Table 1 Summary of bioanalytical and chemical sensors utilizing taste, olfactory, and neural cells or tissues as biological sensing elements.

Cells/tissues	**Molecules/receptors**	**Transduction techniques**	**Stimuli/analytes**	**Performance/notes**	**Ref.**
Primary taste cells of rats	Multiple taste receptors	Electrochemical: LAPSs for extracellular recording	Tastant mixture (NaCl, HCl, $MgSO_4$, sucrose, and glumate)	The responsive extracellular potential changes were recorded.	[14]
	Multiple taste receptors		HCl, tastant mixture*, and exogenous ATP	Distinct temporal firings and firing rates were related to cell types and stimulus concentrations.	[15]
	Acid-sensing ionic channels (ASICs)		HCl	Two types of acid-sensitive taste cells were distinguished by firing spikes.	[16]
	Bitter taste receptors		$MgSO_4$, denatonium, and salicin	Bitter substances were discriminated via extracellular recording and PCA analysis.	[17]
	Multiple taste receptors	Electrochemical: Serotonin-sensitive LAPSs	Tastant mixture*/HCl	Lower detection limit: 3.3×10^{-13} M; Sensitivity: 19.1 mV per concentration decade	[21]
	Multiple taste receptors	Electrochemical: ATP-sensitive LAPSs	Tastant mixture*	A dose-dependent response to ATP at concentrations from 10^{-8} to 10^{-4} M was obtained.	[24]
Bioengineered HEK-293 cells	Bitter taste receptor: hT2R4	Electrochemical: LAPSs for acidification measurement	Denatonium	Sensitivity: 1.0 mV/s; a dose-dependent response at concentrations of 50, 200 and 500 nmol/L.	[27]
Bioengineered human enteroendocrine NCI-H716 cells	Sweet taste receptors: T1R2/T1R3	Electrochemical: carbon screen printed electrodes (CSPEs)	HCl, NaCl, $MgSO_4$, and sucrose	Four basic tastants and different concentrations of sucrose were distinguished.	[28]
Bioengineered human enteroendocrine STC-1 cells	Bitter taste receptors: T2Rs		Quinine, nphenylthiourea, and 6-propyl-2-thiouracil	Different concentrations of bitter substances were distinguished.	[29]
Bioengineered human embryonic kidney (HEK)-293	Polycystic kidney disease (PKD) channels	Electrochemical : MEAs	HCl	Distinct responses to sour stimuli were recorded in a non-invasive way for a long-term.	[35]
Rat taste epithelium	Multiple taste receptors		HCl, NaCl, quinine-HCl, glucose, and sodium glutamate	Different spatiotemporal patterns were recorded for different tastants.	[36]
	Bitter taste receptors		Quinine, denatonium and cycloheximide	Dose-dependent signals were recorded at 10 μM, 1 mM and 10 mM of bitter tastents.	[37]
Primary olfactory cells of rats	Multiple olfactory receptors	Electrochemical: LAPSs for extracellular recording	Acetic acid and glutamic acid	Characteristic signals at a unique frequency (24 Hz) were obtained.	[38]
			Acetic acid, octanal, cineole, hexanal and 2-heptatone	Inhibitory and enhancive effects on olfactory signals were recorded.	[39]
Bioengineered rat olfactory cells	ODR-10		Diacetyl	The amplitude patterns of the temporal firing were obtained at 0.1 μM to 100 μM diacetyl.	[40]
Bioengineered HEK-293 cells	ODR-10	Electrochemical: LAPSs for acidification measurement	Diacetyl	A dose-dependent response: at 10, 50 and 100 nmol/L of diacetyl. Sensitivity: 9.8 mV/s	[27]
Primary olfactory cells of rats	Multiple olfactory receptors	Electrochemical: MEAs	Dl-limonene and isoamyle acetate	Two odorants at different concentrations were distinguished.	[41]
Bioengineered HEK-293 cells	Olfactory receptor I7	Electrochemical: planar microelectrode	Octanal	A dose-dependent extracellular potential response was obtained at 1, 5 and 10 mM of octanal.	[42]
Bioengineered *X. laevis* oocytes	BmOR1, BmOR3, PxOR1, and DOr85b	Electrochemical: capillary Ag/AgCl electrodes	2-heptanone, bombykol, bombykal, and Z11-16:Ald	Dynamic ranges: 10 nM–1 μM; Sensitivity: a few parts per billion (ppb)	[43, 44]

Bioengineered HEK-293 cells	ODR-10	Optical: SPR	Diacetyle	A dose-dependent response was obtained at 0.01, 0.1 and 1 mM of diacetyle.	[45]
	ORI7		Octanal	A dose-dependent response was obtained at 0.1, 1.0, 10 and 100 mM of octanal.	[46]
Bioengineered yeast cells	Olfr226	Optical: fluorometry	2,4-dinitrotoluene	Lower detection limit: 25 μM	[47]
Intact antennae of Colorado potato beetle	Multiple olfactory receptors	Electrochemical: FETs for extracellular recording	(Z)-3-hexen-1-ol	0.1 parts per million (ppm) to 100 ppm/0.1 ppb to 100 ppm	[48, 49]
An olfactory sensillum of a blowfly		Electrochemical: microelectrodes	1,4-diaminobutane, 1-hexanol, and butanoic acid	Diaminobutane: a few ppb-100 ppm; hexanol: 8 ppm-500 ppm; butanoic acid: ppm-200 ppm	[50]
Rat olfactory epithelium		Electrochemical : MEAs	Ethyl ether, acetic acid, butanedione, and acetone	Different firing modes were recorded in response to different odorants.	[51-53]
Intact rat olfactory epithelium and bulb slices			Isoamyl acetate and l-carvone	The frequency of spiking activity was obtained in a concentration-dependent manner.	[54]
Rat olfactory bulb slices	Glutamic acid receptors		Glutamic acid	The amplitudes and firing rates increased with the concentration of glutamic acid.	[55]
Rat olfactory bulb *in vivo*		Electrochemical: microwire electrode array	Carvone and isoamyl acetate	Temporal features and rate features of firing patterns were distinguished. Accuracies: 83-96%.	[56]
Rat olfactory epithelium	Multiple olfactory receptors	Electrochemical: LAPSs for extracellular recording	Acetic acid and butanedione	Different frequencies and firing modes were elicited in response to different odorants.	[57]
The olfactory system of the *D. melanogaster* fruit fly		Optical: fluorometry	Volatiles from five different cancer cell lines	Characteristic response vectors were achieved upon different volatiles.	[58]
W1 and W2 neurons from *L. stagnalis*	5-HT receptors	Electrochemical: glass capillary microelectrodes	5-HT	Responses to 5-HT at concentrations of 10^{-6} M to 10^{-3} M were obtained.	[65]
H19-7 hippocampal neurons	Multiple membrane receptors and channels	Electrochemical : MEAs	Ethanol, H_2O_2, pyrethroids, and EDTA	Lower detection limits: 9 ppm, 19 ppm, 280 ppb and 180 ppm for each analyte.	[61]
			Ethanol, pyrethroid, and H_2O_2	Lower detection limits: 9 ppm, 180 ppb and 19 ppm for each analyte.	[62]
			Diesel and gasoline	Diesel and gasoline were detected at 30 ppb and 280 ppb, respectively.	[63]
Single neuron	Multiple membrane receptors		Ethanol	The background noise was decreased by a factor of 1.3 by increasing the microelectrode diameter.	[64]
Dorsal root ganglia neurons from adult mice			N/A	Changes in the solution temperature had a strong effect on the firing characteristics of the neurons.	[66]
Primary cultures of murine spinal cord neurons	Multiple membrane receptors and channels		Strychnine, biculline, and gpl20	Distinct burst patterns were obtained for distinguishing different chemical substances.	[67]
Neural networks of chick/mouse embryos			Electrical current stimulation	Electrical activity was elicited and distinct amplitude spikes were recorded for several weeks.	[68]
Spinal cord or frontal cortex murine tissue	Sodium/potassium channels		Tetrodotoxin (TTX) and tityustoxin	Lower detection limit: 2 nM; resolve extracellular potentials as small as 40 μV.	[69]

