G0 is

too small to ignore it. k is the constant that means the sensitivity of the sensor transducer would respond to the adsorbed target molecules [75]. |ΔG/G0| affecting factors are device parameters such as transconductance and network structures of the swCNT network channels in the biosensors. Therefore, the sensitivity |ΔG/G0| of the swCNT-FET-based sensor can be written as follows:

$$\mathrm{Cs} = \frac{\mathrm{Cs\ max}}{1/K + C} \tag{12.2}$$

The normalized sensitivity N can be calculated by the Eq. (12.3):

$$\mathrm{Cs} = \frac{\mathrm{C}}{1/K + C} \tag{12.3}$$

The equilibrium constant K for various tastants and gustatory receptors can be obtained by the Eq. (12.3) [76, 77]. Figure 12.9b show the normalized responses of the swCNT-FET-based hT2R38 biosensor to various tastants that were measured to demonstrate the selectivity. The unique binding properties of gustatory receptors showed unique responses of human tongues to tastants. Although propylthiouracil (PROP) and methylthiouracil (MTU) have a similar structure, MTU molecules cannot bind to the hT2R38 gustatory receptor. Nevertheless, PTC can bind to hT2R38 receptor although the structure is completely different to PROP. As shown in Fig. 12.9b, the hT2R38 biosensor began to respond to PTC, PROP solution with 100 and 1 fM concentration, respectively. Although MUT also causes the response of biosensors, the minimum response concentration is as high as 100 μM. Thus, the swCNT-FET-based hT2R38 biosensor can discriminate the tastants by detecting the unique responses of gustatory receptor. Furthermore, it also can mimic the unique selectivity of human taste systems [78, 79].

12.4 Applications of Gustatory Receptor-Based Taste Sensors

12.4.1 Identified the Natural Ligand to the Orphan Receptor

More than 30 bitter receptors have been identified at present. However, some of them remain orphan receptors, such as hTAS2R42, hTAS2R45, hTAS2R48, and hTAS2R60 for no natural ligand of them has been found [80, 81]. Therefore, it is an urgent and pressing need to identify the natural ligand of the orphan receptors from millions of compounds. The procedures of conventional method-based cell assay were time-consuming and tedious [82–84]. The receptor-based assays using biosensor technology show more specificity and sensitivity than cell-based assays in the mechanisms of gustatory receptors [8, 11].

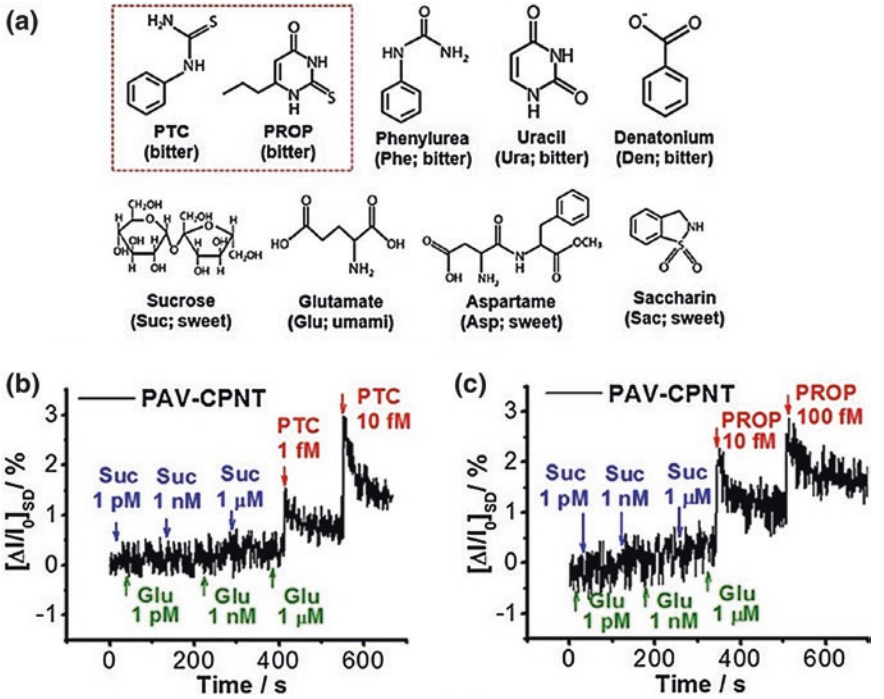

Fig. 12.10 Selective detection of target tastants using CPNT-FET biosensor. **a** The chemical structures of tastants were used for the tastant mixtures. Real-time responses of PAV-CPNT stimulated with the sequential addition of nontarget bitter tastants and target bitter tastants, **b** PTC, and **c** PROP [8]. (Reproduced with permission from Ref. [8]. Copyright 2013 American Chemical Society)

Song and co-workers have used the human bitter receptor hTAS2R38 as the sensing element of CPNT-FET biosensor and applied it in tastant screenings with high sensitivity and selectivity. Figure 12.10 shows the real-time response of CPNT-FET biosensor with functional bitter receptor to different kinds of taste compounds. PTC and PROP are the target molecules of hTASR38, others can not bind to the receptor. In conclusion, the hTAS2R38 receptor-based biosensor allowed to achieve a high sensitivity and selectivity for the detection of target bitter tastants. We believed that this novel biosensor hold great potential as replacement or complement for traditional cell-based assay in gustatory receptor-ligand research in the future.

12.4.2 Development of Novel Sweeteners

Sweet taste is very important for humans as it means the nutrients. Many of the existing sweeteners is very popular for the good taste and high energy [85].

Nowadays, people are struggling with the diseases of affluence, as diets grow richer. Hence, the development of novel sweeteners plays a pushing role in the food industry and human health [86–88]. The conventional methods on the cellular-level studies of sweet mainly include calcium image and electrical physiological technology [23, 89–91]. With the development of technology, gustatory receptor can be applied to the development of human-like bioelectronic tongues based on various transducer. Unlike bitter receptor, sweet receptor is a heterodimeric GPCR which is composed of T1R2 and T1R3 [23, 92]. Therefore, the sensing element of sweet receptor-based biosensor mainly used cells and not proteins. Here, a novel sweet receptor biosensor based on swCNT-FET was developed by Song et al., and was introduced [11]. Figure 12.11 shows the detection mechanism of the device. When sweeteners bind to the sweet gustatory receptor which is located on the cell membrane, the Ca^{2+} influx in the cells will be increased. Finally, it will induce a positive field-effect on the underlying swCNT channel. The normalized sensitivity of the biosensor at different concentrations of various tastants was show in Fig. 12.11b. A dose-dependent response of biosensors to natural sweeteners (sucrose and fructose) and artificial sweeteners (aspartame and saccharin) followed the Hill equation model. This biosensor shows a broader selectivity than the previous cell-based functional assays using the human sweet gustatory receptor in tastants detection [16]. The sweet gustatory receptor-mediated signal transduction can be efficiently monitored by a swCNT-FET sensor platform. This biosensor is used to detect sweeteners in the food and beverage industry and can also overcome the limitations of conventional artificial tongues in terms of its sensitivity and selectivity. In short, the gustatory receptor overexpression cell-based biosensors have very great application prospects in the screening of sweet gustatory receptors and novel sweeteners.

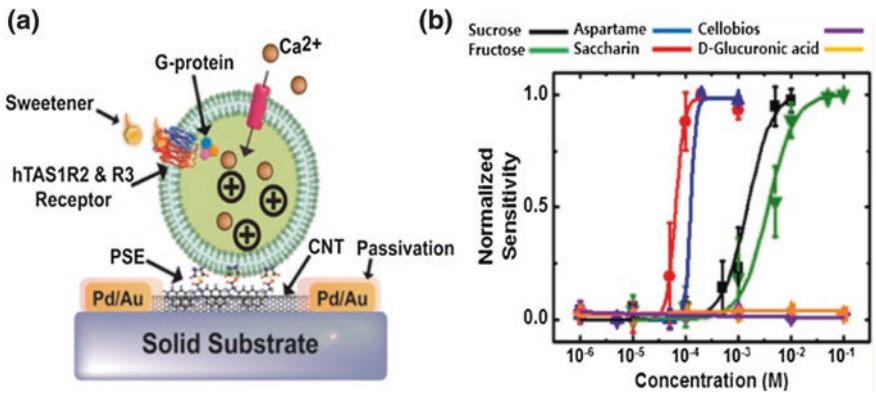

Fig. 12.11 **a** The detection mechanism of the cell-based swCNT-FET device. **b** The normalized sensitivity of the biosensor at different concentrations of various tastants [11]. (Reproduced with permission from Ref. [11]. Copyright 2014 American Chemical Society)

12.4.3 Other Applications

Recent advances in molecular biology have shown us that gustatory receptors is not only expressed in gustatory cells but also in the airways and gastrointestinal (GI) tract [93]. Although much research work have been done on the subject of the mechanism of the taste sense that occurs in the GI tract, it is unclear today. The receptor-based biosensor and cell-based biosensor would offer an efficient method for studying the chemosensation in the GI system. Compared to sweet-tasting compounds, sweet taste inhibitors or blockers which have a potential for the treatment of obesity and diabetes is very limited [94]. Sweet receptor-based biosensors will play a great role in the mechanisms of sweetness inhibition and the possible design of new sweetness inhibitors for pharmaceutical and food development industries.

12.5 Summary

The gustatory receptor and the taste transduction pathway are described in detail in this chapter. Several gustatory receptor-based biosensors and their application are also mentioned. In conclusion, significant advances have been made in understanding the mechanisms of human taste sensation by the development of molecular biology, electrophysiology, and genetics. However, the unique binding properties of gustatory receptor provide a unique selectivity to tastant molecules of human tongues. Thus, human gustatory receptor-based biosensors hold an incomparable advantage to mimic the unique responses of human tongues in terms of selectivity and sensitivity compare to previous taste sensors which used synthetic materials. In recent decades, the advances in the research of gustatory receptors have made a significant impact on the development of gustatory receptor-based biosensors for chemical sensing. At the same time, the development of nanomaterials sciences gives new impetus to improve the performance of gustatory receptor. Several gustatory receptor-based biosensors used carbon nanotubes demonstrated that the combination of receptor-based biosensor and nanomaterials was a powerful tool for basic research on food industry, diagnostics, and drug discovery and the functionality of taste. And yet for all that, there are still many experiences to sum up and many theories to research as well on the study of gustatory receptor-based biosensors. In the near future, the development trends of biomimetic gustatory receptor-based biosensors are fabricate sensor arrays to utilize gene engineering to gain various functional olfactory receptors, which can mimic the responses of human tongues by performing dynamic and noninvasive detection and possess characters of miniature, integration, and intelligence.

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Chapter 13
Biomimetic Gustatory Membrane-Based Taste Sensors

Hao Wan, Da Ha and Ping Wang

13.1 Introduction

Electronic tongue is the classical tool for taste evaluation in different applications such as food evaluation [1], water [2] and process monitoring [3]; biomimetic membrane-based taste biosensor is another important approach for taste evaluation. With the rapid development and intensive study in molecular structure [4] and function of molecules [5, 6], biomimetic membrane has attracted extensive interests due to the exceptional selectivity and high transport rates of the membrane. The study in biomimetic membrane is to mimic functions and mechanisms of biological membranes for different applications. The increasing interest in the mimicry of biological principles, morphology, behavior, and manufacturing has led to extensive studies in biomimetic membranes. Generally, the biomimetic membrane consists of lipid-like polymers, membrane proteins and artificial channels mimicking membrane proteins [7]. Due to the elaborated structure of biomimetic membrane, the membrane can be utilized as a promising material and tool for various applications such as biomimetic membrane-based sensors. Biomimetic sensors are devices integrated of many engineered products, systems, and manufacturing processes to provide feedback, monitoring, safety, and other benefits [8]. Compared to conventional sensors, biomimetic sensors offer superior advantages for improvements to current technologies in terms of high sensitivity and various selectivities. Hence, biomimetic sensor design is achieved in an interdisciplinary area with mimicry in functionality, principle, morphology, and behavior. Different biomimetic

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sensors in acoustic, biological, and chemical sensors, etc. have been designed for applications in many fields to measure strain, force, position, current, and voltage.

Based on artificial lipids [9], various biomimetic membrane-based taste sensors have been developed for evaluation of pharmaceutical products, foods, and drinks. Taste is comprised of five basic qualities: sourness, saltiness, sweetness, bitterness and umami. Traditional sensors are always utilized to determinate specific substances, while the taste is the overall perception by human tongue. Hence, biomimetic membrane-based taste biosensor presents global selectivity to various substances and gustatory perception is achieved with the overall sensing by the taste sensor. Through this approach, the biomimetic taste sensor is utilized to classify enormous kinds of chemical substances into five basic groups.

13.2 Theories of Biomimetic Membrane-Based Taste Biosensors

13.2.1 Biological Performance of Biomimetic Lipid Membrane

Biomimetic lipid membranes have received significant attentions in recent years due to their promising applications in chemistry, material and biomedical fields [10–12]. The fundamental principle of biomimetic membrane-based taste biosensor design is to study the mechanism of human taste receptor and develop biomimetic approaches for taste analysis. The biomimetic lipid membrane with self-assembly is considered as one of the most powerful tools for gustatory perception and study. Generally, lipid membranes consist of two amphiphilic lipid layers arranged with their hydrophilic head region exposed to the surrounding aqueous environment and hydrophobic domains in the core [13]. And in cells, lipid membranes constitute the whole cell structure and form channels for ions transportation. Besides ion channels, some functional proteins are inlaid in the lipid membrane for transportation of specific ions. The membrane structure of a gram-negative bacterial organism [7] is shown in Fig. 13.1. The surface layer (S-layer) proteins are arranged in oblique, square, or hexagonal arrays [14, 15], forming porous membranes of different sizes. S-layer proteins provide functions such as cell protection, cell adhesion, surface recognition, molecular sieving, and molecule and ion traps. Different protein receptors acting as ion channels, ion transporters and ion pump are inlaid in lipid membrane. Ionophore can also mediate transport across lipid bilayer by complexing specific ions. With various ion channels and protein receptors, the lipid membranes can adjust the intra- and extracellular environment by spontaneous and passive ion transportation.

Besides, self-assembly is another remarkable property of lipid membrane which constructs a dynamic and rigid structure. Self-assembled hydrated lipids are physicochemically, not covalently bonded, and the lipids dynamically diffuse in the bilayer with certain orientation [16]. Thus, lipid membranes can be easily fabricated with self-assembly and mixed with other substances to enhance the performance. The

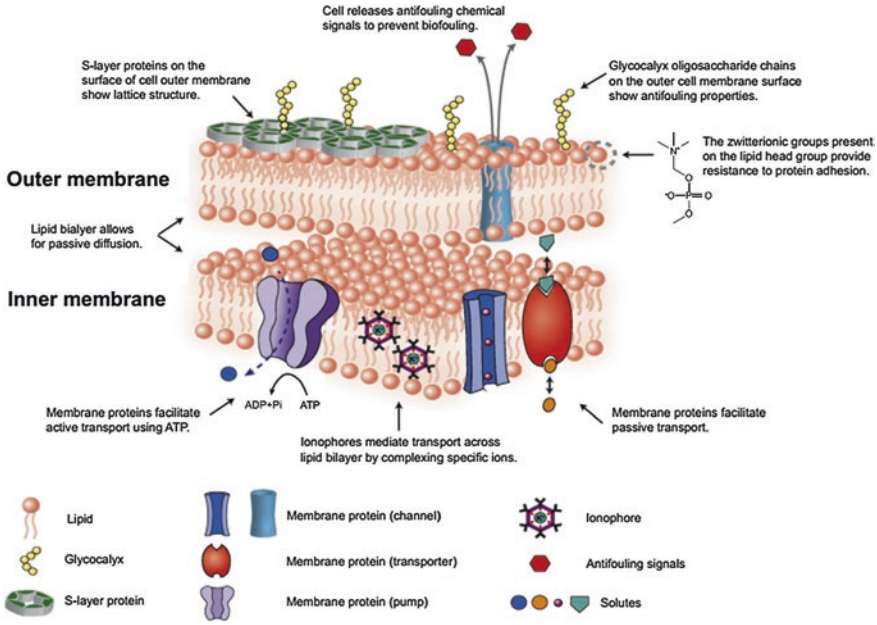

Fig. 13.1 The membrane structure of a gram-negative bacterial organism. Different protein receptors including channel protein, transporter protein and pump protein are inlaid in the membrane for ion transportation. Glycocalyx oligosaccharide chains and surface layer (S-layer) proteins are fixed on the surface of cell outer membrane. Ionophores mediate transport across lipid bilayer by complexing specific ions (Reproduced with permission from Ref. [5]. Copyright 2013 Elsevier)

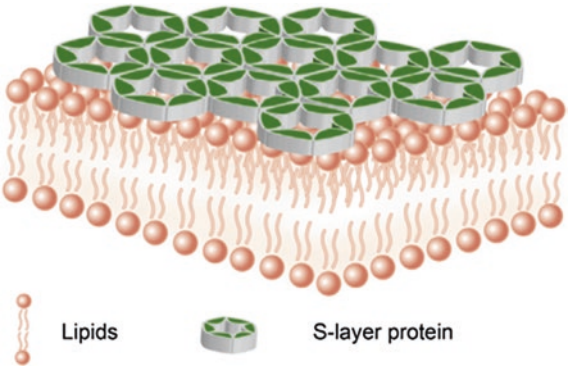

Fig. 13.2 Bilayer lipid membrane structure with surface-layer protein (Reproduced with permission from Ref. [5]. Copyright 2013 Elsevier)

lipid consists of a hydrophilic head and a hydrophobic tail. During the self-assembly in water, the hydrophilic heads orient at the aqueous interface and the hydrophobic tails orient at the core of the aggregate, forming the bilayer membrane structure as shown in Fig. 13.2. When lipid membranes distribute in solutions, a membrane can

be electrically charged due to the ionization of hydrophilic head. That results in a membrane potential under nonequilibrium conditions, while the membrane potential varies according to the difference of ion concentration. Thus, the lipid membrane can be regarded as an excitable model for ion analysis by observing changes in membrane electric potential and electric resistance.