*Tastant mixture: $MgSO_4$, sucrose, monosodium glutamate (MSG)

Table 2 Tastants and corresponding taste receptors/molecules commonly used for tissue- and cell-based biosensors.

Taste sensation	Taste stimuli	Taste receptors/molecules
Sourness	HCl	Acid-sensing ionic channels (ASICs)[15,16], polycystic kidney disease (PKD) channels[35],
Sweetness	Sucrose	T1R2+T1R3[28]
Bitterness	$MgSO_4$, salicin, denatonium, quinine, *N*-phenylthiourea, and 6-propyl-2-thiouracil, cycloheximide	T2Rs[29,17,27,37]
Saltiness	NaCl	Epithelial Na channel (ENaC)[14,28,36]
Umami	Glutamate	T1R1+T1R3[14,36]

ANALYST

MINIREVIEW

Bioanalytical and chemical sensors using living taste, olfactory, and neural cells and tissues: a short review

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Biosensors utilizing living tissues and cells have recently gained significant attention as functional devices for chemical sensing and biochemical analysis. These devices integrate biological components (i.e. single cells, cell networks, tissues) with micro-electro-mechanical systems (MEMS)-based optical and electrical sensors. Various types of cells and tissues derived from natural and bioengineered sources have been used as recognition and sensing elements, which are generally characterized by high sensitivity and specificity. This short review summarizes the state of the art in tissue- and cell-based biosensing platforms with an emphasis on those using taste, olfactory, and neural cells and tissues. Many of these devices employ unique integration strategies and sensing schemes based on sensitive transducers including microelectrode arrays (MEAs), field effect transistors (FETs), and light-addressable potentiometric sensors (LAPSs). Several groups have coupled these hybrid biosensors with microfluidics which offers added benefits of small sample volumes and enhanced automation. While this technology is currently limited to lab settings due to the limited stability of living biological components, further research to enhance their robustness will enable these devices to be employed in field and clinical settings.

1. Introduction

Tissues and cells are complex biological systems which can detect multiple chemical and biochemical signals in complex environments with a high level of performance that currently cannot be matched by artificial devices. For example, vertebrate olfactory systems can recognize and discriminate thousands of odorants at trace levels due to the highly developed sensing capabilities of olfactory cells and epithelial tissues.[1-3] Similarly, taste cells and taste buds can simultaneously sense multiple taste signals elicited by different tastants.[4-7] These capabilities are also exhibited by neurons and neural networks, which can respond to multiple biochemical signals transmitted via neurotransmitters.[8-10] For these reasons, cells and tissues are promising candidates as recognition and sensing elements for bioanalytical and sensors.

Rapid advancements in microfabrication technologies have paved the way for the development of miniature biosensors that can be coupled with living cells and tissues.[11-13] These devices employ transducers that are typically on the order of 10's to 100's of microns in size, which facilitates their coupling with biological elements. Thoughtful device design and integration strategies have enabled the incorporation of cells and tissues onto sensors with high efficiency and a negligible loss of functionality, which can improve the detection of responsive signals from target compounds. The integration of biosensors with microfluidics can offer additional advantages including small liquid volumes, which can significantly reduce sample and reagent consumption, and increased analyte transport (i.e. diffusion). Furthermore, microfluidic biosensors can offer enhanced automation which minimizes the time and error due to manual sample processing. A variety of tissue- and cell-based biosensing platforms have been developed which utilize common analytical detection techniques such as electrochemistry, potentiometry and fluorometry.

In this review, we summarize the state of the art in tissue- and cell-based biosensors for chemical sensing and biochemical analysis focusing on those using taste, olfactory, and neural cells and tissues. A comprehensive comparison of these technologies is presented in Table 1. Since many of these systems employ similar integration strategies, this review is organized according to the types of sensors that are used, mainly microelectrode arrays (MEAs), field effect transistors (FETs), and light-addressable potentiometric sensors (LAPSs). Key innovations and limitations of these systems will be discussed as well as future opportunities and prospects for tissue- and cell-based biosensing systems.

2. Biosensors based on taste sensation

Biological taste systems are natural chemical sensing systems that can distinguish the five "basic" tastes (sweet, bitter, sour, salty, and umami) providing organisms with essential

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information on the quality and nutrition of food. Taste cells and taste buds are key components of biological taste systems and exhibit unique characteristics for the detection of chemical signals in response to tastants. Taste sensation is facilitated through taste receptors, which are located on the surfaces of taste cells and taste buds. The most common taste receptors and corresponding tastants utilized for taste biosensors is summarized in Table 2.

2.1 Taste cell-based biosensors

Taste cells coupled with LAPSs. A LAPS is a semiconductor-based sensor that uses light to detect changes in the surface potential. Due to its ability to perform spatially resolved measurements, LAPSs are useful for monitoring extracellular signals from cells. Fig. 1 shows the basic mechanism of a LAPS based on an electrolyte-insulator[SiO_2]-semiconductor[Si] substrate. Upon illumination of light, the LAPS semiconductor surface produces electron-hole pairs due to the absorption of light energy. A bias voltage is applied to the LAPS chip, via a reference electrode and working electrode, to avoid the rapid recombination of the electron and hole. As a result, a photocurrent is generated which can be detected by a peripheral circuit. When cells or tissues are cultured on a LAPS surface, changes in their extracellular potential will subsequently alter the local surface potential of the sensor which can be detected by measuring fluctuations in the photocurrent.