13.2.2 Production Techniques of Biomimetic Lipid Membrane

In real biological membranes, the compositions are very complicated due to hundreds of lipid species, protein species, different mutual coupling structures, and the mobility of the membranes. For biomimetic lipid membrane, it is almost impossible to mimic the real biological membrane with all compositions in the lipid membrane due to complicated mutual interactions. Thus, only a few components are combined in the membrane to achieve similar membrane functions. However, an inevitable obstacle has to be straightly faced, that is, the stability of the lipid membrane should be carefully considered in the production. And the lipid composition need to be tuned to host different proteins according to specific requirements such as phospoinositides and specific acyl chain length for protein function. Thus, the stability of the lipid membrane should be carefully studied in the production.

The basic principle in the assembly of biomimetic membrane components is to form a membrane across an aperture in a hydrophobic material or on a suitable support. There are mainly two methods in the production of biomimetic lipid membrane: one is the painting method where the lipid components are dissolved in a hydrocarbon and the mixture spread-painted across the aperture in the partition [17], the other is the folding method where the lipid components are spread as monolayers on two partition-separated air−water interfaces using a low-molecular-weight hydrocarbon and subsequently folded across the aperture [18]. For membrane formation on supports, various deposition methods are used to deposit the lipid membrane on a surface including Langmuir−Blodgett (vertical) or Langmuir−Schafer (horizontal) transfers from an air−water interface [19] or via vesicle collapse [20]. Langmuir−Blodgett and Langmuir−Schafer methods can result in micro-sized bilayer lipid membranes easily while thermal conditions should be optimized in vesicle collapse. And vesicle collapse method can be applied in lipid membranes which require large protein reconstitution [21].

In order to achieve biomimetic lipid membrane with good protein reconstitution, different strategies are studied in the production techniques of lipid membranes. As mentioned above, biomimetic lipid membranes are formed on a material or support which is crucial for the formation of membranes. Supports for the formation of biomimetic lipid membranes mainly include two types, solid support and porous support. For biosensors which do not require massive flux of matter across the membrane, the formation of lipid membrane can be realized on a solid surface

[22] such as the smooth surfaces of cleaved mica, silicon as shown in Fig. 13.3. The lipid membrane can be deposited directly on the solid surface as shown in Fig. 13.3a. Despite its simplicity, this method may lead functional proteins too close to the surface and further inactivate the proteins. Also, some polymer

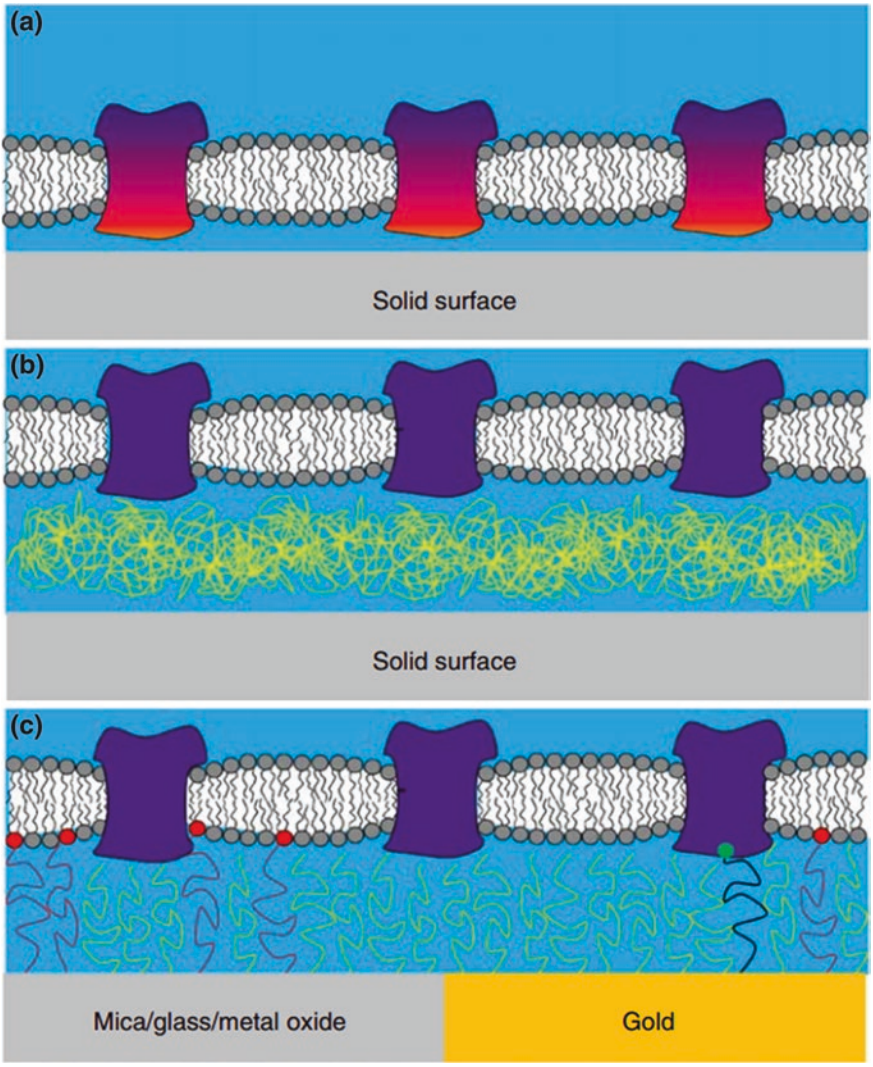

Fig. 13.3 Cross-sectional examples of solid-supported biomimetic membranes. **a** Direct deposit on a hydrophilic surface. This may make membrane proteins (*red*) too close to the surface and inactivate the protein; **b** Cushion-supported biomimetic membrane (*yellow*); **c** Layers grafted covalently on to the support using spacers with silane groups reacting with hydroxyl surfaces or spacers with thiol groups bonding on gold surfaces (*orange*). This cushion can non-covalently interact with lipid membranes (*yellow spacers*) or covalently interact with lipids (*red spacers*) or proteins (*blue*) (Reproduced with permission from Ref. [14]. Copyright 2009 Elsevier)

materials can be deposited on the solid surface in advance as shown in Fig. 13.3b, which can effectively reduce the inactivation of proteins. More complicated surface modification techniques are introduced for the support modification as shown in Fig. 13.3c. Various hydrophilic spacers such as poly (ethylene glycol) are utilized as modification materials in which the cushion can covalently attach with lipids or membrane proteins. With covalent bonding to the support, the biomimetic lipid membrane can be produced with enhanced stability and portability [23–27].

For biomimetic lipid membranes without massive flux, solid-cushion supports can meet the requirements. However, for lipid membranes demanding massive flux across the membrane, the cushion side cannot provide sufficient flux for ion transportation. In these cases, various porous supports are applied for the formation of lipid membranes. The porous material must be sufficiently porous to allow massive flux and be dense enough to support the membrane in the presence of transmembrane pressure gradients. Some hydrogels [28, 29] and bacterial surface [30, 31] layers are studied for formation of lipid membranes which demonstrate good stability for ion transportation. Besides, many nanoporous materials are used as the supports for formation of biomimetic lipid membranes. Some additional materials can also be used for modification on nanoporous supports to enhance the performance in the formation of lipid membranes such as diblock copolymers [32].

13.2.3 Excitability of Biomimetic Lipid Membrane

Solute transportation is the basic function of biological lipid membrane, which maintains the balance of intra- and extracellular environment. Thus, the key question for biomimetic lipid membrane is that whether it is possible to reconstitute functional proteins in the biomimetic membrane. And the approach to assess the excitability of biomimetic lipid membrane is to study the solution exchange capability with different inlaid proteins.

The Na^+/K^+-ATPase together with the sarcoplasmic Ca^{2+}-ATPase and the gastric H^+/K^+-ATPase have been reconstituted on solid supported membranes [33] with functionality demonstrated using photolabile (caged) ATP. This lipid membrane with the inlaid Na^+/K^+-ATPase pump protein can enable rapid solution exchange and this membrane can also be studied in conditions without ATP [34]. By constituting the Na^+/K^+-ATPase and Ca^{2+}-ATPase pump proteins in biomimetic lipid membrane, the current or potential change can be measured due to solution exchange by pump protein transportation, which provides information about the reaction mechanism of enzymes and ion concentrations. This method provides a promising way to incorporate ATPases with other functional proteins such as ion channels. Also this kind of biomimetic ATPase-inlaid membrane can be used for spectroscopic investigation of the protein and its regulation [35]. Other reversible ATPases such as F-type and V-type have also been investigated to be reconstituted with biomimetic lipid membranes because they couple ionic gradients directly to motion.

Besides the ATP-driven transportation in biomimetic membranes, some light-driven transportations also occur with ion pump proteins reconstituted on the membrane [36]. Bacteriorhodopsin and halorhodopsin are examples of light-driven ion pumps for protons [37] and chloride ions, respectively. However, this biomimetic lipid membrane is required to be deposited on a porous alumina substrate and to absorb purple membrane patches onto the membrane. And the biomimetic membrane is stable enough for days and can be used for stationary photocurrent measurements. Also the current information provides insight into how biomimetic ion pumps maintain their function by allowing differential rates of ion flow during pumping and resting states, creating a kinetic value [38].

In contrast to active transportation with pump proteins or carrier proteins, which requires extra driven energy, ion channel proteins are another important protein group in the lipid membrane which passively transports ions according to the concentration gradient. There are mainly two types of ion channel proteins sorted according to the function, one for solute transportation and the other for water transportation. For solute transport, ion channels are water-filled pores which span the bilayer and catalyze selective ion transmembrane movement. Typically, the voltage-gated K^+ channels [39] and proton channels [40] are common channel proteins on the lipid membrane with high selectivity and relative permeability. In terms of water transport, the water transportation can be achieved by a special ion channel, the aquaporin [41]. The solvent such as water can pass through the membrane with the water channel, leaving all other solutes behind. However, some recent studies have presented that the function of aquaporins is dependent on calmodulin [42], phosphorylation [43] and pH [44], etc.

13.3 Design of Biomimetic Membrane-Based Taste Biosensors

13.3.1 Mechanism and Coupling Techniques of Biomimetic Membrane

Following the basic research on lipid/polymer membranes [45–51], Toko group developed a Taste Sensing System correlated with the gustatory perception of living organisms by using artificial lipids as transducers for multichannel taste sensors [9, 52–55]. Further improvements led to successful development of advanced taste sensors capable of evaluating saltiness, sourness, bitterness, sweetness, umami and astringency. These taste sensors are based on very different concepts from the electronic tongue and feature global selectivity and high correlation with human sensory score. They offer satisfactory taste results closer to human sensory evaluation while eliminating the need for multivariate analyses and artificial neural networks. This chapter describes all aspects of these taste sensors based on artificial lipid, ranging from the response principle and optimal design methods to applications in the food, beverage, and pharmaceutical markets.

There are many taste substances but the sense of taste has five qualities: saltiness, sourness, bitterness, sweetness, and umami (savoriness) [56]. These qualities are called basic tastes and each plays an important role in humans. Saltiness, which is caused mainly by ionic materials, is a good indicator of electrolyte balance in foods; sourness, which is produced by organic acids, signals decomposition; bitterness, which is often considered distasteful, prevents intake of poisonous materials; umami, which is evoked by some amino acids, provides information on the presence of amino acids; sweetness, which is produced by sugars or sugar alcohols, has a role in indicating nutrient sources. Astringency, which is produced mainly by tannins, is sometimes considered a taste quality in the broad sense [57, 58].

The "fluid mosaic model" was proposed to explain the structure of biological membranes [59] in the early 1970s. In this model, proteins move in a sea of lipid molecules on cell membranes, including taste-cells. Recent advancements have identified the taste receptor cells on the human tongue for the five basic tastes [60–64]; their signal pathways [65] are shown in Fig. 13.4. There are about 100 taste receptor cells composed of a lipid bilayer in the taste buds of the human tongue. They are distributed across three types of papillae: circumvallate, foliate, and fungiform, located at the back, posterior lateral edge, and anterior of the tongue. Umami, sweet, and bitter compounds are received by seven transmembrane domain receptors interacting with intercellular G proteins, or G protein-coupled receptors (GPCRs). Several types of GPCRs (T1R1, T1R2, T1R3, and T2Rs) are involved in taste transduction. The T1R1 + T1R3 heteromer, T1R2 + T1R3 heteromer, and T2Rs GPCRs function as umami, sweet, and bitter receptors, respectively [60, 62]. In contrast, stimuli evoked by sour materials are thought to be perceived via a candidate sour receptor called the PKD1L3-PKD2L1 channel, which is a member of transient receptor potential (TRP) family [63, 66]. The salt receptor epithelial sodium channel (ENaC), which is an amiloride-sensitive Na^+ channel, allows Na^+ ions to enter the taste-cell membrane. In addition, the amiloride-insensitive channel vanilloid receptor-1 variant, functions as a non-selective

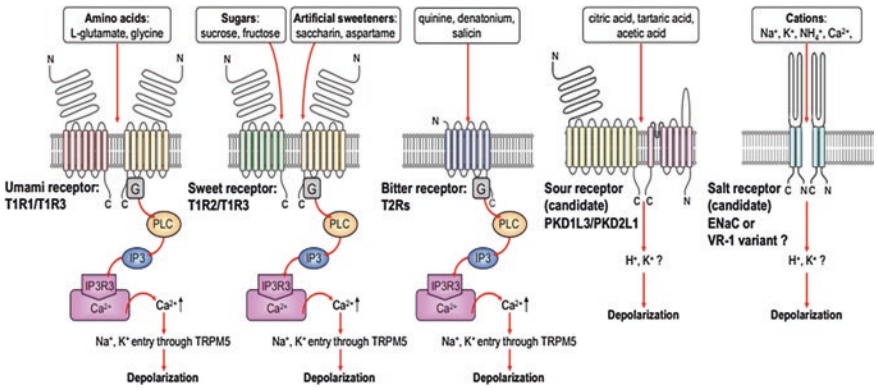

Fig. 13.4 Taste receptors for five basic taste qualities and signal transductions pathways (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

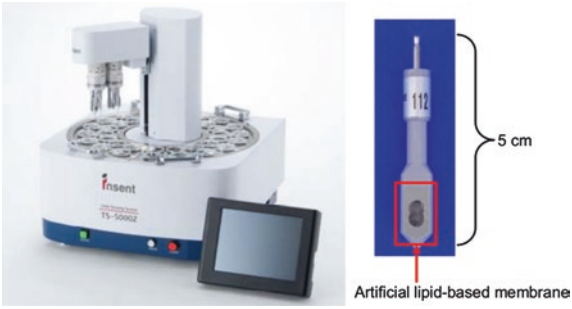

Fig. 13.5 TS-5000Z Taste Sensing System. *Left* TS-5000Z. *Right* Taste sensor (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

cation channel [64, 67]. However, it is still not known whether these channels serve as a salt receptor. All tastes are detected and perceived via these taste receptors, which mediate signal cascades through second messenger molecules [68–71].

The artificial-lipid sensors were made using tetradodecylammonium bromide (TDAB), trioctylmethylammonium chloride (TOMA), oleic acid, 1-hexadecanol, gallic acid, phosphoric acid di-n-decyl ester (PADE), and phosphoric acid di (2-ethylhexyl) ester (PAEE). Dioctyl phenyl-phosphonate (DOPP), 2-nitrophenyl octyl ether (NPOE), bis(1-butylpentyl) adipate (BBPA), bis(2-ethylhexyl) sebacate (BEHS), phosphoric acid tris(2-ethylhexyl) ester (PTEH), tributyl O-acetylcitrate (TBAC), 3-(trimethoxysilyl)propyl me thacrylate (TMSPM), diethylene glycol dibutyl ether (DGDE), and trioctyl trimellitate (TOTM) were used as the plasticizer. The polymer support was polyvinyl chloride (PVC). Tetrahydrofuran (THF) was used as the preparation solvent. Various amounts of lipid and plasticizer were mixed for 1 h in 10 mL of THF, depending on the taste sensor type. The mixture was dried in a Petri dish at room temperature for 3 days to form the transparent membrane. The membrane was attached to the sensor surface using a solution of 800 mg of PVC and 10 mL of THF. The Ag/AgCl electrode with a single ceramic junction is the reference electrode. A solution containing 3.33 M KCl and saturated AgCl was used as the inner solution for the sensors and reference electrode. These electrodes were conditioned for 2 days in a solution of 30 mM KCl and 0.3 mM tartaric acid before measurement. Figure 13.5 is a photograph of the fourth TS-5000Z model composed of a sensor unit and management server. Up to 8 sensors can be connected to the control center and different taste information is collected by taste sensors to analyze and discriminate different tastes.

13.3.2 Taste Sensor Design

There are four requirements for objective taste evaluation: (1) The taste sensor must respond consistently to the same taste like the human tongue (global selectivity); (2) The taste sensor threshold must be the same as the human taste threshold; (3) There must be a clearly defined unit of information from the taste sensor; and (4) The taste sensor must detect interactions between taste substances.

Item (1) eliminates use of multivariate analyses, making it easy to interpret sensor output data with regard to taste quality. Item (2) provides results mimicking the human gustatory sense. Item (3) is essential for objective evaluation of taste. For example, data cannot be interpreted as taste quality or intensity if it is unclear what the graph axes explicitly represent in principal component analysis (PCA). Therefore, if the origins of all the samples are unknown, it is impossible to interpret both taste quality and intensity in the analysis. Item (4) enables sensor data to be consistent with sensory evaluation scores even when interactions between taste materials increase or decrease taste intensity. When the first Taste Sensing System was launched in 1993, all taste sensors had low taste selectivity, causing difficulties in evaluating samples with unknown taste. Although the first Taste Sensing System used PCA to classify samples based on information from the low-selectivity sensors, the result was just the sum of less taste information.