Compared with other types of sensors, such as MEAs or FETs, LAPSs can achieve high spatial resolution by simply focusing the light on the target cells, which avoids the need for complicated cell positioning. One of the earliest demonstrations of a taste-based LAPS was reported by Zhang et al. for extracellular potential recordings of rat taste receptor cells (TRCs) in response to a tastant mixture.[14] It was observed that extracellular signals from the tastant mixture (NaCl, HCl, $MgSO_4$, sucrose and glutamate) generated different burst shapes and amplitudes compared with the signals from a control sample (cell culture media), demonstrating the feasibility of this this technology. To explore the possibility of

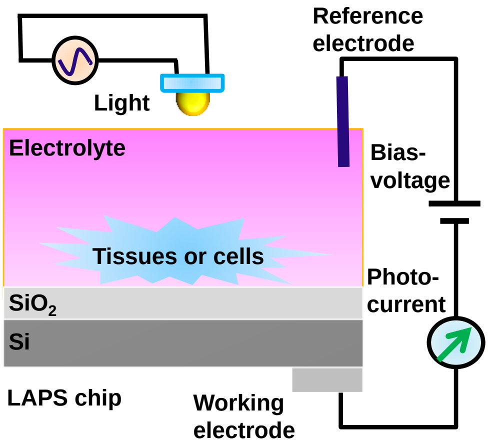

Fig. 1 Schematic illustration of a LAPS coupled with living cells or tissues for chemical sensing.

discriminating distinct tastants from LAPS extracellular recording measurements, Chen et al. developed a LAPS to analyze the temporal firing rate of TRCs in response to HCl and a tastant mixture.[15] Distinct firing responses were observed for HCl, the tastant mixture ($MgSO_4$, sucrose and monosodium glutamate (MSG)) and a control sample. Additionally, the firing rate was observed to be dose-dependent for HCl. This device was also able to distinguish different types of TRCs based on temporal firing responses by employing principal component analysis (PCA) for signal processing, and was used to demonstrate the enhancive and inhibitory effects of exogenous adenosine triphosphate (ATP) on the spontaneous firing rate. This work was further developed by incorporating computational models of acid-sensing TRCs to simulate the action potentials which improved the analysis of the extracellular signals.[16] Bitter-sensitive TRCs have also been coupled with a LAPS device for bitter substance detection.[17] Similar to the approach by Chen et al., signal processing of the extracellular signals was performed using PCA, which enabled the discrimination of three distinct tastants including $MgSO_4$, denatonium, and salicin.

In addition to detecting potential changes from cells directly attached on LAPS surfaces, sensitive membranes have been integrated onto LAPSs to detect specific analytes. Based on this approach, LAPS devices have been used for the detection of neurotransmitters released by TRCs, which play an important role in taste signal transduction and transmission. In particular, serotonin (5-hydroxytryptamine, 5-HT) and ATP are common neurotransmitters associated with taste cell-to-cell communication.[18-20] Chen et al. developed a LAPS which was modified with a thin serotonin-sensitive polyvinyl chloride (PVC) membrane for the detection of 5-HT by TRCs.[21] The serotonin-sensitive PVC membrane exhibited inhibitory effects to Na^+, K^+ and quaternary ammonium ions and good stability in solutions with pH 2–9, which is very important for taste cell measurements that need to be performed in complex microenvironments (e.g. acid-sensitive cells need to be stimulated by solutions with low pH values).[22,23] This biosensor could detect 5-HT released from TRCs upon the application of HCl and a tastant mixture ($MgSo_4$, sucrose and MSG) with a lower detection limit of 3.3×10^{-13} M and a sensitivity of 19.1 mV per concentration decade. In addition to serotonin detection, the detection of ATP secreted from TRCs using LAPSs has also been explored. Wu et al. developed a LAPS functionalized with ATP-sensitive aptamers for the detection of ATP released from TRCs during cell-to-cell communication.[24] Compared with using an analyte-specific PVC membrane, ATP-sensitive aptamers are more stable since they are less sensitive to environmental, chemical and temperature changes. Local ATP secretion from a single TRC could be detected in response to a simulated tastant mixture ($MgSO_4$, sucrose, MSG) by monitoring the working potential shifts of the LAPS. This biosensor exhibited a dose-dependent response to ATP concentrations from 10^{-8} to 10^{-4} M. Measurements were also performed in response to octanol, an inhibitor of TRCs. These results showed a significant decrease in the working potential, validating the inhibitory effects of octanol on ATP secretion from TRCs.

While isolating primary TRCs from rats is a relatively straightforward process,[25,26] collecting a sufficient amount of cells is challenging due to the limited number of cells in a rat tongue and the low efficiency of cell isolation. In addition, the types of receptors expressed in primary taste cells are not well defined, which can result in inconsistent responses from similar taste substances. To address these issues, bioengineered TRCs have been used as sensing elements which are generated by expressing defined taste receptors in a heterologous cell system. Compared with primary taste cells, bioengineered taste cells can respond to specific tastants in a more stable and repeatable manner due to their homogeneity. Du et al. coupled bioengineered TRCs with a LAPS device for label-free functional assays of chemical receptors.[27] Human embryonic kidney (HEK)-293 cells, engineered to express hT2R4 taste receptors, were cultured on the LAPS surface. The specific ligand binding function of the receptors was monitored by localized extracellular acidification measurements, which detects changes in proton generation by the cells. A dose-dependent response to the bitter compound denatonium was observed at concentrations of 50, 200 and 500 nmol/L. Since LAPSs are sensitive to changes in the electrical charge of the surface, the main advantage of localized extracellular acidification measurements over extracellular potential recording measurements is that the sensor surface does not need to be modified which greatly simplifies device fabrication.

Taste cells coupled with carbon screen-printed electrodes. Carbon screen-printed electrodes (CSPEs) are a type of electrochemical sensor which can be used to detect changes in electrical impedance at an electrolyte-electrode interface. When cells or tissues are coupled to the surface of a CSPE, morphological changes in response to specific stimuli will alter their impedance, which can be detected by the electrodes. Compared with LAPS extracellular measurement, which are only suitable for electrically excitable cells, impedance measurements using CSPEs can monitor extracellular signals from non-electrically excitable cells.

Bioengineered tasted receptor cells have been combined with CSPEs for the detection of sweet and bitter substances. Human colorectal carcinoma NCI-H716 cell lines expressed with α-gustducin and the sweet taste receptor T1R1/T1R3 were coupled with CSPEs to develop a sweet cell-based biosensor.[28] The response of the cells to HCl, NaCl, $MgSO_4$ and different concentrations of sucrose solutions was monitored by electrochemical impedance spectrum measurements. Bistable stochastic resonance (BSR) was employed for data processing and signal amplification, which couples additional noise to a bistable nonlinear system and enables weaker signals to be distinguished from the background noise. Using BSR analysis, the four basic tastants and sucrose concentrations from 17-200 mM could be distinguished from each other. Based on a similar platform, G protein-coupled receptors (GPCRs) and type 2 member (T2R) receptors expressed in human enteroendocrine STC-1 cells were used as recognition elements for the detection of different concentrations of bitter substances including quinine, *N*-Phenylthiourea and 6-propyl-2-thiouracil.[29] This biosensor could selectively respond to various concentration of these bitter compounds while generating a negligible response to sucrose.

Hui et al. also developed a CSPE biosensor for impedance measurements of taste cells in response to sweet and bitter tastants.[30] In contrast to prior works using this technology, this device employed NCI-H716 cells expressing GPCRs and T1R1/T1R3 receptors and STC-1 cells expressing GPCRs and T2R receptors. A unique double-layered cascaded series stochastic resonance (DCSSR) method was used for data processing and signal amplification, where several stochastic resonance systems are connected in series so the output of the first single-layered stochastic resonance signal is used as the input of the second single-layered stochastic resonance signal. This approach was able to achieve improved discriminating abilities and higher sensitivity for sucrose/quinine tastant mixtures than the more commonly used bistable stochastic resonance method.

Taste cells coupled with MEAs. A MEA is a device comprised of multiple microelectrodes to perform simultaneous measurements at multiple sites on the sensor surface. Fig. 2 shows the coupling of cells or tissues with a MEA chip for extracellular recording measurements of cell membrane potential. The physical mechanism behind MEA biosensors is based on the principle that cells and tissues generate a transmembrane current in response to specific stimulations, which is caused by the opening of ion channels in the cell membrane. This alters the cell membrane potential, which subsequently changes the electric field across the cellular membrane and polarizes the microelectrodes that are in contact with the cells. The charge distribution at the interface between the electrode and electrolyte can be measured by the microelectrodes, which are commonly coupled with external electronics for signal amplification, processing and analysis. A major advantage of MEA biosensors over LAPSs or FETs is that they enable high throughput measurements due to having a large number of electrodes. This is particularly useful when they are coupled with bioengineered cells which generally suffer from poor

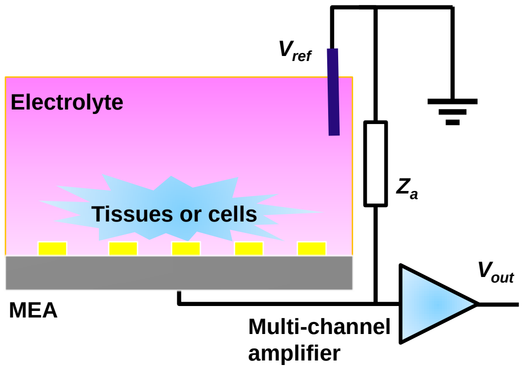

Fig. 2 Schematic illustration of a MEA coupled with cells or tissues for extracellular recording measurements.

transfection efficiency.

As with all taste sensations, there are a variety of taste cells responsible for the sour sensation. Polycystic kidney disease (PKD) channels, which belong to a family of transient receptor potential ion channels, are generally used as molecular sensors for sour sensation because of their acid sensing capability and well-known biological mechanisms.[31-34] Wu et al. developed a cell-based biosensor using HEK-293 cells expressing PKD channels coupled with a 6 × 6 MEA.[35] Extracellular recording measurements were monitored in response to HCl and a tastant mixture containing $MgSO_4$, sucrose and MSG. Extracellular recording signals from the sour tastant were up to 4× as large as those from the mixture sample and control sensors, which contained HEK-293 cells without PKD channels. Compared with the onset response of acidic stimuli recorded by LAPSs,[16] this biosensor is able to record the unique “off-response” of PKD channels when stimulated by a set of sour stimuli (pH 7.0-2.5-7.0-4.0), which indicates that acid-activated PKD channels do not generate a transmembrane current until the removal of acid stimulus.

2.2 Taste tissue-based biosensors

Taste epithelium coupled with MEAs. Epithelia tissue from the tongue contains various types of taste buds, which are specialized structures that can simultaneously respond to multiple tastants. Upon application of a tastant, the detected signals are converted into cellular responses, such as changes in the membrane potential or the release of neurotransmitters. Due to its unique capability for taste sensation, taste epithelium has been used as a sensitive element for biosensors. While this approach has the potential to achieve multiplexing and high sensitivity measurements, the responsive behaviors of taste epithelium are complicated and unclear, which makes it difficult to obtain distinct and stable sensing signals, and interpret the data. As a result, there are only a few studies that report on the utilization of taste epithelium as sensitive elements for bioanalytical and chemical sensors.

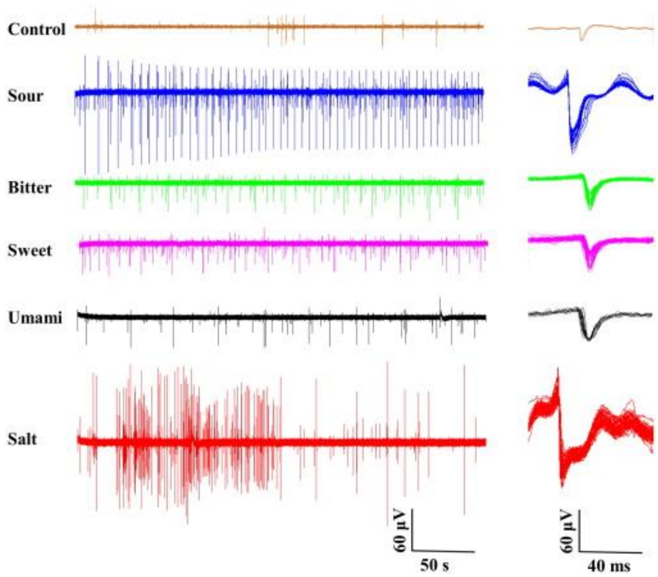

Fig. 3 Representative recorded electrophysiological signals from a tongue epithelium MEA biosensor in response to the five basic tastants. The recorded potentials of the complete waveform are shown on the left and the spike sorting maps are on the right. (Reproduced with permission from ref. 36. Copyright 2013 Elsevier)

Liu et al. developed a MEA biosensor using epithelium from a rat to detect the five basic tastants via extracellular potential recordings.[36] Representative electrophysiological signals recorded by one channel of the MEA, in response to HCl, NaCl, quinine–HCl, glucose and MSG are shown in Fig. 3. This data shows that the presence of taste stimuli generates significant action potentials compared with the native activities from the control sample. Additionally, the response patterns and waveforms from different stimuli are unique, which reflect the distinct properties of these tastants.

A similar MEA taste epithelium-based biosensor was developed by Liu et al. for the detection of bitter compounds.[37] Electrophysiological activities, including the firing rate, amplitude and power spectrum, of taste epithelium before and after application of quinine, denatonium and cycloheximide were measured. Each of these tastants exhibited unique field potentials with respect to the duration and amplitude of the signal. In addition, dose-dependent responses for three concentrations (10 µM, 1 mM and 10 mM) of these tastants were observed. Specifically, the amplitude and firing rates of extracellular potentials increased with higher tastant concentrations. The authors also noted that measurements could be performed up to 24 hrs after tissue isolation with a negligible loss in the signal.

3. Biosensing based on olfaction

The olfactory system is a biological sensory system capable of recognizing and discriminating thousands of odorants even at trace levels. The fundamental elements of the olfactory system are olfactory cells which contain numerous olfactory receptors. Olfactory cells are located in olfactory epithelium in mammals and olfactory sensilla in insect antennae. Due to their unique sensing ability, olfactory cells and sensilla have been utilized in biosensors for various applications including the detection of drugs, toxins and explosive residues. Researchers have also focused on the development of electronic noses that can recognize and detect odors and flavors. These devices typically consist of an olfactory-based biosensor array coupled with pattern recognition systems to mimic the human olfaction process. The responsive signals from an olfactory biosensor usually exhibit characteristic features in the time or frequency domain, which can be extracted and analyzed by pattern recognition or classification techniques such as PCA, artificial neural networks (ANN), and genetic algorithm (GA).