Two methods were proposed to improve the selectivity and sensitivity of taste sensors: modulating the electric charge density of the membrane and the hydrophobicity of the membrane surface. To meet the first requirement for global selectivity (i.e. like the human tongue, the taste sensor must respond consistently to the same taste), modulating electric charge density of the membrane is quite effective for improving selectivity and sensitivity to bitter and astringent materials [72]. Figure 13.6 shows the relationship between lipid concentration in membrane and relative value for a bitterness sensor composed of the positively charged lipid, tetradodecyl ammonium bromide (TDAB), and the plasticizer, 2-nitrophenyl octyl ether (NPOE). The sensor is very sensitive to bitter materials, such as iso-alpha acid, which is negatively charged in a solution. As shown in Fig. 13.3, the relative value for NaCl increases negatively with the lipid concentration due to the screening effect of the electrolyte, Cl^- anions. The findings suggest that achieving high sensitivity to bitter or astringent substances requires incorporating appropriate amounts of lipid into the membrane to cause the maximum shift in membrane potential by changing the electric charge density. Sensitivity and selectivity to salty and sour substances can be achieved by incorporating more lipids

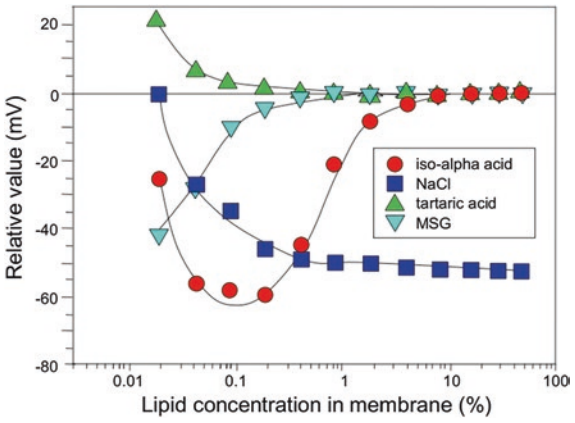

Fig. 13.6 Relationship between lipid concentration in membrane and relative values of bitterness sensor. The concentrations of each sample are: iso-alpha acid, 0.01 vol. %; NaCl, 300 mM; tartaric acid, 2.7 mM; MSG, 10 mM. All samples include 30 mM KCl and 0.3 mM tartaric acid as supporting electrolyte [65] (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

into the membrane, helping reduce sensitivity to bitterness and astringency [73] as described above. A medium amount of membrane lipids shows highest selectivity for umami [73].

Another approach to meeting the first requirement for global selectivity is optimizing the hydrophobicity of the membrane surface. An example of developing another bitterness sensor using this approach is described below. Bitter substances are sensed by the T2Rs bitter taste receptors, but are also thought to be adsorbed on the surface membrane of taste cells [74]. To control adsorption, LogD was focused on, which is known to be correlated with hydrophobicity [75, 76]. Therefore, taste sensors based on 8 plasticizers with different hydrophobicity were examined for sensitivity and selectivity to several taste substances (Fig. 13.7) [65, 77]. The sensors with BBPA, BEHS, PTEH and TBAC plasticizers were very selective for quinine hydrochloride although all were based on PADE lipid, suggesting that hydrophobicity of the membrane significantly affects sensitivity and selectivity to bitterness produced by positively charged bitter substances. Interestingly, sensors with no lipid did not respond to bitter substances at all, even when the membrane contained any of the four plasticizers. This indicates that both substantial lipid content and a plasticizer with appropriate hydrophobicity are needed for high selectivity and sensitivity and selectivity.

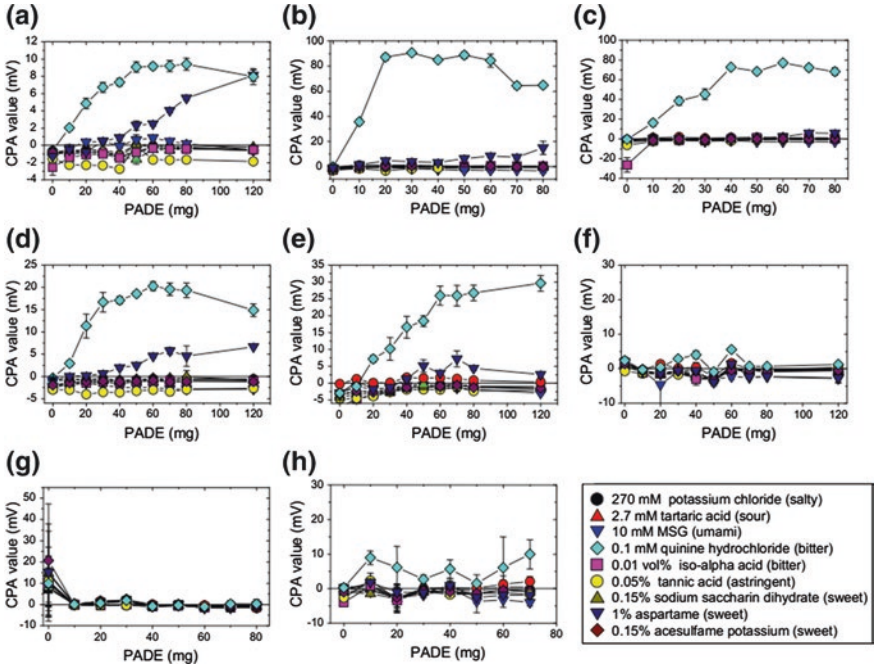

Fig. 13.7 Sensor responses to basic taste substances. The x-axis represents PADE contents in the membrane, while the y-axis shows the CP A value [65] (Reproduced with permission from Ref. [70]. Copyright 2009 Elsevier)

13.3.3 Sensor System and Data Processing

Compactness, portability, high specificity, and global sensitivity are the main advantages of biomimetic membrane-based taste biosensor in bionic taste analysis. Individual taste biosensor demonstrates global sensitivity to various analytes instead of high selectivity to specific substances. Nevertheless, taste is comprised of five basic qualities: sourness, saltiness, bitterness, sweetness and umami taste, in which one taste can be generally caused by many substances. For example, sourness taste can be perceived in solutions with hydrochloric acid, acetic acid, citric acid, etc. Thus, individual taste biosensor cannot satisfy the demands for taste analysis, and taste sensor system consisting of multiple taste sensors is proposed to overcome the barrier. Multiple taste biosensors are cross-sensitive to various substances in samples and different sensitivity of sensors should be guaranteed to ensure distinction of acquired signals.

The performance of biomimetic membrane-based taste biosensor depends on the constitution of the sensor system to a great extent, generally by immobilization of different membranes or proteins on the lipid sensor. The sensor system generally comprises of the working electrode, the reference electrode, measuring circuit, and computer for data management. Figure 13.8 shows a diagram of the taste sensing system with the taste sensor acting as the working electrode [77]. The Ag/AgCl electrode with a single ceramic junction is the reference electrode. A solution containing 3.33 M KCl and saturated AgCl is used as the inner solution for the sensors and reference electrode. These electrodes are conditioned for 2 days in a solution of 30 mM KCl and 0.3 mM tartaric acid before measurement.

After data acquisition by sensor system, the results are always processed with various data processing methodologies, thus obtaining the taste results and visualizing them on a plot. The main aim of data processing is to recognize different

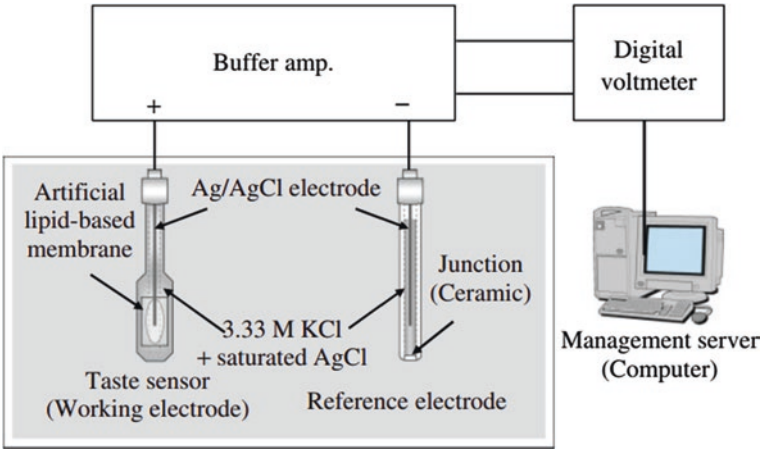

Fig. 13.8 Diagram of taste sensing system (Reproduced with permission from Ref. [70]. Copyright 2009 Elsevier)

tastes, to discriminate among various objects and to evaluate different quality of objects. Data processing including chemometrics, pattern recognition, and multivariate calibration are used to deconvolute complex signals from sensor system and produce qualitative and quantitative information about multicomponent solution. Generally, data processing methods consist of two main steps [78]. First, data is always preprocessed in order to normalize the data, remove redundant information and extract independent factors in data. Thus, irrelevant and redundant information is eliminated from all signals. After that, the model between variables and target variables should be constructed for qualitative and quantitative analysis.

Mainly, the processing of the data form the sensor system is performed using principal component analysis (PCA), multivariate regression analysis (MRA), partial least square (PLS) regression, artificial neural network (ANN), etc. PCA is a conventional linear feature extraction method that consists of projecting the *m*-dimensional dataset, *m* being the number of sensors, in a dimension smaller than m. The uncorrelated and orthogonal coordinates of this reduced space are the eigenvectors (principal components) of the covariance matrix of the dataset. These new variables are more descriptive because they are chosen to describe the maximum amount of variance in a data matrix. The eigenvalue of a principal component is directly related to the percentage of "information" contained in the corresponding component, so that only the most relevant components can be preserved. PCA is a very useful classification and evaluation technique for taste biosensor analysis. Taste discrimination can be realized on a PCA plot with different directions in the pattern space. The extracted principal components after PCA may also be used as inputs in further data processing such as ANN.

MRA is another useful method to obtain the experimental predicting or controlling criterion variables by determining a linear expression explaining the relationship between the criterion and explanation variables. The correlation coefficient between the experimental Y_{exp} and predicted Y obtained from the computed multiple regression models is designated as the multiple correlation (R). R_2, a multiple determination coefficient, express the explained ratio of variation in Y from the multiple regression models. Combined with different data processing, the acquired data from taste biosensor can be extracted and used for taste analysis.

PLS and ANN are supervised methods in which models between two variable metrics should be constructed. Only after modeling with training data, the models can be used for qualitative and quantitative taste analysis. PLS modeling is performed to find the multidimensional direction in the independent variable space that explains the maximum multidimensional variance direction in predicted variable space. Compared to conventional MRA, PLS modeling can be used in variable matrices with multiple correlation problems, which always exist in taste biosensor results. And noisy signals in data can be easily distinguished and eliminated by PLS. ANN is an approach for data processing by mimicking the behaviour of real neural systems. The nonlinear correlation between two variable metrics can be trained and modeled after ANN analysis and ANN is also widely applied in taste analysis. Other methodologies such as Canonical correlation analysis (CCA), canonical discriminant analysis (CDA), and linear discriminant analysis (LDA)

are also used for taste analysis in some cases. It should be noted that there is no approach that can be applied in all taste data with high reliability and accuracy. Considering the critical role of data processing in taste analysis, different data processing methodologies should be optimized for best performance in taste analysis.

13.4 Applications of Biomimetic Membrane-Based Taste Biosensors

13.4.1 Food Taste Measurement and Quality Control

Taste sensors have applications in manufacturing of beverages, including beer, wine, green tea, sake, coffee, soybean paste, milk, and soy sauce, as well as in production of foodstuffs, such as rice, pork, and tomatoes. The so-called "radar chart" is one method for understanding multivariate taste information at a glance. Figure 13.9 shows radar charts from taste sensors for beer and green tea. All the taste information have the same meaning, where the difference of 1 unit corresponds to the smallest taste difference that a person can distinguish. Also, the "reference solution," tasteless sample composed of 30 mM KCl and 0.3 mM tartaric acid is used with all related taste information set to zero. In this case, when the taste information value is 12.6, it is equivalent to the same degree of taste intensity as the concentration of the standard sample used for calculating the conversion factor. For example, when the "saltiness" taste information is 12.6, the saltiness intensity is considered to be equivalent to 270 mM of potassium chloride. In other words, when a tasteless sample is used as the control, this analysis is an "absolute comparison." Figure 13.9a shows that all beer samples have strong sourness, bitterness, and

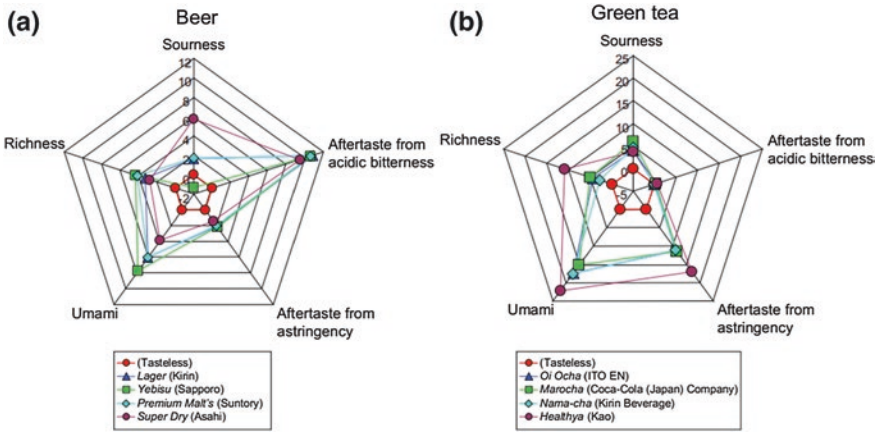

Fig. 13.9 Radar charts for beer and green tea (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

umami, clearly reflecting the taste of beer. By contrast, in Fig. 13.9b, all green tea samples have strong astringency, umami, and richness, demonstrating that taste sensors provide explicit information on the taste of green tea.

Figure 13.10 shows a taste map with various beers from different countries [79]. The taste sensor is designed for evaluation of bitterness and sourness of the beers and to discriminate different brands of beers. In the taste map, the ordinate indicates the bitterness or malt taste tested with different beers and the abscissa represents the sourness or dry taste of beers. With the two sets of data provided by the taste sensor, the taste of different beers can be evaluated and discriminated, which also provides information about beer tastes preferred in different countries. Tastes of various foods and beverages can be evaluated and discriminated based on different signal responses using taste sensing systems, such as milk [80], rice [81], pork [82], table salt [83], and ginseng [84].

Food safety is the focus of consumer attention worldwide and the food and beverage industry requires strict quality control. Taste sensors can play an important role in the food and beverage industry by detecting deteriorated taste qualities. Figure 13.11 shows changes in the taste of commercial PET-bottled green tea due to heat aging. Six types of taste information: "acidic bitterness", "astringency", "aftertaste from acidic bitterness", "aftertaste from astringency", "umami", and "richness" were measured, and all taste information for control samples without heat deterioration was set to zero. "Aftertaste from acidic bitterness" increased

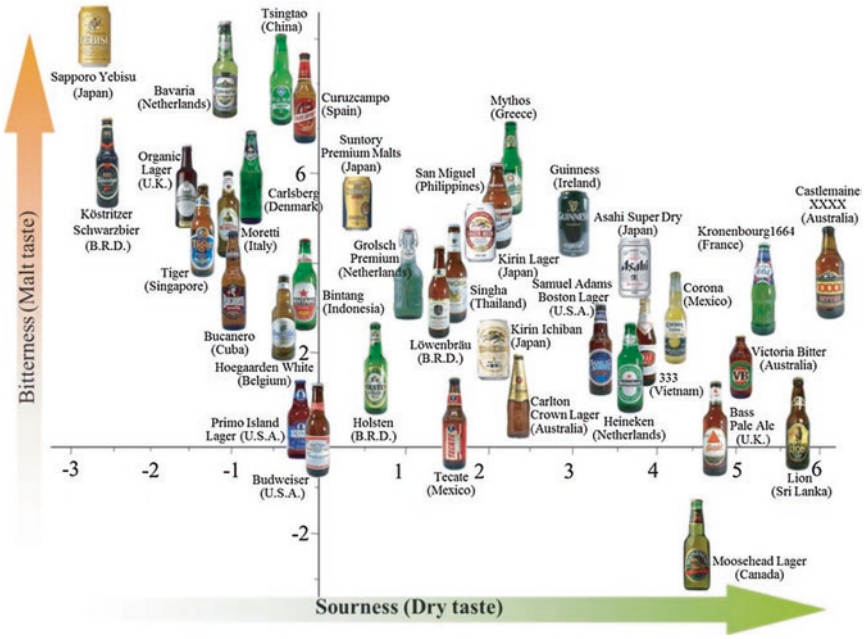

Fig. 13.10 Taste map of beer (Reproduced with permission from Ref. [72]. Yusuke Tahara and Kiyoshi Toko)

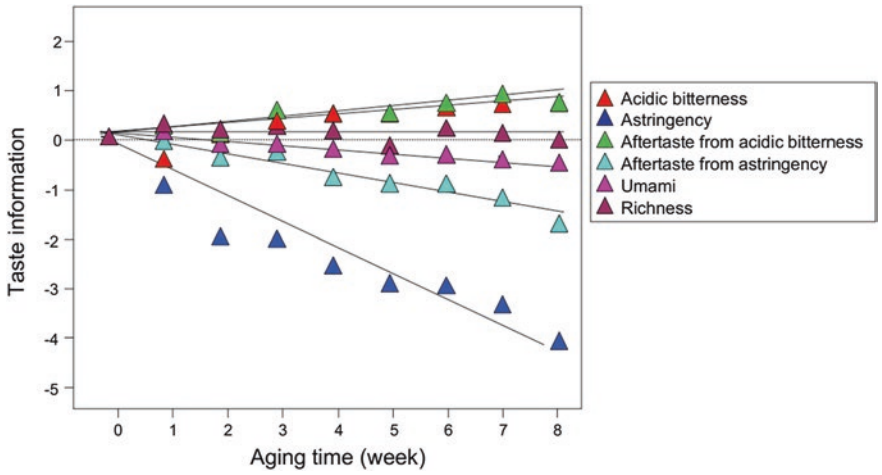

Fig. 13.11 Change in taste qualities for green tea with aging. All green tea samples were stored in a temperature bath at 60 °C for up to 8 weeks (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

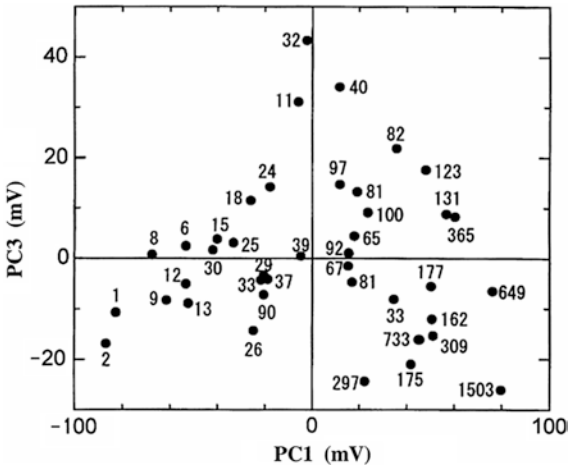

Fig. 13.12 Taste map of 41 kinds of mineral water (Reproduced with permission from Ref. [78]. Copyright 2000 Elsevier)

with aging, while "astringency" decreased. Astringency is usually an appreciated quality while bitterness is deprecated because it is not found in fresh tea. The results indicating deterioration of green tea with aging show how taste sensors can be effective in quality control.

Besides assessment of flavor change to different food and beverage, quality control of drinking water is also another important topic for people due to its crucial role in daily life. However, no convenient approaches are available to evaluate the quality of drinking water. Biomimetic taste sensor can solve this problem with specific taste sensor system. Figure 13.12 shows the result of PCA applied to

response patterns for 41 kinds of commercial mineral water [85]. The right-lower plane contains mineral water with strong hardness and the left-lower plane represents soft water. Though it is actually very hard for human to discriminate different brands of mineral water, the taste sensor have very good response to different kinds of water in spite of the subtle difference. Based on the information, different water can be discriminated and evaluated, which provides a promising way to control the quality of waters for both producers and consumers. And because the taste sensor is very sensitive to ions in water, some toxic substances in drinking water can also be measured such as CN^-, Fe^{3+}, and Cu^{2+}. So far, some taste sensors have already been applied to measure the contamination of factory drains [86].

13.4.2 Suppression Effect Evaluation

Taste substances interact with each other, increasing or decreasing the intensity of the six taste qualities, including astringency. This is called the synergistic/suppression effect. As a result, even when all taste materials in a product have been quantified by chemical analysis, the actual taste still cannot be evaluated, explaining why a taste sensor must detect interactions between taste substances.

In the pharmaceutical industry, evaluating the bitterness of drug products is very important because almost all active pharmaceutical ingredients in drug products are bitter. Therefore, drugs are usually formulated with sweeteners, such as sucrose, to suppress bitterness. Taste sensors can be used to evaluate both drug bitterness [87–90] and bitterness suppression effects [91–93]. Further, taste sensors are presently being studied for specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, and robustness for drug products [94], according to International Conference on Harmonisation guideline Q2.

Figure 13.13 shows the bitterness suppression effect of the bitter-masking materials sucrose, α-cyclodextrin, and BMI-40 on quinine hydrochloride using

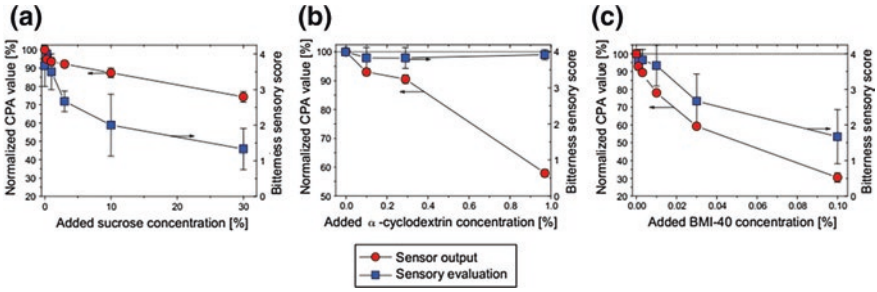

Fig. 13.13 Bitterness suppression effect of bitter-masking materials on quinine hydrochloride using BT0 bitterness sensor. CPA values are normalized to 100, and expressed as mean ± SD (n = 4). The standard deviation for sensory evaluation score is the difference between volunteer taste panels (n = 3). All samples include 10 mM KCl as supporting electrolyte (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

the BT0 bitterness sensor. Sucrose is a sweet substance used widely to suppress drug bitterness; α-cyclodextrin is a hydrophilic compound with a hydrophobic cavity and forms an inclusion complex by including hydrophobic compounds in the cavity [95]; BMI-40 is composed mainly of phosphatidic acid and suppresses drug bitterness by trap and masking effects [96]. In Fig. 13.13a, addition of sugars to quinine solution decreases CPA values by 20 % as sucrose concentration increases and sensory evaluation bitterness scores decrease greatly with addition of sugars. This suggests that the sensor can detect the suppression effect of sucrose. Addition of α-cyclodextrin greatly decreases the CPA value despite little decrease in the sensory evaluation score (Fig. 13.13b). The sensory test has also confirmed that the bitterness sensory score of the quinine solution with addition of 9.7 % α-cyclodextrin is decreased to 2.83, suggesting that α-cyclodextrin has low ability to suppress bitterness. This demonstrates that the sensor has a better ability to detect the suppression effect of α-cyclodextrin. With BMI-40, the CPA value decreases greatly with increasing BMI-40 concentration (Fig. 13.13c), indicating that BMI-40 has the highest ability among the tested bitter-masking materials to suppress the bitterness of quinine hydrochloride. The corresponding decreased sensory evaluation score indicates a good agreement between the sensor and sensory evaluation score.

Interestingly, this bitterness sensor does not respond to such bitter-masking materials. The sensor detects the suppression effect because it responds to drugs based on various interactions between the sensor and the bitter-masking materials. Figure 13.14 shows some possible mechanisms for sensor response to the suppression effect. This bitterness sensor has a negatively charged lipid that reacts strongly by hydrophobic interaction with the positively charged quinine hydrochloride. Sucrose does not interact directly with bitter substances, so it is believed to inhibit adsorption of bitter substances by the sensor by covering the

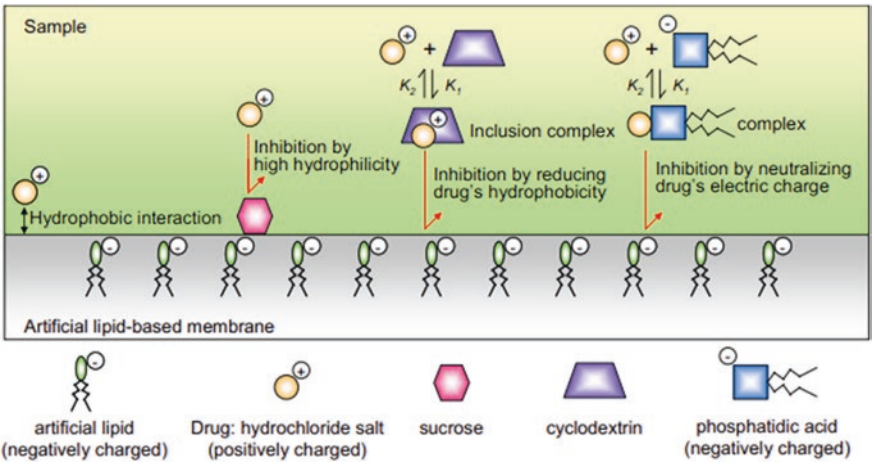

Fig. 13.14 Possible mechanisms of suppression effect of bitter-masking materials (Reproduced with permission from Ref. [58]. Yoshikazu Kobayashi)

sensor surface. As mentioned above, cyclodextrins interact selectively with bitter substances, so cyclodextrins are believed to inhibit the adsorption by inclusion. Since a CPA value cannot be observed for the BMI-40 solution, which contains some phospholipids such as phosphatidylinositol, phosphatidic acid, phosphatidylethanolamine, and phosphatidylcholine, it is considered to suppress bitterness by binding and neutralizing bitter substances in an aqueous solution, as shown in Fig. 13.14. However, a negatively charged sensor immersed in a solution of BMI-60 also showed the suppression effect on the bitterness of quinine hydrochloride, suggesting that some of the phospholipids in the BMI-40 and BMI-60 suppress bitterness by partial covering on the sensor membrane.

13.5 Summary

Biomimetic membrane-based taste biosensor demonstrates global sensitivity to enormous chemical substances and can transform this information into gustatory perception in taste intensity and quality. From this perspective, this kind of taste biosensor mimics the functionality of human tongue, which is hardly achieved by other traditional sensors. Hence, the biomimetic membrane-based taste biosensor is widely utilized in food and pharmaceutical industry for taste analysis. In these applications, biomimetic taste biosensor can successfully discriminate different tastes and species. Especially in pharmaceutical industry, bitterness suppression is the result of interactions among various chemical substances. It is impossible to evaluate the suppression effect by detecting the quantity of different substances. Biomimetic membrane-based taste biosensor exhibits extraordinary merits in taste evaluation. More and more studies are ongoing to apply the sensor to wider fields. Biomimetic membrane-based taste biosensor will provide a reliable approach for taste discrimination and evaluation in various areas.

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Chapter 14
In Vivo Bioelectronic Tongue

Zhen Qin, Bin Zhang and Liang Hu

14.1 Introduction

Over the past two decades, the development of biomimetic techniques for chemical sensing has been promoted by research in chemical signal transduction mechanisms. Much work has been done in the development of bioinspired sensors which combined biological functional components with various secondary sensors [1]. Taking advantage of mammalian chemical sensing mechanisms, many kinds of biological components originating from gustatory system have been used as recognition elements, including gustatory cells, gustatory tissues, and taste-related proteins [2, 3]. Comparing to conventional sensitive materials such as lipid membranes, biological taste components have the merits of fast response, high sensitivity, and excellent specificity for potential applications in many fields.

Despite the growing interests of bioinspired taste sensors, there are so far limited number of systems using in real applications. The reasons for this mainly include: (1) we still lack basic understanding of taste stimulus space. Mechanisms of different basic tastes and function of gustatory receptors are not very clear; (2) isolated cells and tissues requires specific conditions, which makes the sensor complicated; besides; (3) biomaterials are easily inactivated in the artificial environment [4]. However, progress in the field of neural recording and decoding methods offers another way to set up an in vivo bioelectronic tongue using whole animal as sensing element. Recording neural activities in the gustatory-related

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brain regions and assessing taste by neural decoding methods should overcome shortcomings and difficult points of in vitro bioinspired taste sensors [5].

The present chapter first makes an introduction of the anatomy of mammalian gustatory system, elaborates upon principle gustatory pathways from oral cavity to brain and roles of important relays in taste representation. Second, type of detection methods, animal training and surgery protocol will be discussed. We also address issues such as coding and decoding of taste signals, discrimination among tastants, as well as some potential applications of in vivo bioelectronics tongue.

14.2 Theories of In Vivo Bioelectronic Tongue

14.2.1 The Human Sense for Taste

The sense of taste is a common ability shared by all organisms and is used to detect nutrients as well as potentially harmful compounds. The five basic taste qualities, bitter, sour, salty, sweet, and umami, composing the sense of taste characterize food, both in solid and liquid state, in various ranges. Bitter detects potentially toxic compounds that usually should be avoided; sour detects protons which indicate spoiled food; salty is used to identify ions that are needed to maintain ionic balance; sweet is used to detect energy rich carbohydrates and umami detects glutamate and other amino acids. Descending from ancestors, human beings, and other creatures, like rodents, inherit the fast response, high sensitivity, and specificity gustatory system, which are crucial for survival, and its impairment often leads to malnutrition and perhaps death.

Human sense for taste has already come into service in food industry to monitor quality and maintain a strict standard. Occupations have been derived from this demand such as sommeliers and other tasters for chocolate and vinegar. However, these occupations require sky-high sensitivity of taste for practitioners, and often involve training and screening, hence, such practitioners have been scarce resources and situation called for improvement. Taste sensor arrays have been proposed to alleviate this shortage of personnel and individual difference by imitating human beings' gustatory system, and lipid membranes and chemical sensors have been adopted as sensitive elements in analyses of solutes and their corresponding concentrations in solutions, whereas dilemma appeared when the taste of the mixture did not conform to the linear addition of separate tastants due to the lack of in vivo interactions between receptors or neurons.

As mentioned above, the application of human taste taking advantage of natural gustatory system can be traced back to the emergence of sommeliers, but the concept of in vivo bioelectronic tongue has not been presented since decades ago with rodents substituting for human beings. Electrophysiology signals replace artificial judgments which narrate the characteristics of tastants in the solution. In vivo bioelectronic tongue, based on similar theories of human sense for taste, was almost perfectly realized for both physiological researches and some elementary applications.

14.2.2 Biology and Electrophysiology of the Gustatory System

As is mentioned in former chapters, gustatory processing is first achieved at the level of gustatory receptor cells (TRCs) that are assembled into taste buds (TBs) distributed among different papillae of the tongue, palate, larynx, pharynx, and epiglottis [6]. TBs contain about 100 TRCs that protrude through the lingual epithelium into a taste pore (Fig. 14.1a). Upon tastant binding to receptors on microvilli of TRCs, transduction machinery is activated and neurotransmitters are released which cause the excitation of afferent nerve fibers. The two afferent branches of the facial nerve (VIIth) that innervate the anterior tongue and the palate is chorda tympani nerve (CT) and greater superior petrosal (GSP), respectively. The posterior and lateral tongue areas are innervated by the lingual–tonsilar branch of the glossopharyngeal (GP or IXth) nerve, while in the larynx, pharynx

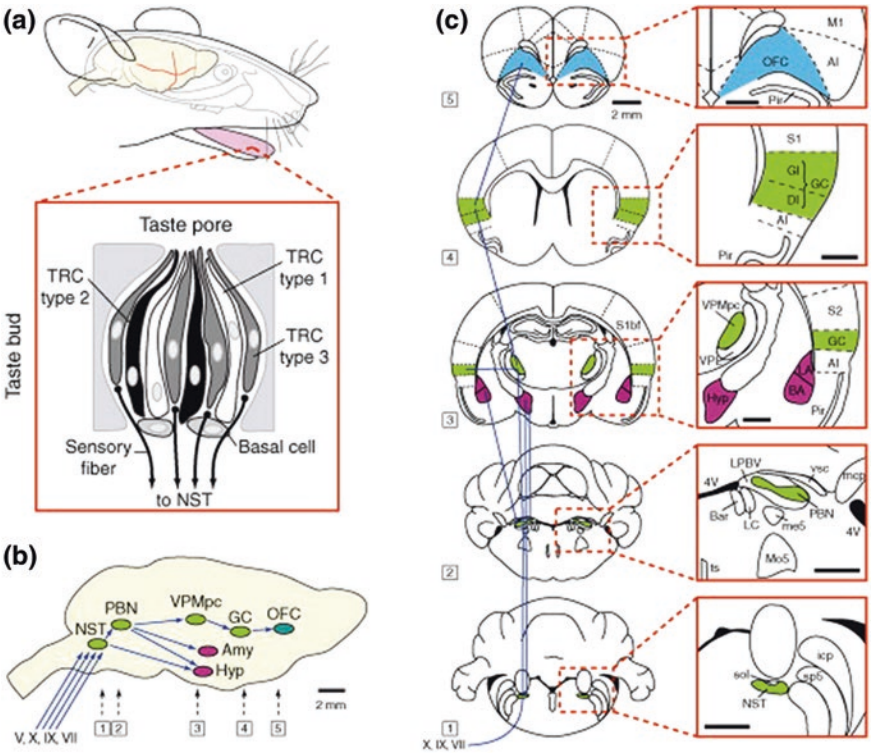

Fig. 14.1 Schematic representation of the rodent taste-pathway organization. **a** TRCs are the chemical sensors and exist in taste buds distributed into different papillae of the tongue and the oral cavity. **b** Three cranial nerves innervate different parts of the oral cavity and convey taste information to the brain. **c** Anatomical overview of the central taste pathways. Scale bar 1 mm in red boxes (Reproduced with permission from Ref. [7]. Copyright 2010 Elsevier)

and on the epiglottis, TRCs are innervated by the superior laryngeal branch of the vagus nerve (Xth) [7]. The two discrete branches of CT project, respectively, to the rostral part of nucleus of solitary tract (rNST) that is involved in taste processing, and to the medullary reticular formation (RF), which a caudal brainstem pathway leading to reflex oromotor functions [8]. The GP and vagus nerves are known to function in swallowing, gagging, salivary secretions, and motor responses involved during eating [9].

These three cranial nerve (CN) branches converge together with the lingual branch of the trigeminal nerve (Vth) in the medulla to synapse in the rNST (Fig. 14.1b), where the rodent and primate gustatory systems differ. In rodents, fibers from the rNST projects ipsilaterally to the PBN (Fig. 14.1b, c), whereas rNST fibers project directly to the parvocellular division of the ventroposterior medial nucleus of the thalamus (VPMpc) in primates. From the PBN there are reciprocal projections to the ventral forebrain, the bed nucleus of stria terminalis, the lateral hypothalamus (LH), the basolateral amygdala (BLA) [10]. These structures are believed to be involved in the processing of taste-related tasks, such as feeding and taste memory formation.

The gustatory fibers from the VPMpc terminate in the primary gustatory cortex (GC) which is a brain structure responsible for the perception of taste. It consists of two substructures: the anterior insula on the insular lobe and the frontal operculum on the inferior frontal gyrus of the frontal lobe [11]. The primary gustatory cortex is sometimes referred to in the literature as the AI/FO because of its composition (Anterior Insula/Frontal Operculum) [12].