3.1 Olfactory cell-based biosensors

Olfactory cells coupled with LAPSs. Similar to taste cells, olfactory cells can be obtained from animals or bioengineered methods. Primary olfactory cells are generally isolated from rodents, which is convenient, but limited due to the fact that olfactory cells contain many different types of olfactory receptors. This could potentially influence the performance of the biosensor since different types of olfactory receptors can generate different responsive signals to the same odorant. In contrast, bioengineered olfactory cells can be generated by expressing specific olfactory receptors in a heterologous cell system, which offers better-defined sensing capabilities. Cells obtained from both methods have been coupled with LAPS devices for odorant detection and studies on olfactory signal transduction.

Liu et al. employed primary olfactory cells isolated from rats, which were directly cultured on a LAPS surface for the detection of acetic acid and glutamic acid.[38] Changes in the membrane potential of the olfactory cells were monitored in response to these two chemical stimuli via extracellular recording measurements. The recorded signals were processed using fast Fourier transform (FFT) analysis which resulted in characteristic signals at a unique frequency (24 Hz). To better understand the mechanisms behind the generation of extracellular signals using LAPSs, Wu et al. used a similar biosensing platform to perform measurements on olfactory signal intracellular transduction pathways using MDL12330A and LY294002, which are compounds that inhibit and enhance the adenylyl cyclase and phosphatidylinositol 3-kinase (PI3K), respectively.[39] Extracellular recordings of rat olfactory receptor neurons (ORNs) revealed that the application of MDL12330A significantly decreased the number of firing spikes compared with control samples. Conversely, application of LY294002 significantly increased the number of firing spikes compared with the control measurements. This work showed that in addition to odorant detection, olfactory cell-based biosensors can be useful for studying the biological mechanism behind olfactory signals transduction.

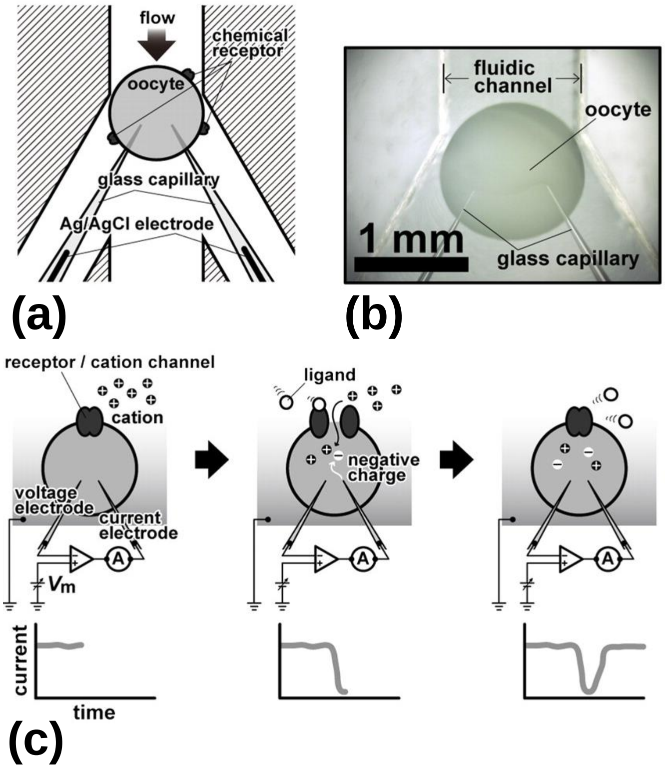

Fig. 4 Schematic illustration (a) and photograph (b) of a *X. laevis* oocyte trapped inside a microchannel and connected to two capillary electrodes. (c) Principle of cell membrane potential monitoring by the two-electrode voltage clamp method (TEVC). (Reproduced with permission from ref. 43. Copyright (2010) National Academy of Sciences, U.S.A.)

Olfactory cell-based biosensors have also been coupled with bioengineered olfactory cells, which are expressed with well-defined olfactory receptors and can offer improved sensitivity, repeatability and device stability. Toward this end, Du et al. developed a LAPS-based platform for odorant detection using bioengineered ORNs.[40] ODR-10, an olfactory receptor of *C. elegances*, was expressed in rat ORNs which were cultured on the sensor surface. Extracellular recordings of the ORNs were performed in response to different concentrations of diacetyl, a natural ligand of ODR-10. A dose-dependent response was observed from 0.1 μM to 100 μM, where the amplitude patterns of the temporal firing corresponded to the concentration of diacetyl. Additionally, specific firing patterns were observed under low/high concentrations. ODR-10 has also been expressed in HEK-293 cells and coupled with a LAPS for localized extracellular acidification measurements.[27] A dose-dependent response was observed for diacetyl concentrations of 10, 50 and 100 nmol/L. Additional studies were performed using MDL12330A, an inhibitor of adenylyl cyclase, which resulted in diminished cellular signals compared with measurements using only diacetyl.

Olfactory cells coupled with microelectrodes. Ling et al. employed a 60-channel MEA to monitor the membrane potential changes of primary ORNs upon the application of odorant stimuli.[41] ORNs were cultured on the MEA surface and extracellular signals of ORNs were monitored in response to increasing concentrations of dl-limonene and isoamyle acetate. The firing spikes occurred when the odor concentration exceeded 1.9×10^{-5} 3.3×10^{-5} mol/L for dl-limonene and 4×10^{-6} - 1.6×10^{-6} mol/L for isoamyle acetate. Lee et al. developed a microfabricated planar electrode coupled with HEK-293 cells expressing the olfactory receptor I7 and transfected with the gustatory cyclic nucleotide gated (CNG) channel.[42] CNG channels were used to amplify the membrane potential. Measurements of HEK-293 cells expressing I7 and cells co-expressing I7 and the CNG channel were performed upon application of a 10 mM octanal solution. A 2.5× larger field potential (~10 mV) was observed for co-expressed cells compared with cells only expressing I7 (~4 mV). Measurements were also performed using 1, 5 and 10 mM octanal solutions, which exhibited a dose-dependent extracellular potential response.

To improve the integration of cells with microelectrodes, Misawa et al. developed a microfluidic biosensor for odorant sensing.[43] *X. laevis* oocytes expressed with four insect olfactory receptors (BmOR1, BmOR3, PxOR1, and DOr85b) were immobilized inside a microchannel trap. Measurements were performed by the two-electrode voltage clamping (TEVC) method using two glass capillary Ag/AgCl electrodes, as shown in Fig. 4a and b. Changes in the cell membrane potential were observed in response to different concentrations of 2-heptanone (odorant) and three pheromones (bombykol, bombykal, Z11-16:Ald)(Fig. 4c). For all of these analytes, the sensor exhibited dynamic ranges of 10 nM–1 μM and a sensitivity of a few parts per billion (ppb). This platform was further developed by Tomida et al. by incorporating microfabricated gold electrodes within the microchannels.[44] The microfluidic network was designed to separate and trap single oocytes at individual electrodes for TEVC measurements. A dose-dependent response upon application of 0.3, 1.0 and 3.0 M KCl solutions was observed.