14.2.3 Modeling of the Gustatory System Electrophysiological Activity

There are presently two mainstream hypotheses on how the gustatory information is carried by innervated nerve fibers, labeled line (LL) and across-fiber pattern (AFP) theories [13, 14]. The former one refers to a model in which peripheral or central neurons that respond the most robustly to a certain taste quality carry the overall information via segregated pathways, which is put forward based on observations that gustatory receptors that transduce disparate taste qualities into electronic signals are found in their own dispersed clusters of gustatory receptor cells. Although peripheral nerve fibers are known to be broadly tuned, which means they respond to more than one taste quality. Under such circumstance, the quality that evokes the largest response, the so-called best stimulus, predicts the relative response magnitude evoked by other taste qualities [15]. In contrast, the latter one asserts that taste qualities are represented by the activation across a population of fibers rather than in dedicated subsets of fibers as in the LL theory. In effect, all members of the array of broadly tuned nerve fibers can convey information about any taste stimulus. As shown in Fig. 14.2, in hypothetical LL model, input from gustatory receptor cells that express bitter receptors is encoded along a labeled

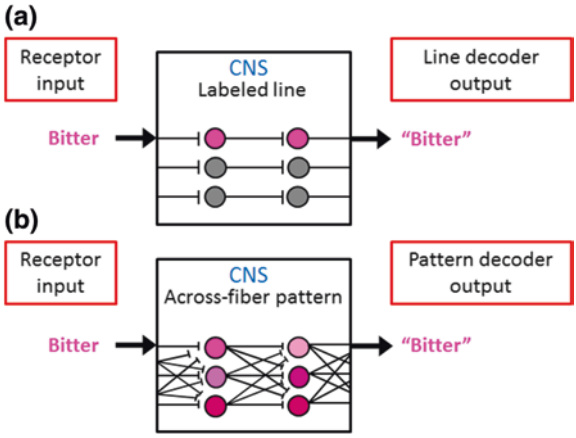

Fig. 14.2 **a** Schematic of labeled line theory. **b** Schematic of across-fiber theory

line in the central nervous system (CNS): information about a bitter stimulus is received exclusively by central neurons that respond only to bitters. A central labeled-line "decoder" could then know that a "bitter" stimulus is present when the bitter "line" is active. In AFP model, input from gustatory receptor cells that detect bitterness is distributed across neurons and represented by a pattern code in the CNS. Here, a bitter stimulus produces a unique pattern of activation across cells. A central pattern decoder could recognize that a bitter stimulus is present through knowledge of this pattern. Under either coding strategy, the stimulation of bitter receptor cells results in the correct recognition of a bitter stimulus.

The discovery that cells in taste buds which express taste quality-specific receptors are innervated by taste quality-specific fibers was originally one of the footholds of the LL theory. Evidences, such as the finding that sodium deprivation has been shown to affect responses to NaCl in NaCl-best fibers only [16, 17], indicates stimulus-specific fibers may have unique behavioral functions. Furthermore, a recent study showed that in marmosets, compounds which stimulated sucrose-best fibers were preferred, whereas compounds that stimulated quinine-best fibers were avoided [18]. However, the most convincing evidence for the LL theory comes from recent research utilizing transgenic animal models. The gene knockout mice with deletion of the five basic gustatory receptors, sweet, sour [19], bitter [20], salty [21], umami [22], are found to show no response in CT nerve when stimulated with corresponding tastant, while no obvious influence in response to other qualities of taste. A more intriguing phenomenon was observed when transgenic mice with bitter receptors expressed in gustatory cells natively expressing sweet receptors, had a preference for bitter compounds compared with pure water [20]. In another research performed by Zhao and colleagues [22], receptors for opioid, which is a normally tasteless substance to rats, were expressed in rats gustatory cells that originally express sweet receptors, which resulted in the rats' preference for opioid agonist. Inversely, if the receptors for opioid were expressed in bitter-responsive TRCs, rats rejected opioid agonist as if it tasted bitter. Together, these

data has strongly indicated that the gustatory system may adopt the quality-specific LL model, at least in the periphery.

Meanwhile, there is mounting evidence for the AFP theory. Unlike the LL theory deduced from genetically modified technologies, the AFP theory was proposed based on the findings that TRCs and innervated nerve fibers are broadly tuned, which means they respond to more than one quality of taste [23, 24]. This broad range of responses bring about the ambiguity of messages loaded on the nerve fibers, which renders the difficulty in identifying the taste quality of stimuli by observers. To solve this puzzle, the AFP theory was proposed advocating that the qualities of taste stimuli are coded by the neural activity distributed across the population of taste-responsive cells, so-called "patterns." With that conceptualization, it can be easily associated with the condition that patterns of activity generated by stimuli that taste alike should resemble each more than patterns generated by stimuli that taste very different. As a matter of fact, early researches have proved this is the truth [25].

At a glance of these evidences, we may favor due to the striking findings through transgenic animal models that the signals pass along nerve fibers adopting the LL theory. However, we still ought to be cautious of potential caveats when drawing such a conclusion. It should be noted that the CT is not the sole nerve that transmits information from TRCs to the rNST. In addition, both of the LL and AFP theory are posited on account of spatial coding theory, which ignore the role of temporal coding, whose core is that the response dynamics may conceal information about taste quality and quantity [26].

14.3 Design of the In Vivo Bioelectronic Tongue

14.3.1 Animal Training and Surgery Protocol

Adult female Sprague-Dawley rats (200–300 g) were used as subjects and were maintained on a 12 h light/dark schedule, and all experiments and training were performed in the light portion of the cycle. Chow and water were available ad libitum.

Anesthesia was induced and maintained using intraperitoneal injections of ketamine, xylazine, and acepromazine mixture (100, 5, and 1 mg/kg, respectively, for induction; 20–30 % of induction dose for maintenance). Once anesthetized and head shaved, rats were placed on a stereotaxic frame, at which time holes were bored in the skull for reference screws, ground screws, and microwire electrode array. The site of transcranial hole for electrode bundles introduced in Chap. 9 depends on the electrophysiological activity of which relay on the gustatory pathway was to be researched, for gustatory cortex, for example, the coordinates are anteroposterior (AP) 1.4 mm and mediolateral (ML) 5 mm from bregma; dorsoventral (DV) 5 mm from dura [27]. After implanting electrode array to preconcerted position, the hole was first sealed by medical glue, then the electrode array

were cemented to the skull with dental acrylic, as was a bolt for restraining head movements.

Besides microwire electrode array, intraoral cannulas (IOCs) were also inserted unilaterally or bilaterally for liquid deliver. The IOCs were flexible plastic tubing inserted close to the tongue through the cheek and extending upward to the top of the skull, as shown in Fig. 14.3a [28]. The cannula consists of a 4-in length of polyethylene (PE) 90 tubing with a heat flare at one end buried under skin, an "L-shaped" 14-mm section of 19-gauge stainless steel tubing, a piece of PE 10 tubing preventing blockage by food particles and a 5 mm section of PE100 tubing to ensure the PE 10 filler is not worn off by rats.

Rats were given 7 days to recover from surgery and afterwards entered the training period to adapt to the test chamber and liquid deliver from IOCs during which mild water restriction was often imposed to subjects (45 min per day). Sapid solutions for five basic taste qualities were chosen at appropriate concentrations. Tastes were selected randomly without replacement so that the rats would not form expectations about which quality of taste was about to be delivered [29], and taste deliveries were interleaved with an adequate aliquot of water; the time between each fluid delivery was set to 10 s or longer.

In those experiments importing training sessions, the lever-pressing self-administration was often employed to take expectations of liquid (not quality) into consideration. Two consecutive trials of a typical regime of such training are depicted by Fontanini and Katz as demonstrated in Fig. 14.3b [30]. The rat is free to press the lever at any time, but for the first 30 s of the trial lever presses do not result in water delivery (depicted in gray dots); under these conditions the rat quickly learns to withhold from lever pressing for approximately the first half of the period. The first lever press made after the end of this 30 s interval causes a 40 μl aliquot of water to be delivered directly into the rat's mouth (depicted in black dots) through IOCs and resets the clock, starting the next trial.

Another frequently used training method often involved conditioned taste aversion (CTA) or Garcia effect due to studies on conditioned taste aversion which involved irradiating rats were first conducted in the 1950s by Dr. John Garcia [31]. Conditioned taste aversion refers to the circumstance under which an animal associates the taste of a certain food with symptoms caused by toxic, spoiled, or

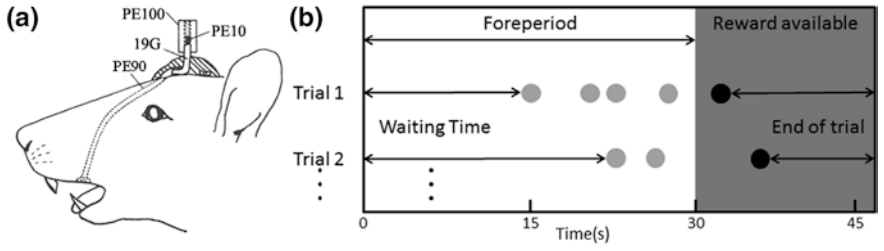

Fig. 14.3 **a** Details of the intraoral cannula (IOC) in place on the skull (Reproduced with permission from Ref. [28]. Copyright 1970, Psychonomic Society, Inc.). **b** Timed lever-pressing self-administration task

poisonous substances. Generally, taste aversion is formed after ingestion of food that causes nausea, sickness, or vomiting. This ability to form a taste aversion is considered as an adaptive trait or survival mechanism that could train the body to avoid toxic substances before they cause any harm. The association reduces the probability of consuming the same or similar substance in the future, thus avoiding further poisoning. It is an example of classical conditioning or Pavlovian conditioning. Conditioned taste aversion sometimes occurs when sickness was totally coincidental and had nothing to do with the substance that caused the sickness. For example, a person who becomes very sick after consuming vodka-and-orange-juice cocktails may then become averse to the taste of orange juice, even though the sickness was caused by the overconsumption of another content in the cocktails, alcohol. It remains unknown for the formation mechanism for CTA upon the neurophysiological level and electrophysiological experiments seem to be an optional technique to solve this conundrum. The parabrachial nucleus [32], amygdala [33], and gustatory cortex [34], those nuclei and cortices concerning information procession in gustatory system, have been examined in search for reasonable excuses for CTA.

14.3.2 Application of Patch Clamp in In Vivo Bioelectronic Tongue

Apart from microelectrode arrays emphasized in Chap. 9, patch clamp technique is another method to record electrophysiological activity, especially for nerve fibers in gustatory system. The patch clamp technique is a refinement of the voltage clamp. Erwin Neher and Bert Sakmann developed the patch clamp in the late 1970s and early 1980s. This discovery made it possible to record the currents of single ion channels for the first time, proving their involvement in fundamental cell processes such as action potential conduction [35].

Patch clamp recording is comprised of an electrode, a glass micropipette with an open tip of about 1 μm in diameter, a size enclosing a membrane surface area or "patch" that often contains just one or a few ion channel molecules [36]. Other than inserting through cytomembrane and into cytoplasm, the clamp is sealed onto the surface of the cell membrane without impalement. The interior of the pipette is filled with a solution matching the ionic composition of the bath solution, and a chlorided silver wire is placed in contact with this solution and conducts electric current to the amplifier. The micropipette is pressed against a cell membrane and suction is applied to assist in the formation of a high-resistance seal between the glass and the cell membrane, which makes it possible to electronically isolate the currents measured across the membrane patch reducing the competing noise, and providing high mechanical stability to the recording process. The interior of the pipette can be filled with different solutions depending on the requirements of the experiments, in the case of cell-attached recording without impalement, the solution should match the composition of the bath solution and in the case of

whole-cell recording, the solution should match the cytoplasm. Meanwhile, the content or corresponding concentrations of these solutions can be changed by adding extra ions or drugs for the research of studying the ion channels under different conditions.

Instead of using true voltage clamp as the amplifiers, many patch clamp circuitries involve differential amplifiers which set the zero current (ground) level by the bath electrode. This setting allows researchers to keep the voltage constant while observing only the changes in current. The current in the patch pipette is compared to the ground electrode in recording signals. Current is then injected into the processing system to maintain a constant, set voltage. However, much current is needed to clamp the voltage is opposite in sign and equal in magnitude to the current through the membrane [37]. Alternatively, the cells can be also current clamped in whole-cell mode, by keeping current constant while observing changes in membrane voltage.

Automated patch clamp systems have recently proposed and been developed in order to collect high throughput of data inexpensively in a shorter period of time. In need of capture single cell or cell population, such systems typically include a single-use microfluidic device, which can be either an injection molded or a PDMS cast chip, and an integrated electrode. Depending on the specific demand of the researchers experiment, several variations of the basic techniques can be applied. Among these techniques, the inside-out and outside-out techniques are also called "excised patch" techniques, because of the patch being excised (removed) from the main body of the cell. Cell-attached and both excised patch techniques are usually used to study the behavioral and electrophysiological characteristics of individual ion channels in the section of membrane attached to the electrode.

Namely, whole-cell patch and perforated patch allow the researcher to study the electrical behavior of the entire cell, rather than single channel currents. The whole-cell patch, which emphasizes low-resistance electrical access to the inside solution of a cell, has now been gradually replacing high-resistance microelectrode recording techniques to record currents across the entire cell membrane.

In one form of such automated systems, a pressure differential is utilized to force the cells being studied to be drawn toward the pipette opening until they form a gigaseal as shown in Fig. 14.4a. Then, the portion of the membrane protruding from the pipette bursts and the membrane is now in the inside-out conformation at the tip of the pipette by briefly exposing the pipette tip to the atmosphere [37–39]. In a completely automated system, the pipette and the membrane patch can then be rapidly moved through a series of different test solutions, allowing different test compounds to be applied to the intracellular side of the membrane during recording [40].

In view of its vigoroso preponderance of patch clamp in investigating excitable cells, it has been playing a significant role in examining gustatory periphery TRCs' and nerve fibers' response characteristics to various stimuli [41]. Researches have been done to ascertain the mechanisms of taste signal conduction [41, 42]. In a more recent study, with auxiliary of transgenic mice, different TRCs for sweet,

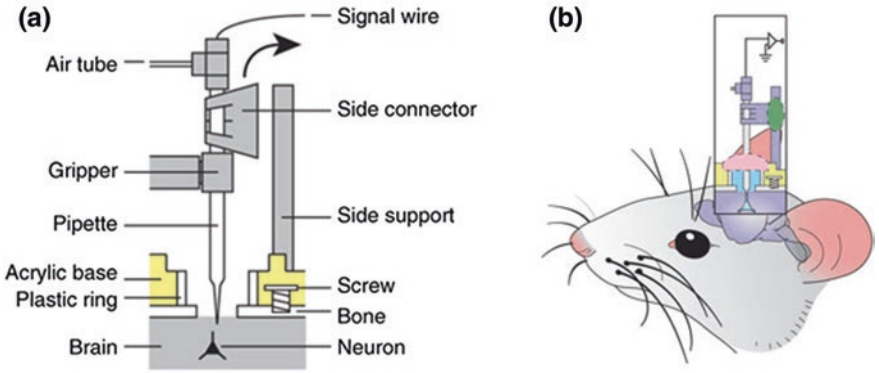

Fig. 14.4 **a** *Side view* of the arrangement of head-mounted items and whole-cell recording equipment (Reproduced with permission from Ref. [38]. Copyright 2009, Nature Publishing Group). **b** Whole-cell patch clamp recording of a hip neuron in a rat, and an electrode holder is mounted on the rat cranium and holds an electrode that is cemented in place at two anchoring sites once a recording is made (Reproduced with permission from Ref. [37]. Copyright 2009, Nature Publishing Group)

bitter, and umami were found to share similar signaling pathways through patch clamp detection [43]. Whereas noticeable imperfections exit in view of low throughput and demanding operation compared with electrode arrays.

14.4 Realization of the In Vivo Bioelectronic Tongue

14.4.1 Recording of Taste Electrophysiological Signals

Merging with the burgeoning technique brain-computer interface (BCI), the subjects are able to yield relevant electrophysiological signals for analysis and identification, considering the subject a black box with tongue as sensing material and brain regions as processing elements. The electrical output of brain regions is reconnoitered by implanted microelectrode arrays and an emblematic system is depicted in Fig. 14.5.

Simultaneous recordings could be obtained by attaching the connector of microelectrode to preamplifier through headstage cable, and data stream to OmniPlex Data Acquisition System (Plexon, Inc., Dallas, TX). Neuronal signals from microelectrode were sampled at 40 kHz, amplified by 1500 × gain, and filtered from 0.5 Hz to 8 kHz. More micromesh bandpass filters are imposed to split the wideband signal into high frequency part, the spikes, and low frequency part, the local field potential. Raw data were saved for offline sorting and further analysis. Meanwhile the triggering of tastant delivery solenoids for pumping liquid through IOCs should also transmit to the data acquisition computer [44]. Offline, the data should be adjusted for physical delays between this signal and the time at which fluid hits.

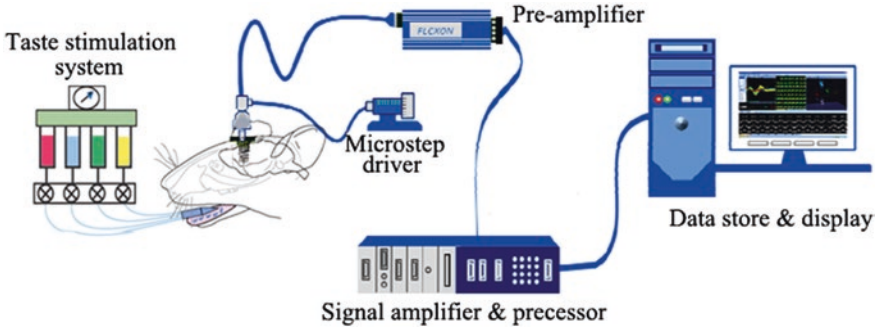

Fig. 14.5 Schematic diagram of a typical instance of in vivo biosensing system. Microwire electrode arrays are implanted by microstep driver to appointed depth and set to detect electrophysiology signals, which are further transmitted through the Plexon system. Sapid solutions are dropped onto tongues as taste stimuli by the taste stimulation system

14.4.2 Information Processing of Taste Signals at Consecutive Relays

Since the studies of the pathways of gustatory system, as mentioned above, have made great progress in recent decades, the electrophysiological features of different relays and coding strategy they take advantage of in processing taste information have become research hotspots. Neural networks between consecutive relays have also attracted colossal attention.

In the periphery, the innervated nerve fibers function as hinges that concatenate the TRCs to brainstem, which gives rise to research interest in expeditions of decoding the messages passing along them. Research had been done to investigate multiple sensitivity of chorda tympani fibers of rats to taste stimuli, impulse discharges in single chorda tympani fibers in response to taste stimuli representing the four basic qualities of taste were recorded, as shown in Fig. 14.6a [45]. The results indicated the existence of certain types of units which can be categorized statistically.

In another study probing into the taste sensitivities of the hamster's soft palate, proportional responses to sweet (0.3 M sucrose), salty (0.3 M NaCl), bitter (10 mM quinine), and sour (10 mM HCl) were recorded from four different nerve cell-types in the hamster gustatory system: CT nerve, greater superior petrosal nerve, glossopharyngeal nerve and the superior laryngeal nerve (SLN, from CN X) fibers [46]. As demonstrated in Fig. 14.6b, each pie represents the response to each stimulus as a proportion of the sum of the responses of that nerve to all stimuli. The results manifested that GSP is relatively more responsive to sucrose than the CT nerve, which also appeared in the subjects. Together, these two branches of the facial nerve provide the vast majority of the taste information to the brainstem about sucrose and NaCl.

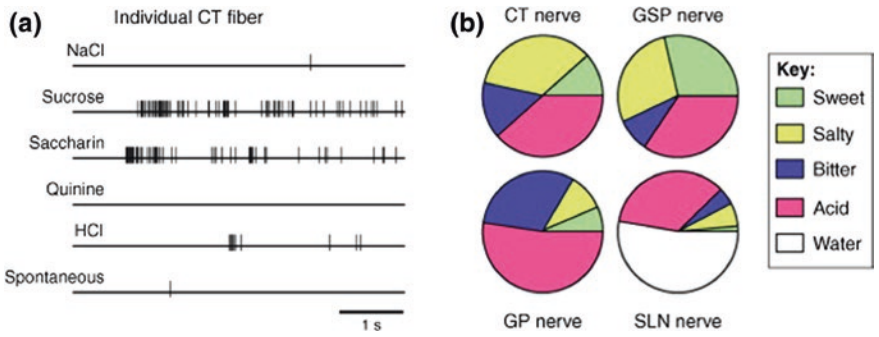

Fig. 14.6 **a** A single-unit recording from the hamster chorda tympani (CT) nerve illustrating a neuron that was selectively responsive to super-threshold concentrations of both sweet tastants (sucrose and saccharin) (Reproduced with permission from Ref. [45]. Copyright 1968 the Physiological Society) **b** Proportional responses to sweet (0.3 M sucrose), salty (0.3 M NaCl), bitter (10 mM quinine) and sour (10 mM HCl) recorded from four different nerve cell-types in the hamster gustatory system (Reproduced with permission from Ref. [7]. Copyright 2010 Elsevier)

The fact that the rNST is the confluence of aforementioned taste nerves suggested researchers that the content and quantity of information in this structure should be more comprehensive. Research had been done to examine how a variety of bitter stimuli were represented by neural activity in central gustatory neurons [47]. Taste responses evoked by a variety of tastants, half of which produce bitter taste, were recorded from neurons in the nucleus of the solitary tract of anesthetized rats. Cluster analysis was used to categorize neurons into types based on responses to different tastants. Multivariate analyses revealed that across-neuron patterns of response among bitter stimuli were strongly correlated. The consequence, shown in Fig. 14.7a, indicated that central neurons most responsive to bitter substances receive significant input from receptors that mediate other tastes, indicating that bitter stimuli are not represented by activity in specifically tuned neurons. As single neurons in the nucleus of the solitary tract responded selectively to bitter taste stimuli, their responses were recorded from the rat nucleus of the solitary tract during whole mouth stimulation with a variety of bitter compounds in another study [48]. Similar research has been done to investigate single neuron response to different stimuli and the result is shown in Fig. 14.7b [49]. The stimulus is indicated on the top of each trace. Full oscilloscope sweep is 2 s. The neuron responded with a fixed latency of 420 ms after stimulus onset (indicated on the bottom trace). The neuron responded significantly to high concentration NaCl, glucose and fruit juice.

Studies have provided evidence that temporal coding contributes significantly to encoding taste stimuli at the first central relay for taste, the rNST. Afterwards, researches moved onto the coding mechanism used at the next relay in the central taste pathway, the parabrachial nucleus of the pons (PBN). In a previous study, electrophysiological responses to taste stimuli (sucrose, NaCl, HCl, and quinine) were recorded from 44 cells in the PBN of anesthetized rats [50]. In 29 cells, the contribution of the temporal characteristics of the response to the discrimination

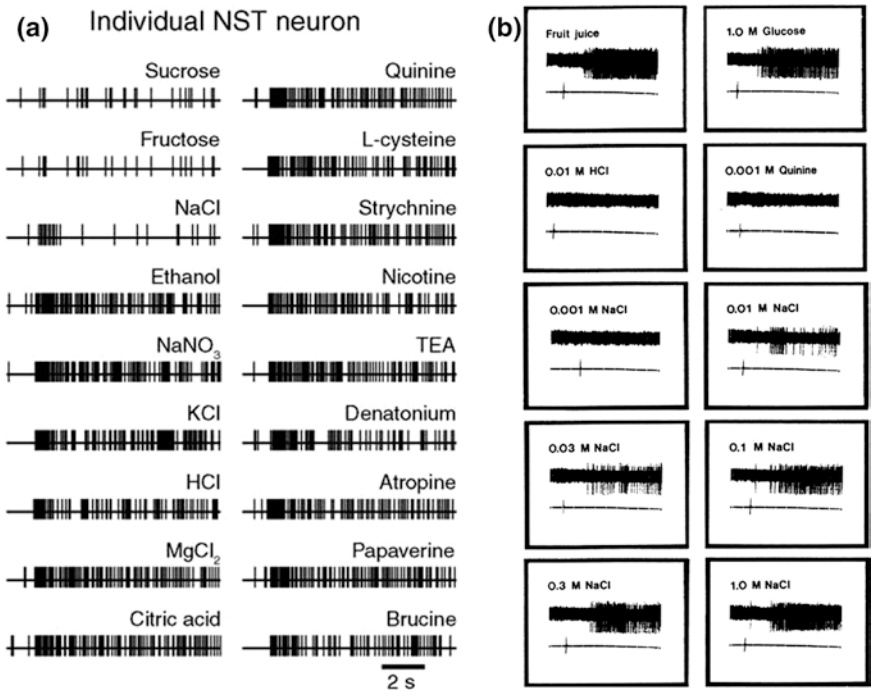

Fig. 14.7 **a** Single-unit recording from individual neurons of the rat rNST illustrating a broadly tuned neuron that responded to a variety of perceptually distinct tastants but apparently not to NaCl, sucrose or fructose (Reproduced with permission from Ref. [7]. Copyright 2010 Elsevier). **b** Raw electrophysiological records (voltage vs time) showing responses to the prototypical stimuli in rNST neuron. (Reproduced with permission from Ref. [49]. Copyright 1998 Elsevier)

of various taste qualities was assessed, which distinctly manifested the theory of electrophysiological research in PBN region. The data acquired suggest that information about taste quality conveyed by the temporal characteristics of evoked responses is transmitted with high fidelity from the NTS to the PBN. Also, the study revealed the existence of two different categories of cells in the PBN region, narrowly tuned and broadly tuned cells. The narrowly tuned cells, like cell A, have high specificity and respond to only one or two kinds of tastants, while the broadly tuned cells, represented by cell B, respond to multifarious tastants but differ in intensity. Besides, studies indicated that the PBN region is involved in the formation of conditioned taste aversion [51]. Example of taste response in PBN neuron in the control group is depicted in Fig. 14.8a. Arrows indicate onset of each stimulation. This unit shows good response to 0.1 M NaCl and 0.01 M HCl infusions. Figure 14.8b shows the mean response profiles to 13 taste stimuli in both groups. It is clear from this figure that sodium salts and monosodium glutamate + IMP elicited higher responses compared with other stimuli.

The primary gustatory cortex (GC) is main structure in brain responsible for the perception of taste. By using extracellular unit recording techniques, scientists have elucidated that neurons in GC respond to sweetness, sourness, saltiness,

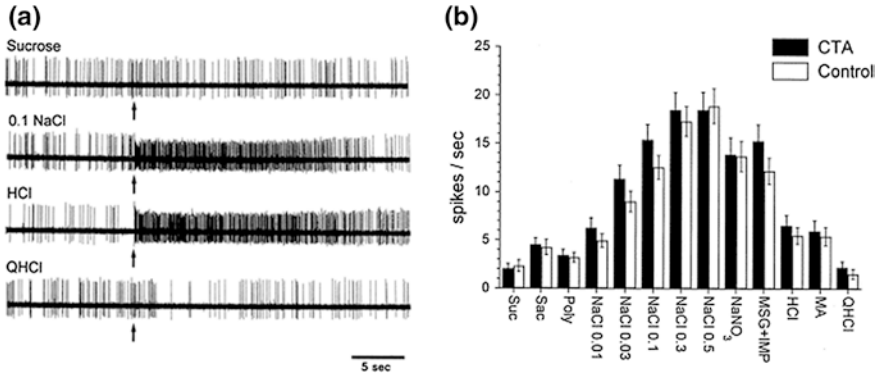

Fig. 14.8 **a** Example of taste response in PBN neuron. **b** Mean response profiles to 13 taste stimuli of taste-responsive neurons in both groups. (Reproduced with permission from Ref. [50]. Copyright 1997 Elsevier)

bitterness, and umami, and they code the intensity of the taste stimulus. Basically, the responses of individual GC neurons to tastants exhibit the same patterns of activity as those described for brainstem neurons in that some have been reported to be quite selective to tastants, whereas others are more broadly tuned. GC chemosensory neurons exhibit concentration-dependent responses. In a study done on GC responses in rats during licking, an increase in monosodium glutamate (MSG) concentration lingual exposure resulted in an increase in firing rate in the rat GC neurons, whereas an increase in sucrose concentration resulted in a decrease in firing rate. GC neurons exhibit rapid and selective response to tastants. Sodium chloride and sucrose elicited the largest response in the rat gustatory cortex in rats, whereas citric acid causes only a moderate increase in activity in a single neuron. Chemosensory GC neurons are broadly tuned, meaning that a larger percentage of them respond to a larger number of tastants as compared to the lower percentage responding to a fewer number of tastants. In addition, the number of neurons responding to a certain tastant stimulus varies. In the rat gustatory complex study, it was shown that more neurons responded to MSG, NaCl, sucrose, and citric acid (all activating approximately the same percentage of neurons) as compared to the compounds quinine (QHCl) and water [52].

Studies using GC of the rat model have shown that GC neurons exhibit complex responses to changes in concentration of tastant. For one tastant, the same neuron might increase its firing rate whereas for another tastant, it may only be responsive to an intermediate concentration. Studies have shown that few chemosensory GC neurons. In these studies it was evident that few chemosensory GC neurons monotonically increased or decreased their firing rates in response to changes in concentration of tastants (such as MSG, NaCl, and sucrose), the vast majority of them responded to concentration changes in a complex manner. In such instances with several concentration tastants tested, the middle concentration might evoke the highest firing rate (like 0.1 M sucrose), or the highest and lowest concentrations might elicit the highest rates (NaCl), or the neuron might respond to only one concentration.

14.4.3 Taste Discrimination and Its Potential Application

The research findings from decades ago to nowadays have cast on electrophysiology characteristics of consecutive relays in gustatory system, to some extent have uncovered possible coding mechanisms. Connections in mind are made between coding and decoding the taste signals, and further discriminating signals evoked by disparate qualities of taste stimuli. However, discrimination seems to be a conundrum in light of dimness of whether the LL or the AFP theory is adopted in the periphery and the coexistence of narrowly and broadly tuned cells in encephalic regions. Still and all, conceivable solutions have been visualized. Utilizing the specificity of narrowly tuned cells in brain nucleus, the stimulus corresponding to the best response can be apparently distinguished from other stimuli as demonstrated in Fig. 14.8a. With regard to broadly tuned cells, pattern recognition algorithms referred in Chap. 9, are indispensable in differentiating between taste stimuli. However, the efficacy of discrimination is barely satisfactory (Fig. 14.9).

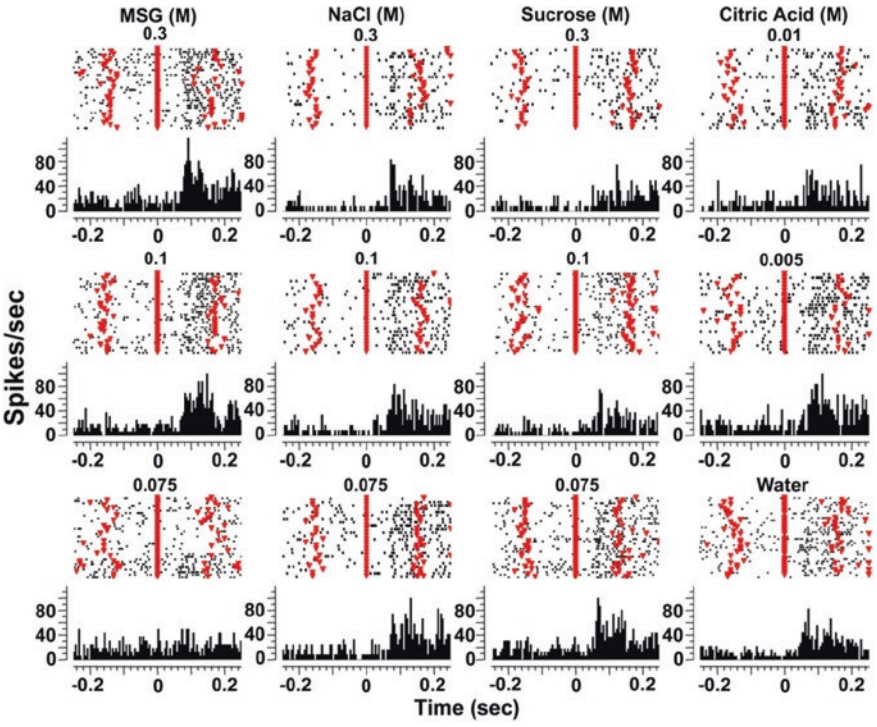

Fig. 14.9 Sucrose and quinine evoke responses in the same GC neuron. Shown are the responses of a neuron to four tastants at multiple concentrations. (Reproduced with permission from Ref. [52]. Copyright 2010 Elsevier)

Chen and colleagues found a new path to resolve this dilemma when taking the temporal coding into consideration [53]. Metric space analysis, as they defined, not only pays attention to spike counts in certain time bins, but also temporal feature of spikes. The similarity of two spike trains are gauged by the cost of adding or deleting spikes as necessary, and moving spikes in time, such that the two spike trains are identical. This cost is called Dspike and is calculated at a variety of levels of temporal precision, called q. After projecting into a virtual space, as demonstrated in Fig. 14.10, results showed that the precise timing of spikes within a taste-evoked spike train was a better predictor of taste quality than spike count alone, even when the concentration of the stimulus was varied. For all plots, dots indicate the location of individual responses; asterisks indicate the centroid of the clusters of responses to a given taste stimulus. Axes are labeled in arbitrary units.

Chasm remains to thoroughly understand gustatory system in spite of progresses being made in getting a clear insight into the operation methods of it. Nonetheless, value of potential applications of the in vivo bioelectronic tongue has revealed its importance in domains like food industry, combining the biosensing system with hedonic or palatability to manufacture goluptious foodstuff [54].

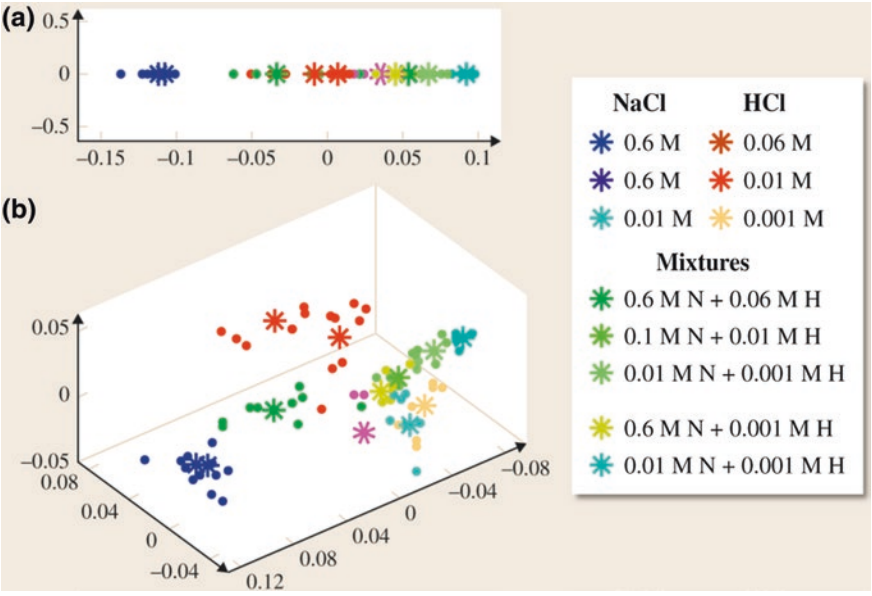

Fig. 14.10 Temporal coding analyses of one cell tested with three concentrations each of NaCl and HCl and five mixtures. **a** The one-dimensional response space created by MDS of the spike count distances. **b** The three-dimensional response space created by the MDS of the spike time distances (Reproduced with permission from Ref. [14]. Copyright 2014, Springer-Verlag)

14.4.4 Summary

Benefitted by advancement in neurophysiology, genetic and cellular biology, light has been cast onto the pathway and mechanism of animals' gustatory system, and the concept of the in vivo bioelectronic tongue has been proposed several decades ago with endogenous merits as fast detecting, high sensitivity, and specificity. Intended to turn the animal model into utility transducers, studies of coding mechanism, and corresponding decoding strategy of electrophysiology signals in neural networks recorded by implanted microelectrode array or patch clamp have been hot issues contemporarily and attempt has been made to discriminate between signals evoked by different tastants. In virtue of importance mainly attached to fundamental research probing into gustatory mechanisms, applications are not available in primary stage. Future studies ought to highlight temporal coding as well as spatial coding to develop full-scale understanding about the gustatory system, thus enables imaginable applications of the in vivo biosensing system.

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Chapter 15
Future Trends of Bioinspired Smell and Taste Sensors

Ping Wang, Liujing Zhuang, Yingchang Zou and K. Jimmy Hsia

15.1 Rehabilitation of Smell and Taste Function for Human

Anosmia and ageusia are both harmful to the quality of life. However, the common view of anosmia and ageusia as trivial can make it more difficult for a patient to receive the same types of medical aid as someone who has lost other senses, such as sight or hearing. In order to achieve those goals, electronic noses and electronic tongue should be designed to mimic human's sense rather than outputting electrical parameters only. Unfortunately, most commercial electronic noses (PEN3, zNose) detect volatile compounds in samples [1]. But for human beings, our nose can determine whether the sample is fragrant or not instead of determining what compounds are in the sample.