Olfactory cells coupled with surface plasmon resonance (SPR) sensors. SPR biosensors are based on the principal of surface plasmon resonance, where a plasmon wave is generated at the interface of a negative and positive permittivity material by incident light. Fig. 5 shows the physical mechanism of a SPR biosensor coupled with cells or tissues. The typical configuration of a SPR sensor consists of a prism with one face covered by a thin metal film, a light source, and an optical detector. The refractive index at which the surface plasmon resonance occurs is measured to monitor the physical property changes on the sensor surface (usually within a few hundred nanometers). When the tissues or cells respond to specific stimuli, changes in the intracellular components within the basal portion of the cells will subsequently shift the refractive index of the reflected light. Compared with LAPSs or MEAs, SPR biosensors can directly measure changes in intracellular components in regions near the sensor surface.

J.Y. Lee et al. developed a SPR biosensor using bioengineered HEK-293 cells for odorant detection.[45] ODR-10 was expressed in HEK-293 cells and cultured on the gold SPR sensor, which was precoated with poly-D-lysine to aid cell adhesion. The binding of odorant molecules initiates a cascade of intracellular signal transduction, resulting in an increase of cytosolic Ca^{2+} within the basal portion of the cells. This increase in ion concentration results in changes in the cell morphology near the sensor surface which can be detected by monitoring the shift in the resonance angle of the SPR waves. Measurements were performed using 0.01, 0.1 and 1 mM of diacetyle, a natural ligand of ODR-10, which revealed a dose-dependent response. S.H. Lee et al. developed a similar SPR biosensor which employed HEK-293 cells expressing ORI7, another olfactory receptor.[46] This device exhibited a dose-dependent response to octanal, a natural ligand of ORI7, at concentrations of 0.1, 1.0, 10 and 100 mM. Octanal solutions above 100 mM (i.e. 1 M) resulted in non-reproducible [illegible] signals, which the authors attributed to toxicity effects of the olfactory cells. The authors noted that the sensitivity of the biosensor could be improved by incorporating high influx ion channels into the cell membrane or modifying the plasmonic structure.

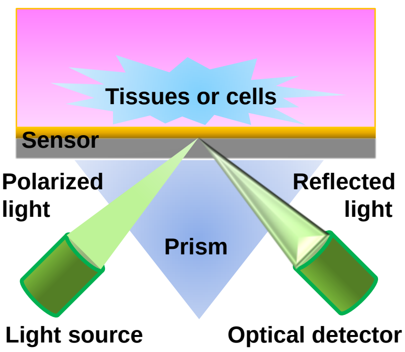

Fig. 5 Schematic illustration of a SPR sensor coupled with tissues or cells for chemical sensing.

Olfactory cells coupled with fluorometry. A fluorescence-based olfactory biosensor was developed by Radhika et al. for chemical sensing of explosive compounds.[47] *S. cerevisiae* yeast cells were constructed and expressed with Olfr226, a olfactory receptor, which was coupled with a green fluorescent protein (GFP) reporter system. The expression of the GFP gene is driven by the cyclic adenosine monophosphate (cAMP) response element binding protein promoter, which is sensitive to changes in intracellular cAMP levels. The binding of odorant molecules to olfactory receptors increases the concentration of cAMP, which promotes the expression of the GFP gene and generates a fluorescence signal. Bioengineered cells expressed with R7, a specific olfactory receptor responded to octylaldehyde at concentrations down to 25 μM. Additionally, the fluorescence signals showed a time-dependent response which steadily increased after 1 hr and reached the maximal intensity at 3 hr. Measurements were also performed using bioengineered cells expressed with Olfr226 to sense 2,4-dinitrotoluene, an explosive residue mimic, which could be detected at concentrations down to 25 μM.

3.2 Olfactory sensilla and tissue-based biosensors

Insect antennae combined with FET devices. FETs are another type of commonly used transducer for extracellular recordings of cells or tissues that can detect membrane potential changes in response to specific stimuli. Fig. 6 shows the configuration of a FET device coupled with cells or tissues on the gate surface via an electrolyte solution, where a reference electrode is placed in the electrolyte solution. Upon exposure to specific stimuli, the membrane potential of the cells or tissues changes and alters the channel conductance

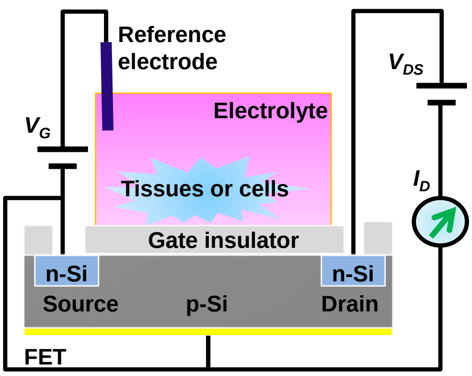

Fig. 6 Schematic illustration of a FET biosensor with tissues or cells cultured on its gate insulator for extracellular recording measurements.

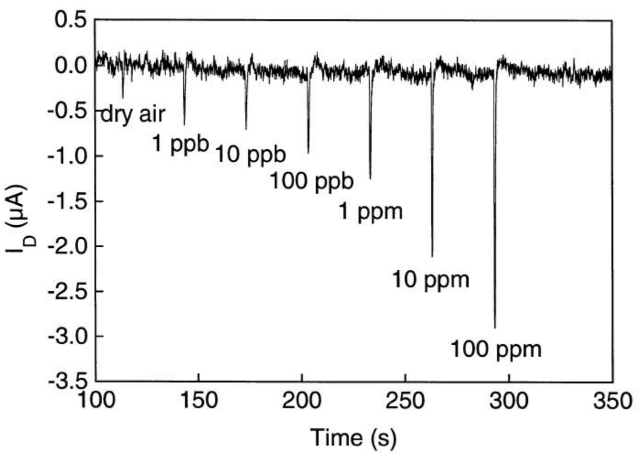

Fig. 7 Representative signals from an insect antenna-based FET biosensor for the detection of detecting (Z)-3-hexen-1-ol, a volatile biomarker for plant damage. (Reproduced with permission from ref. 49. Copyright 2000 Elsevier)

under the gate surface. Changes in the channel conductance are detected by monitoring the drain current with respect to the electrolyte conductance. An advantage of FETs over LAPSs for extracellular recording measurements is their high input impedance, which enables direct coupling of the cells with the gate surface of the FET sensor. This simplifies device fabrication and improves the mechanical and electrical stability of the cell-sensor surface.

Antennal olfactory sensilla are specialized organs found in many insects which enable them to sense environmental chemical compounds. Intact antennae of *L. decemlineata* (Colorado potato beetle) have been used as sensitive elements for the detection of specific odorants.[48] The antenna was connected to the gate of a FET device using a hemolymph Ringer solution as an electrolyte. This device was used to detect (Z)-3-hexen-1-ol, a volatile biomarker for plant damage, which could be detected from 0.1 parts per million (ppm) to 100 ppm. Additionally, this device exhibited a response time of < 1 sec and high reversibility in air. A similar biosensor was employed by Schütz et al. for monitoring plant damage in a greenhouse setting.[49] This biosensor could detect (Z)-3-hexen-1-ol from 0.1 ppb to 100 ppm in air (Fig. 7), which the authors claimed can distinguish a single mechanically or beetle-damaged plant among 1,000 undamaged plants in a greenhouse. The authors also noted that the lifetime of their biosensor was ~ 4 hrs.

Olfactory tissue coupled with microelectrodes. Rather than using intact insect antennae, which can be difficult to prepare, olfactory cells isolated from antennae have been employed for olfactory biosensors. Huotari coupled microelectrodes with ORNs in an olfactory sensillum of a blowfly for the detection of several odorants including 1,4-diaminobutane, 1-hexanol, and butanoic acid.[50] Measurements were performed by analyzing the action potential rates in response to the application of these odorant compounds. This device could detect 1,4-diaminobutane from a few ppb to 100 ppm, 1-hexanol from 8 ppm to 500 ppm and butanoic acid from 20 ppm to 200 ppm. The author noted that the upper detection limit of this biosensor is due to odorant saturation of the ORNs, which hinders action potential production.

MEA biosensors for electrophysiological recording measurements using olfactory epithelium isolated from the noses of rats were developed by Liu et al.[51-53] The use of MEAs enabled simultaneous measurements at multiple sites on the tissue for spatio-temporal analysis. Analysis of electrophysiological recording measurements revealed that different firing modes were elicited in response to different odorant stimuli, such as ethyl ether, acetic acid, butanedione, and acetone. In addition to intact olfactory epithelium, rat olfactory epithelium sagittal slices with intact connection to the olfactory bulb were coupled with a 8 × 8 MEA device for spatial odor detection.[54] Parallel multi-site extracellular recordings showed that the application of isoamyl acetate or l-carvone increased the frequency of spiking activity in a dose-dependent manner. The enhancive effects of forskolin and 3-isobutyl-1-methylxanthine were also observed using this biosensor, which resulted a unique, partially overlapping spatial distribution pattern compared with those of isoamyl acetate and l-carvone. Olfactory bulb slices from rats have also been coupled with a MEA device for multi-site electrophysiological recording measurements of neural networks.[55] The recorded electrophysiological activities were evaluated by spike detection and cross-correlation analysis in response to glutamic acid. These results revealed that higher concentrations of glutamic acid increased the amplitude of the signals as well as the firing rates. Additionally, preliminary results showed that different sites of the bulb slice elicited different electrophysiological characteristics and firing patterns in response to glutamic acid, which could be detected at concentrations down to 100 μM. The authors noted that further studies are needed to improve device sensitivity and to determine a possible correlation between odorant stimulation and site-specific response.

While the majority of tissue and cell-based biosensors employ biological materials isolated from animals, Dong et al. undertook an alternative approach by implanting a 16-channel microwire electrode array into the olfactory bulb of a rat for *in vivo* extracellular potential monitoring.[56] The extracellular potential of mitral/tufted (M/T) cells was monitored in response to carvone and isoamyl acetate at concentrations from 10^{-15} M to 10^{-5} M. From these experiments, the firing patterns showed noticeable differences in temporal and rate features in response to different odorant stimuli and concentrations. An algorithm based on population vector similarity and support vector machine (SVM) was employed to classify the odorants, which exhibited accuracies between [illegible] 96%. Based on these results, the authors claimed a detection limit of some odorants as low as 1 ppm, which is ~10× lower than the detection limit of biosensors using *in vitro* olfactory bulb as sensing elements.[55] However, the utilization of *in vivo* olfactory tissues requires much more complicated surgery on animals and well-controlled animal status during measurements. While further characterization is required, this *in vivo* biosensor represents a promising technology for the detection of various drug and explosive compounds and for brain-machine interface (BMI) research.

Olfactory tissue coupled with LAPS. Liu et al. combined olfactory epithelium with a LAPS for odorant detection.[57] Olfactory mucosa epithelium was affixed to the surface of a LAPS, and measurements were performed in response to acetic acid and butanedione. The results showed that different frequencies and firing modes were elicited in response to these two odorants. Specifically, butanedione stimulation elicited an increase in signal at 6.1 Hz and 9.2 Hz, while a characteristic peak at 7.6 Hz was registered for acetic acid stimulation. When using fresh isolated tissue, the authors noted that their biosensor exhibited a lifetime of up to 2 hrs. Compared with using olfactory cells, obtaining precise extracellular recording measurements using olfactory epithelium is complicated due to the superposition of extracellular potentials from adjacent cells in the tissue, which can lead to difficulties in analyzing the recorded signals.

Olfactory tissue coupled with fluorometry. Recently, Strauch et al. developed a biosensor utilizing the olfactory system of the *D. melanogaster* fruit fly for the discrimination of cancer cells from non-cancer cells.[58] This approach is based on the detection of distinct volatile compounds emitted by cancer cells, which has been previously reported as a non-invasive technique for cancer screening.[59,60] Briefly, GCaMP, a green fluorescence reporter protein with a Ca^{2+} binding domain, was expressed in antennal ORNs. Fruit flies were then exposed to different odorants taken from the headspace of the culture media of five different cancer cell line samples. The presence of odorants from cancer cells resulted in an increase in Ca^{2+} concentration, generating a higher fluorescent signal. Multidimensional analysis was performed on the recorded responses of the antenna, which indicated that characteristic response vectors could be achieved upon stimulations by volatiles elicited from different cancer cell types. Furthermore, it could be used to discriminate healthy mammary epithelial cells from different types of breast cancer cells. This proof of concept work shows that olfactory-based biosensors could be an effective technology for non-invasive diagnosis of cancer or other diseases. Compared with insect antenna FET biosensors, which can only provide measurement from a single location on the sensor surface (i.e. the gate of the FET), this fluorescence biosensor is able to read out multiple olfactory receptors from various sites on an insect antenna. However, the time resolution of fluorescence imaging is several orders of magnitude lower compared with extracellular recordings measurements using electrical sensors.

4. Biosensors based on neural cells and tissues

While biosensors based on taste and olfactory cells and tissues are the most common due to their natural capabilities for chemical sensing, many other types of cells and tissues have been employed in biosensors for chemical and biochemical analysis. In this section, we focus on devices that employ neurons and neural networks integrated with electrical transducers such as microelectrodes, MEAs, and FETs.

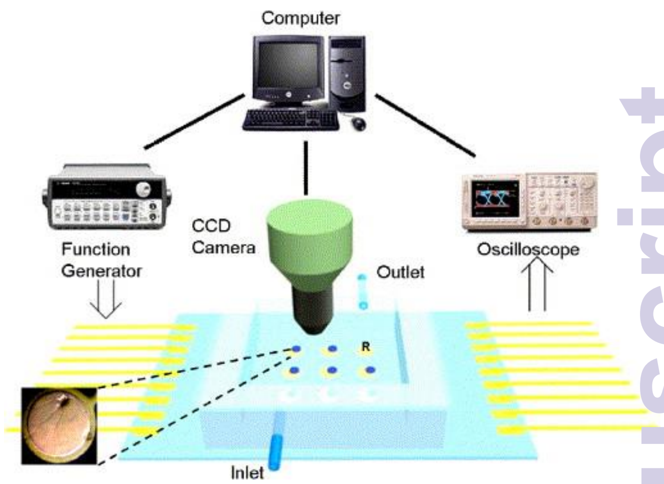

Fig. 8 Schematic illustration of a neuron-based MEA biosensor for chemical sensing. This system employs dielectrophoretic traps to manipulate single neurons, which are monitored electrically and optically in response to chemical stimuli. (Reproduced with permission from ref. 61. Copyright 2004 Elsevier)