For human beings, the sense of taste consists of five basic tastes including sourness, saltiness, umami, bitterness, and sweetness [2]. When tasting a food or beverage, a human perceives each type of taste on sensory organs called taste buds on the tongue. Taste buds are composed of approximately 50–100 cells. Many kinds of gustatory receptors present in gustatory cells. Each gustatory receptor receives multiple chemical substances constituting a single taste. Namely, gustatory receptors exhibit semi-selectivity rather than rigid and high selectivity. While those researches on the molecular and cellular biology of taste reception has been

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carried out, sensing technologies for discrimination and quantification of tastes which mimic the biological taste reception have been developed since 1990. For imitation of biological taste, a taste sensor with global selectivity is needed. It is impossible to measure the taste of foods containing several hundreds of types of taste substance by chemical analysis methods, such as liquid chromatography, although their measurements are precise. In addition, interactions between different tastes and between different taste substances should be considered. Toko [3] made an example to explain that. He said the bitterness of coffee is suppressed by adding sugar and a synergetic effect for umami can be obtained by mixing glutamine acid, an amino acid and nucleotide-derived inosinic acid. Global selectivity is a term originally proposed by Toko et al. They [4] developed a series of taste sensors with global selectivity which are composed of several kinds of lipid/polymer membranes for transforming information of taste substances into electric signal. The output of sensor shows different patterns for chemical substances which have different taste qualities such as saltiness and sourness. Taste interactions such as suppression effect, which occurs between bitterness and sweetness, can be detected and quantified by using the taste sensor. Amino acids and peptides can be classified into several groups according to their own tastes from sensor outputs. Bitter-tasting amino acids such as l-tryptophan have response electric patterns similar to a typical bitter substance, quinine. The taste of foodstuffs such as beer, sake, coffee, mineral water, milk and vegetables can be discussed quantitatively. The taste sensor with lipid membranes provides the objective scale for the human sensory expression and will contribute to clarification of the reception mechanism at gustatory cells.

Toko's taste sensor with global selectivity has been put into production and widely applied in food industry. However, it is far away from being used for taste rehabilitation. In addition to global selectivity, equipment for taste rehabilitation should be portable and its output signals are required to be read by human brains. Such huge volume made the present electronic nose unavailable to be used as taste aids. Considering eyeglasses and hearing aids, a wearable design may be better for rehabilitation equipment. What does a wearable electronic tongue look like? How could it work safely in human mouth? And how does the output signal transform into brain-reading signals? Only when these problems were solved can electronic tongue be used for taste rehabilitation. So does the situation of olfactory rehabilitation.

Due to the strong desire of smell and taste rehabilitation, researchers should make attempts to solve those problems. Some researchers or designers have put forward some conceptual products about wearable smell or taste devices (Fig. 15.1). As shown in figures, these wearable devices are flexible circuits or chips. Japanese scientists have developed a flexible electrical circuit one-fifth the thickness of food wrap and weighing less than a feather (Fig. 15.2). Its developers said it could improve the movement of artificial limbs by tapping into signals from the brain [5].

In addition to flexible circuits, brain–computer interface (BCI) technology is also very important for the development of rehabilitation of smell and taste function. BCI gives users communication and control channels that do not depend on the brain's normal output channels of peripheral nerves and muscles [6]. BrainPort (Fig. 15.3a) is the device being developed by neuroscientists

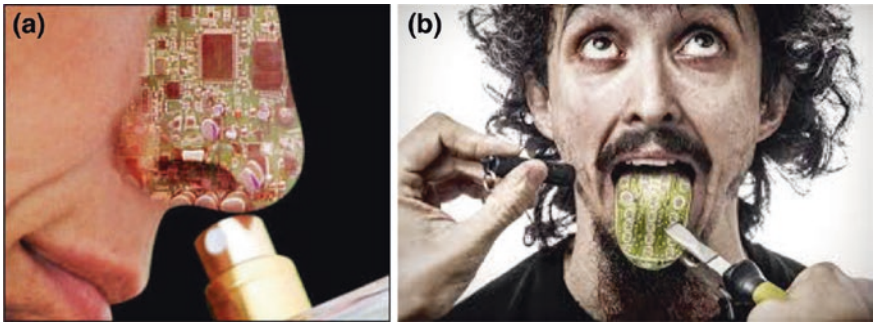

Fig. 15.1 Conceptual designs of rehabilitation devices for **a** olfaction (inventorspot.com) and **b** taste (scienceroll.com)

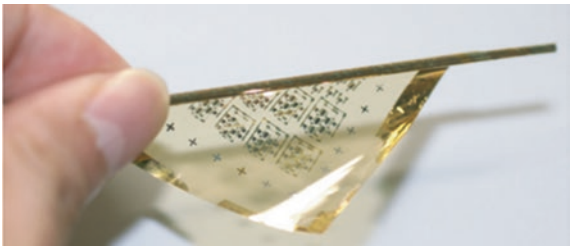

Fig. 15.2 Flexible circuit [5] (Reproduced with permission from Ref. [7]. Copyright 2010 Nature Publishing Group)

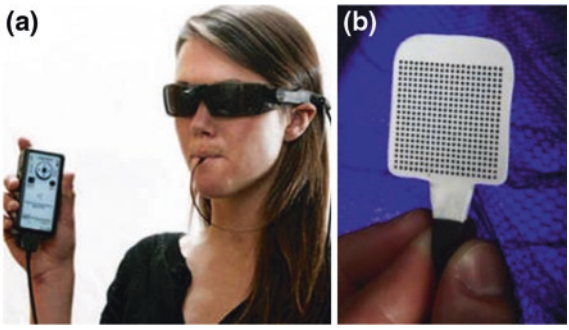

Fig. 15.3 **a** Brain Port and **b** its "lollipop" (engadget.com)

at Middleton, Wisconsin–based Wicab, Inc. (a company co-founded by the late neuroscientist Paul Bach-y-Rita) that helps users "see" without using their eyes [7]. Bach-y-Rita hypothesized in the 1960s that "we see with our brains not our eyes." Using the BrainPort device, visual data is collected through a small digital video camera, about 1.5 cm (5/8 in) in diameter, housed in the center of a pair of sunglasses worn by the user. From there, the data is transmitted to a handheld base unit with the size of a cell phone. The unit converts the digital signal into electrical pulses—replacing the retina's function. The base unit also incorporates features like zoom, light settings, and shock intensity levels as well as a

central processing unit (CPU). Signals are sent from the CPU to the tongue via a "lollipop" (Fig. 15.3b), and an electrode array about 9 cm^2 (1.4 in^2) that sits on top of the tongue. Each electrode corresponds to a set of pixels: white pixels, for example, deliver a strong electrical pulse whereas black pixels have no signal. Densely packed nerves on the tongue surface receive the incoming electrical signals, which users describe as feeling a little like Pop Rocks or champagne bubbles. For instance, if the camera detects light fixtures in the middle of a dark hallway, corresponding electrical stimulations will occur along the center of the tongue. There exist other commercial products for seeing aids [8]. Considering commercial visual rehabilitation products, such as BrainPort, BCI should be involved in these designs. Because of anosmia and ageusia, odors and tastes have to be sensed by chemical sensors. Signals obtained by chemical sensors should be processed by CPU and then transformed to electroneurographic signal. Thus, rehabilitation of human functions can be performed.

In our opinion, BCI technology and flexible circuit materials are two of the most critical techniques for applying electronic noses and tongues in rehabilitation of human function. When those products come to the world, it might be promising production boosting economy and benefiting human beings.

15.2 Bioinspired Smell and Taste Hybrid System

In recent years, a large number of researchers dedicated to the study of bioinspired smell system and taste system. Electronic noses and electronic tongues have been applied in many fields, including food, environment, medicine, and security. However, the sole use of an electronic nose or electronic tongue has become inadequate in some industries. For example, for some flavor sensing system, it seems necessary to develop some hybrid systems to collect related information about both gaseous phase and liquid phase.

There are mainly two methods to integrate olfaction system with taste system. One of them is data fusion. In detail, the sample was detected with electronic nose and electronic tongue independently. Then these two parts of data were combined for further processing. The other method is to develop a hybrid system which can be used to detect gaseous compounds and liquid compounds simultaneously.

Most researchers chose to use the first method, because it is more convenient to be performed. Bougrini et al. [9] elaborated an innovative analytical technique to differentiate between different pasteurized milk brands and for the exact recognition of their storage days through data fusion of the system—a hybrid electronic nose with a voltammetric electronic tongue. The hybrid electronic nose system consists of two sensor arrays. One comprises four micromachined gas sensors. The other consists of four commercial tin dioxide gas sensors, including TGS 815, TGS 822, TGS 824, and TGS 842, obtained from Figaro Engineering, Inc. (Osaka, Japan) plus a temperature sensor (LM 335Z) and a relative humidity sensor (HIH4000-01) from National Semiconductor in order to monitor the temperature

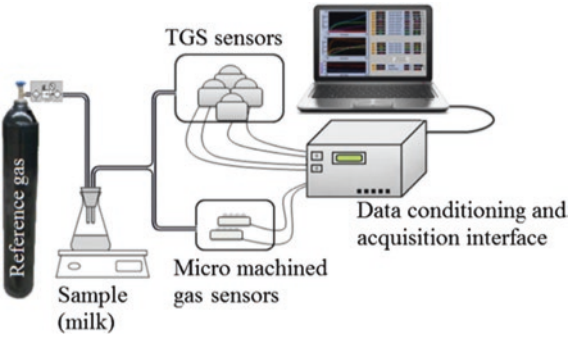

Fig. 15.4 Hybrid electronic nose setup for headspace evaluation of pasteurized milk [9] (Reproduced with permission from Ref. [11]. Copyright 2014 Elsevier)

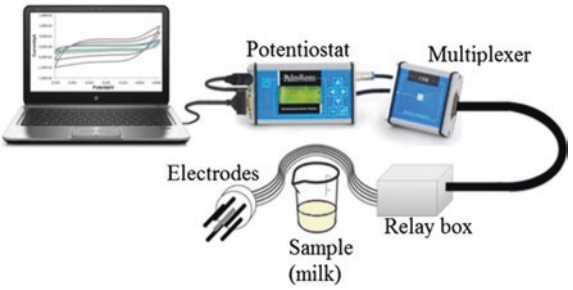

Fig. 15.5 Voltammetric electronic tongue setup for the evaluation of pasteurized milk [9] (Reproduced with permission from Ref. [11]. Copyright 2014 Elsevier)

and the hygrometry into the sensor chambers. Figure 15.4 shows a schematic representation of the experimental setup used in the measurements. 20 ml milk was put in a 50 ml vial and heated to 35 °C. The dynamic headspace was sampled and transferred into the sensor chamber. The variation of sensor conductivity was acquired and then digitized using a data acquisition board (PCL 812PG, Advantech). A program in LabView was developed to control the data acquisition process. In total, 16 features were extracted from the hybrid electronic nose.

The voltammetric electronic tongue used for this study consisted of four working electrodes (Platinum, Gold, Glassy Carbon, and Silver), a reference electrode (Ag/AgCl), and a Platinum electrode as the auxiliary. In Fig. 15.5, the electrodes were assembled in stainless steel tubing. The wires from the electrodes were connected via a relay box to a portable potentiostat PalmSens (PalmSens BV, The Netherlands). The cyclic voltammetry is applied as the measurement principle in the electronic tongue. The measurements were carried out at a temperature of 35 °C. After a series of electrochemical cleaning steps, electrodes were put into the detected milk. For response of every electrode, three features were extracted. Since there were four working electrodes within the array, each voltammetric measurement was described by 12 variables.

At first, every sensor system (i.e., electronic nose or electronic tongue) was considered separately and, in the second step, the information of the two systems was combined. For individual electronic devices, differentiation between

five pasteurized cow's milk brands has been conducted. PCA results dealing with the hybrid electronic nose data indicated that three pasteurized milk brands share similar characteristics, which made their discrimination quite difficult. On the other hand, PCA results from the voltammetric electronic tongue showed a clear distinction of the milk brands on the first storage day. Another study has been conducted in order to exactly recognize the storage day that a pasteurized milk (Jawda) has undergone in a refrigerator maintained at 4 °C. When the electronic devices were applied separately to the study of the different spoilage states of the pasteurized milk, no sufficient difference was found for the storage days. To exploit the combination of the two instruments, the mid-level of abstraction data fusion has been conducted and therefore yielded perfect classification of all the storage days.

Cole et al. [10] developed a hybrid system which combined electronic nose with electronic tongue. In Cole's system, electronic nose and electronic tongue work together. Information about headspace and information about liquid can be recorded simultaneously. Their sensing system was developed for the flavor analysis of liquids. Flavor is the sensory impression of a food or other substance, and is determined mainly by the chemical senses of taste and smell [11].

The system comprises both a so-called "electronic tongue" based on shear horizontal surface acoustic wave (SH-SAW) sensors analyzing the liquid phase and a so-called "electronic nose" based on chemFET sensors analyzing the gaseous phase. Thus, by combining two types of microsensors, an artificial flavor sensing system has been developed (Fig. 15.6). Tests conducted with different liquid samples, i.e. water, orange juice, and milk (of different fat content), resulted in 100 % discrimination PCA. In addition, experiments were conducted on low vapor pressure taste-biased solutions and high vapor pressure smell-biased solutions. Only the combined flavor analysis system could achieve 100 % discrimination between all the different liquids.

Coles's design assembled gaseous sensors and liquid sensors into a single chamber instead of using more than two sample chambers. The hybrid structure could provide simultaneous detection which guaranteed the matching degree between gaseous data and liquid data. In addition to that, it also decreased the detection time and simplified the procedure (Fig. 15.8).

The advent of electronic nose and tongue makes it possible now to extract information about the necessary features of the samples, in a similar way to that of an experienced taster using his perception of smell and taste. These hybrid systems provide better discrimination and classification of samples than separated electronic tongue or electronic nose. In recent years, more and more hybrid systems were studied, especially in food industry [12–14]. Considering both taste and odor are natural properties of food, we believe combination of electronic nose with electronic tongue could provide sufficient information of food only if suitable methods for model recognition are used.

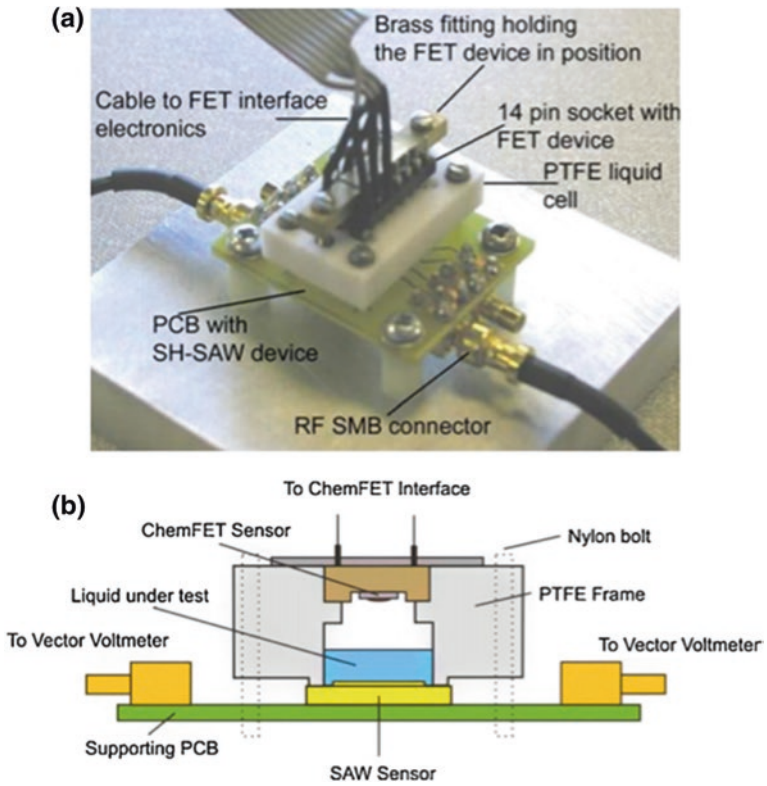

Fig. 15.6 **a** Photograph and **b** scheme of the hybrid electronic nose and electronic tongue [10] (Reproduced with permission from Ref. [12]. Copyright 2011 Elsevier)

15.3 Cyborg Nose and Tongue

A cyborg (short for "cybernetic organism") is a theoretical or fictional being with both organic and biomechatronic parts. The term was coined by Clynes and Kline in 1960 to refer to their conception of an enhanced human being who could survive in extraterrestrial environments [15]. The term first appears in print 5 months earlier when The New York Times reported on the Psychophysiological Aspects of Space Flight Symposium where Clynes and Kline first presented their paper: A cyborg is essentially a man-machine system in which the control mechanisms of the human portion are modified externally by drugs or regulatory devices so that the being can live in an environment different from the normal one [16]. Cyborg technologies take four different forms: restorative, normalizing, reconfiguring, and enhancing. It is often applied to an organism that has restored function or

enhanced abilities due to the integration of some artificial component or technology that relies on some sort of feedback [17].

Animal cyborg is a term used to describe an animal whose physical or mental form has been augmented with a piece of technology for the purposes of research, control, experimentation, or rehabilitation. It is a promising area of study for several reasons. First off, one of the biggest hurdles in the development of human cyborgs is the restrictions on human testing. For example, it is ethically permissible to completely control the movements of a fly or beetle, which allows us to understand how nervous systems can be synthesized with circuitry to advance fields such as bionics. Animals have also evolved some capabilities that defy our most advanced technologies. The flight systems of a fly or hummingbird far outmatches even our most deft aircrafts. By building off of these advanced organisms, we avoid having to start from scratch and can instead focus on novel combinations of animals' natural abilities and our technological augmentations. Animal cyborgs can also offer glimpses into nonhuman intelligences. Intelligence is a notoriously anthropocentric concept, and is often used as the elastic category by which we differentiate ourselves from mere "animals." Animal cyborgs allow us to understand and appreciate animals in new ways. For example, by embedding salmon with tracking devices, we have started to appreciate the strange intelligence of this species that allows it to travel thousands of miles and return to the exact stream it spawned in.