4.1 Neuron-based biosensors

Neurons are electrically excitable cells that generate action potentials in response to electrical or chemical stimuli. This makes them ideal candidates for the development of electrical biosensors for chemical sensing. Prasad et al. developed a neuron-based MEA biosensor which employed positive dielectrophoretic traps to position single neurons on individual electrodes.[61] As shown in Fig. 8, this biosensor is able to monitor single neurons electrically and optically in response to chemical stimuli. The MEA was encapsulated in a silicone microfluidic chamber and the entire device was enclosed in an environmental chamber maintained at 37°C and 5% CO_2. The extracellular potential signals of H19-7 hippocampal neurons were processed and analyzed using FFT and wavelet transform analysis. The lower detection limits for a single neuron was 9 ppm, 19 ppm, 280 ppb and 180 ppm for ethanol, hydrogen peroxide, pyrethroids and ethylene diamine tetra acetic acid (EDTA), respectively. An additional study was carried out to determine if this single neuron-based biosensor could distinguish chemical agents in an unknown sample by exploiting the unique electrical identifiers generated by the neurons.[62] The authors showed that their biosensor exhibited a prediction capability for identifying ethanol, pyrethroid, and hydrogen peroxide in an unknown test sample. Furthermore, their device exhibited lower detection limits of 9 ppm, 180 ppb and 19 ppm for ethanol, pyrethroid and hydrogen peroxide, respectively. Prasad et al. further applied this single-neuron MEA biosensor for the detection of unburned fossil fuel compounds.[63] Two types of fuels, diesel and gasoline, were measured, which could be detected at concentrations down to 30 ppb and 280 ppb, respectively.

While neuron-based biosensors have shown tremendous promise for bioanalysis and chemical sensing, their performance can be influenced by multiple factors including the size of the microelectrodes and the local temperature of the neurons. Studies were performed by Yang et al. to investigate the influence of electrode geometry and environmental parameters on the performance of single-neuron MEA biosensors.[64] They observed a ~1.3× decrease in the background noise by increasing the diameter of microelectrodes from 80 µm to 110 µm. In addition, fully immersing the microelectrodes in media solution and achieving a good microchamber seal reduced the noise level by a factor of ~1.5. To investigate the influence of temperature on neural sensing properties, Kurdikar et al. developed a biosensor integrating W1 and W2 neurons from *L. stagnalis* (great pond snail) with glass capillary microelectrodes.[65] Changes in the maximum firing frequency in response to 5-HT at concentrations of 10^{-6} M to 10^{-3} M were measured between 20-32°C. An increase in maximum firing frequency and sensitivity with higher temperatures was observed up to 32°C, which demonstrate the capability of neural biosensors to operate at elevated temperatures.

Alternatively, Pearce et al. developed a microfluidic MEA biosensor to measure the electrical activity of neurons under controllable fluid conditions.[66] Dorsal root ganglia neurons from adult mice were cultured onto a MEA. The local temperature of neurons was dynamically controlled by the fluid flow inside the microchannels. Extracellular recording measurements indicated that changes in the solution temperature had a strong effect on the firing characteristics of the neurons. Warm solution (35°C) resulted in the firing rate of neurons to drop to almost 0 spikes/s. Conversely, cold (16°C) solution caused the firing rate to increase to ~1 spikes/s.

4.2 Neural network-based biosensors

Biological neural networks consist of a series of neurons that are interconnected via synapses to dendrites on other neurons. Since neural networks retain the connectivity between neurons, they can provide measurements with improved sensitivity compared with individual neurons. In addition, neural networks can provide faster response signals than those from individual neurons. Generally, these devices monitor changes in the action potential patterns based on extracellular potential recordings generated from MEAs, FETs or LAPSs.

Gross et al. developed a neural network-based biosensor using MEAs for odor, drug and toxin analysis.[67] Primary cultures of murine spinal cord neurons were coupled with a 64-channel MEA for electrophysiological recording measurements. Measurements of neural networks upon applications of strychnine (synaptically active agents), biculline (competitive antagonist of gamma-aminobutyric acid A receptors), and gpl20 (protein of the AIDS virus) resulted in distinct burst patterns, demonstrating its ability to distinguish different chemical substances. The authors also noted that cultured neural networks exhibited different burst patterns in response to different strychnine application protocols (e.g. gradual vs. sudden application). Morin et al. developed neural network biosensing platforms comprised of three-dimensional PDMS microwells and channels integrated with MEAs.[68] Neural networks of primary cells from chick or mouse embryos were cultured on the sensor surface. Extracellular recordings of the cell cultures were monitored upon electrical stimulation, which resulted in distinct amplitude spikes. It was noted that electrical activity from primary cultures could be elicited for more than four weeks, which demonstrates the stability of the technique for long term-measurements.

Towards a fully integrated, portable biosensing platform with temperature and flow control, Pancrazio et al. developed a neural network MEA biosensor for neurotoxin detection.[69] A two-stage thermal control system with integrated fluidics was employed to maintain a temperature of 36-37°C for neural network cultures. Cells from spinal cord or frontal cortex murine tissue were cultured on MEA surfaces within PDMS microstructures. Extracellular recording measurements were performed in response to tetrodotoxin (TTX) and tityustoxin (ion channel blockers) which could be detected at concentrations down to 2 nM. Furthermore, this recording system could readily resolve extracellular potentials as small as 40 µV. While the authors suggest that further developments are needed to integrate additional neural networks and improve signal analysis, this work demonstrates one of the most functional and portable biosensing systems using living cells/tissues.

5. Conclusions

Tissue and cell-based biosensors are a promising biomedical technology which can be used to detect and analyze a wide spectrum of targets with a high degree of sensitivity and specificity. Many of these devices employ natural cells and tissues isolated from animals in order to preserve the recognition and sensing capabilities of these elements. Efforts have also focused on the use of bioengineered cells and tissues which can enable greater flexibility in regards to analyte recognition and signal transduction. Biosensors have also been developed which employ intact biological structures (e.g. insect antenna) to maintain their natural functionality. While many promising proof of concept devices have been demonstrated, there are several issues that need to be addressed before these platforms can be used outside of lab settings. For instance, scalable methods to generate high quality living cells and tissues for use as recognition and sensing elements are needed. Current advancements in stem cell and tissue engineering may provide useful approaches to address this issue through the development of efficient bioreactors.[70,71] In addition, methods for improving the integrity and stability of living tissue- and cell-based biosensors are required. Progress in micro-/nanofabrication and surface chemistry to improve the biocompatibility of sensor surfaces can improve device stability and facilitate signal transduction.[72] Lastly, new approaches to integrate biosensors with microfluidic components are desired to enhance automation and make these systems more user-friendly. Specifically, researchers are devising new methods for simplifying the integration of biosensors with microfluidic components and systems.[73,74] With the emergence of new micro-/nanofabrication technologies and biotechnologies, we anticipate that next generation tissue- and cell-based biosensors will offer enhanced robustness, sensitivity and scalability. These efforts, combined with the use of different types of tissues and cells, will help to make this technology more useful for high impact applications such as environmental and food quality monitoring, toxin detection and disease diagnosis.

Acknowledgements

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