Military organizations' research has recently focused on the utilization of cyborg animals for the purposes of a supposed tactical advantage. DARPA (Defense Advanced Research Projects Agencyhas) announced its interest in developing "cyborg insects" to transmit data from sensors implanted into the insect during the pupal stage. The insect's motion would be controlled from a microelectromechanical system (MEMS) and could conceivably survey an environment or detect explosives and gas (Fig. 15.7a). Similarly, DARPA is developing a neural implant to remotely control the movement of sharks. The shark's unique senses would then be exploited to provide data feedback in relation to enemy ship movement or underwater explosives [18, 19].

Rats equipped with radios that transmit their brainwaves could soon be helping to locate earthquake survivors buried in the wreckage of collapsed buildings (Fig. 15.7b). Rats have an exquisitely sensitive sense of smell and can crawl just about anywhere. This combination makes them ideal candidates for sniffing out buried survivors. For that the animals need to be taught to home in on people, and they must also signal their position to rescuers on the surface. In a project funded by DARPA, the Pentagon's research arm, Linda and Ray Hermer-Vazquez of the University of Florida in Gainesville have worked out a way to achieve this. First the researchers identified the neural signals rats generate when they have found a scent that they are looking for. "When a dog is sniffing a bomb, he makes a unique movement that the handler recognizes," says John Chapin, a neuroscientist at the State University of New York in Brooklyn who is collaborating on the project. "Instead of the rat making a conditioned response, we pick up the response immediately from the brain." Each rat has electrodes implanted in three areas of

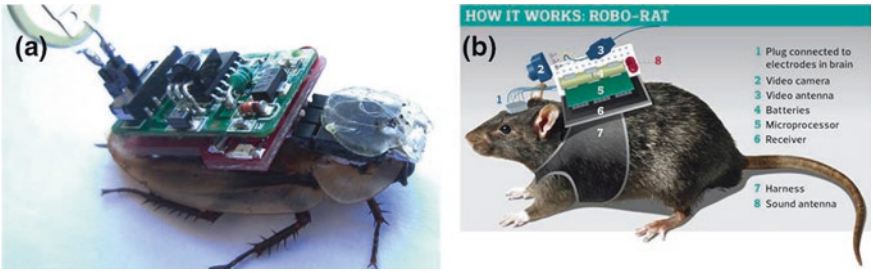

Fig. 15.7 Cyborg nose. **a** Insect-cyborg used to detect traces of explosive; **b** Rat-cyborg to the rescue with sense of smell (theguardian.com)

the brain: the olfactory cortex, where the brain processes odor signals; the motor cortex, where the brain plans its next move; and the reward centre, which when stimulated gives the rat a pleasurable sensation. The electrodes, each consisting of an array of up to 32 stainless steel wires 75 μm in diameter, are permanently implanted in the brain and can give accurate signals for up to 9 months. The researchers trained the rats to search for human odor by stimulating the reward centre when it found its target smell. Once the rats were trained, they were set to forage for the target smell, while electrodes recorded their neural activity patterns. This allowed researchers to identify the brainwave patterns associated with finding that smell. They were also able to train the rats to sniff out the explosives TNT and RDX–key after terrorist attacks that may leave buildings harboring unexploded bombs. "There are two neural events that we believe are hallmarks of the 'aha!' moment for the rat," says Linda Hermer-Vazquez. These are high-frequency activity in one subset of neurons, and decreased activity in two other areas, she says. Signals from the rat's brain will be relayed to a radio transmitter pack strapped to the animal's back, which Chapin is developing. Rescuers will be able to follow the rat's position by tracking these signals. They are also developing software that will recognize the 'aha!' moment when the rat has found its target, so rescuers will know where to start digging. The team hopes to create a working system within 9 months. Other teams looking at ways to seek people trapped under debris have designed wheeled, tracked, or even snake-like robots that can slither into wrecked buildings (New Scientist, 10 November 2001, p. 22). But rats have several advantages. "Artificial noses don't work well when there are other smells around," says Christiane Linster, an olfaction expert at Cornell University in New York. "Rats are good at that." Rats are also adept at navigating over unexpected obstacles, and of course they do not need an electricity supply [20, 21].

What is more, the far-out researchers at DARPA appear to have a unique approach to the problem: they want to combine dog nose with machine in a new research effort. "The goal of the DARPA RealNose program is to build a nose constructed from actual olfactory receptors that further leverages the components of the canine olfactory system to create a breakthrough detection system with potential capabilities beyond that of a canine," the broad agency announcement notes.

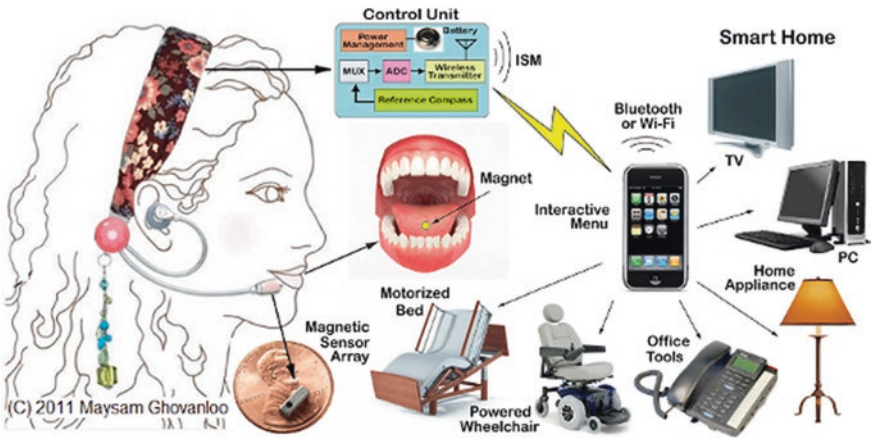

Fig. 15.8 Cyborg taste (thesocietypages.org)

"The key to the program concept is that by simulating the entire mammalian olfactory system (from air intake to pattern recognition), revolutionary detection capabilities will be created, demonstrating canine-comparable specificity, distance, and detection thresholds." (http://www.wired.com/2007/11/darpa-to-build/).

Figure 15.8 is a recent example of the new cyborg body. It is a new form of wheelchair mobility through the use of a tongue piercing. The Tongue Drive System in mouth uses a dental plate that captures the movement of the tongue piercing below, which is fashioned with a tiny magnet on top. The information is then sent to an iPod Touch or iPhone, where software installed on the Apple device works out the relative position of the magnet with respect to the array of sensors in real time and interprets the user's commands. This information is then used to control the movements of a cursor on the computer screen or to substitute for the joystick function in a powered wheelchair. This cyborg taste technology is also a recent example of medical technology incorporating popular body modifications into new assistive technologies, blurring the boundaries between medicalization and body modification.

15.4 Biomimetic Human with Olfaction and Taste Function

Biological systems perform sensing and actuation functions at many different levels, from individual cells to complex cell constructs to tissues to organs to organisms. The sensing and actuation are accomplished extremely efficiently in biological systems with very low energy consumption. Research on using engineered systems to mimic sensing and actuation functions of biological systems,

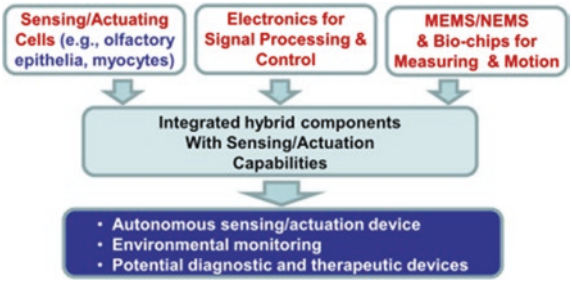

Fig. 15.9 Schematic of the cell-based biosensors and actuators

often referred to as biomimetics, has been one of the fastest growing research areas in the past decade. Majority of the work in this area has been focused at the systems level, advances in biomimetics at the cellular and molecular levels have been made only recently, largely due to the recent availability of nanotechnologies [22]. It is commonly accepted that, even though engineered systems (biomimetic or not) are extremely good at well-defined specific tasks such as identifying a particular toxin through chemical binding of a molecule, they are not nearly as good as at performing versatile functions. Take, for example, olfactory sensing and taste sensing. Smell and taste sensor systems can play very important roles in food security and environmental protection. They also present opportunities for potential commercial applications. Tremendous advances have been made in research of biomimetic methods for smell and taste sensing. But these devices still have their limitations in sensitivity and specificity compared to the binding of specific odorants and tastants by olfactory and gustatory receptor cells. To date, only a few smell and taste sensing systems are in commercial use. Thus the research of bioinspired artificial smell and taste sensing is still a very active and promising field.

Recently, some researchers are focus to utilize the remarkable capabilities of living cells by building novel components consisting of biological cells and engineered systems with unique sensing and actuation functionalities. Specifically, they proposed to investigate creating an integrated sensing and actuation system using cell-based biosensors and cell-driven actuators (Fig. 15.9). The biosensors will perform *smell or taste sensing functions*. The signals from the cell-based sensors would then be used to activate the cell-driven actuation components. The responses of the actuation components could result in either releasing of mitigating chemicals or directional locomotion toward (away from) targets. Development of such hybrid, autonomous systems represents a grand challenge and a critical emerging frontier in biosensing and bioactuation.

As shown in Fig. 15.10, a self-contained, battery-powered hybrid smell neuroelectronic system consisting of cells encapsulated in a polymer matrix with the following operational functions is built. The system would be triggered by smell sensing through olfactory receptor cells. Olfactory cells from primary cultured cells, mucosal tissue, and even from differentiated stem cells are used in cell-based biosensor. The sensing potentials of olfactory cells induced by odors can be recorded neurochips, such as microelectrode array (MEA), light-addressable

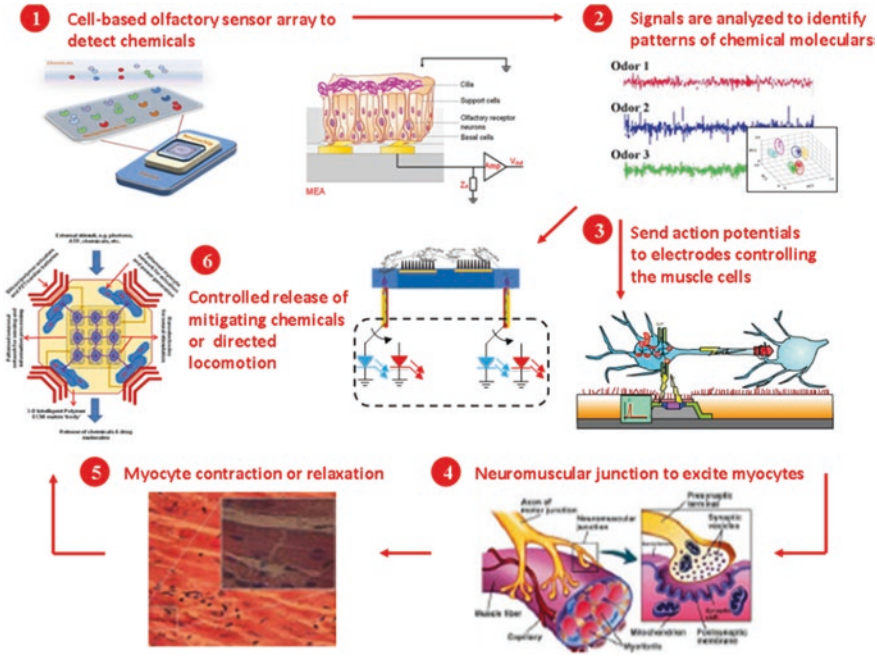

Fig. 15.10 A flowchart depicting the operations and the sub-components of a hybrid sensing-actuation system

potentiometric sensor (LAPS), or field effect transistor (FET) array. What is more, to activate contraction in muscle cells, motor neurons are used [23]. However, before integrating all the components to form the system, the basic questions at the component level must be studied.

15.4.1 Neural Cells, Nanoelectrodes, and Biosensor Chips

The basic approach of cell-based olfactory sensing is to combine the biomolecular functional units with microelectronics to realize the desired olfaction. This technique takes advantage of receptor cells for odors detection and, at the mean time, collects the electrophysiological data related to cellular functions using microelectronics sensor chip directly. It has been shown that the olfactory cells can be extracted and cultured on sensors for recording the electrical signals upon odorant-receptor binding. When microelectrode arrays (MEA) and olfactory epithelium hybrid systems are used, MEA can record the multichannel signals simultaneously, with the recording in the intact olfactory epithelium mimicking the in vivo process of gas sensing by well-preserved primary cells. Unlike in most solid state systems, this cell-based sensor enables us to detect not just a particular type of odor molecules, but many different types of odor molecules.

15.4.2 Activation of Muscle Cells

Myocytes (muscle cells) can be categorized into three different types: cardiomyocytes, skeletal, and smooth muscle cells. It is known that skeletal muscle is a voluntary muscle that contracts with conscious control by electrical muscle stimulation or electromyo stimulation. In fact, automatically creating action potential pulses to activate skeletal muscle cells in a controlled manner is an enormous challenge. Now there are two alternative options. One option is a solid state specialty chip with built-in functions. Such chips can be designed and made by computer engineers nowadays with relative ease. The other option is to produce engineered neuromuscular junctions, in which the mixture of neurons and skeletal muscle cells will be co-cultured on the hydrogel chemically linked with laminin or synthetic peptides containing YIGSR sequence [24]. Using the hydrogel, it is able to further modulate both substrate stiffness and density of cell adhesion molecules to control the properties of the neuromuscular junctions.

15.4.3 Cell-Mediated Locomotion

Biological cardiac and muscle cells are highly efficient chemical factories capable of converting nutrients to mechanical energy [25]. The mechanical energy can be further harnessed to produce physical actuation. For example, cardiac cells use the glucose from media to power its internal actin–myosin machinery thereby producing a continuous pulsation profile. Researchers have used this pulsatile nature of cardiac cells to drive microdevices, pump fluid in microchannels, and actuate microcantilevers. On the other hand, muscle cell contraction can be well controlled by electric signals. Utilizing these features of biological cells together with solid-state systems, actuation functions and locomotion of hybrid machinery test bed can be accomplished.

Directional movement of an object requires a nonsymmetric actuation of subcomponents of that object [26]. Coordinated actuation of two cantilever structures (with a timing delay between the actuations) can result in directional motion of the hybrid device on a surface (walking) (Fig. 15.11). The anticipated mechanism of directional motion for this device is as follows. As the front leg bends forward caused by cell contraction, it raises itself away from the surface. This will reduce the adhesion and friction between this leg and the surface the device crawls on (Fig. 15.11b). If the rear leg bends backward while the front leg remains bent, the platform will move forward while being lowered such that the front leg will resume contact with the surface (Fig. 15.11c). Then straightening up of the front leg will further move the platform forward and lift the rear leg from the surface (Fig. 15.11d). The straightening up of the rear leg will bring the system to its original state with the platform advanced a distance forward. Continued operation of this process will cause the device to move forward steadily.

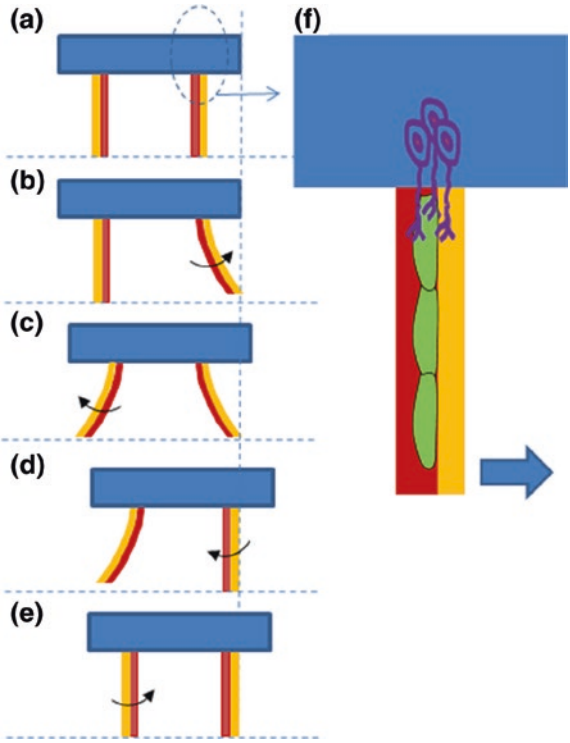

Fig. 15.11 Proposed mechanisms for directional locomotion

15.4.4 Integration of Different Components

To achieve the complete system integration, there are consecutive steps in the integration of various components. (1) Cell-based olfactory sensors and signal processing devices will be integrated to produce a smell sensing system. (2) Solid-state devices including nanoelectrode array and locomotion cantilever legs will be integrated together. The micro and nanofabrications of the proposed solid-state devices are compatible with conventional semiconductor electronics and micro-electromechanical device processes and thus they can be monolithically manufactured and integrated together. (3) The actuation components made of muscle cells and cantilever-based devices will be developed by plating the cells onto the surface of the solid state components. (4) A control algorithm for triggering desirable actuation will be integrated into the system to connect the sensing and actuation modules of the system.

15.5 Summary

Similar to biological olfaction and gustation systems, the bioinspired smell and taste sensors have high sensitivity, fast response, and good selectivity characteristics. The study of the transmission mechanism of biological olfaction and gustation signals are constantly exploration, extraction and olfactory and gustative tissues, cells, receptors, and ion channels and other biological materials still exist some difficulties and restrictions in terms of reliability, ease of use, and other aspects of lifetime and, therefore, bioinspired smell and taste sensing technology is still at an early stage of research, promotion applications are still far carried out. However, because the technology has broad application prospects in the future, such as providing important safety information and food intake of toxic and hazardous substances to our, monitoring of human survival and a healthy environment are all important, therefore, the development of this technology will with a wide range of needs and get more and more intense human desire to explore.

In recent years, the rapid development of brain science, cognitive science, and brain–computer interface techniques provides a new way of thinking for exploring bioinspired smell and taste sensor. The use of minimally invasive surgery to micro and nano electrodes implanted in animals, smell and taste were identified by the encoding and decoding of brain systems approach, it does not require the traditional electronic nose and electronic tongue-stringent restrictions on the testing conditions, while the use of animals healing ability to extend the lifetime of bioinspired smell and taste sensors. In addition, the light gene biotechnology and micro-nano optical technology combined, the future can realize the control of animal and human sense of smell and taste behavior. The creature with the latest developments in medicine and engineering technology combined with the cross will develop a new generation of bioinspired smell and taste sensors.

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