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Review

Development of planar patch clamp technology and its application in the analysis of cellular electrophysiology

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Abstract

A patch clamp chip, as a novel cell-based chip for electrophysiological recordings, has many prominent advantages such as high resolution, accuracy, high throughput and automation. It can be used to perform multivariate and real-time measurements of cell networks *in situ*. Therefore, this technology will dramatically promote the research on ionic channels, neuronal networks and the application of this technology in drug screening. This paper reviews the development of planar patch clamp technology and its applications in detail. The latest progress in the research of taste cells electrophysiology and taste transduction is also presented. Finally, this paper analyzes the methodology of neural chips. Based on the current research of our laboratory, the prospective applications of a patch clamp chip in the research of taste sensation and transduction mechanisms at molecular and cellular levels are discussed.

Keywords: Patch clamp chip; Cellular electrophysiology; Taste; Taste cell; Taste transduction

1. Introduction

Conventional patch clamp technology, as the golden standard of cellular electrophysiology research, has the quality of large information content and high resolution. However, the intrinsic limitations of this technology such as low throughput, complicated operations and the requirements of delicate experimental conditions, have hampered its applications in cellular physiology and drug discovery. A patch clamp chip can form a high seal resistance automatically and perform high throughput cellular electrophysiology recordings. Combined with optical measurements, the patch clamp chip will facilitate biophysical and neurobiological research on ionic channels and promote drug discovery.

Taste is one of the chemosensations. Taste buds are peripheral sensory organs that respond to a great variety of taste stimuli. The five basic taste modalities are categorized as salty, sweet, bitter, acid and umami. Each utilizes different receptors, ionic channels and transduction mechanisms to transmit taste information. Due to the complications of the gustatory system, the understanding of taste has fallen behind our understandings of other chemosensations. Recently, the development of patch clamp technology, microelectronic chip technology, and cellular and molecular biology, has greatly promoted the research of taste at the cellular level. Furthermore, communications among taste cells within the taste buds, i.e. encoding and transmitting taste information to the afferent nerve and the brain, have been found. Cell-based chip technology is mainly being utilized to investigate signal transductions

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in the neural network. However, a potential problem of this technology is that the relationship between the extracellular and intracellular signals has not been well understood. By inheriting the advantages of the intracellular recordings employed in conventional patch clamp technology, a patch clamp chip will be a new tool in the research of information transduction in the taste cell network.

This paper firstly presents the development of planar patch clamp technology, and its applications in cellular electrophysiology and pharmaceutical screening. Then, the latest progress of taste electrophysiology and transduction mechanisms is reviewed. Based on analysis of neural chips, the prospective applications of a patch clamp chip in taste sensation and transduction studies are discussed.

2. Conventional patch clamp

Conventional patch clamp technology is a powerful technology which has been used to study ionic channels. The basic principle of whole-cell recording is holding the membrane potential of a cell to study its function by way of recording the ionic currents. The key point of patch clamp is to form giga-Ohm seal resistance between a microelectrode and cell membrane. It can reach up to 10–100 GΩ. Meanwhile, the distance between the microelectrode and cell membrane is less than 1 nm so that the membrane patch is electrically isolated from the surroundings (Fig. 1).

Resolution of the patch clamp recording has been greatly improved with continuous applications. The cell attached mode has been used to obtain the information about the ion selectivity, kinetics and types of ionic channels. Combined with the whole-cell mode, the physiology of ion channels, and the amount and open probability of ion channels on the membrane have been studied. Using an excised patch, it is possible to obtain information about the regulation of the transmitters and the second messenger on the channels. Conventional patch clamp technology can directly measure the gating, permeability, selectivity and voltage sensing of ionic channels at the molecular level, showing its great potential in the research of neuroscience and electrophysiology.

However, patch clamp technology has certain inevitable limitations, such as a very low throughput. In this respect, it can hardly be applied to the research of cellular communication in neural networks. Moreover, during the process of recording, the intracellular solution cannot be exchanged conveniently, such that a large number of experiments need to be carried out. In addition, a highly skilled and experienced operator is needed to accomplish the suction, exchange of solution or drug, and perform recordings under a microscope.

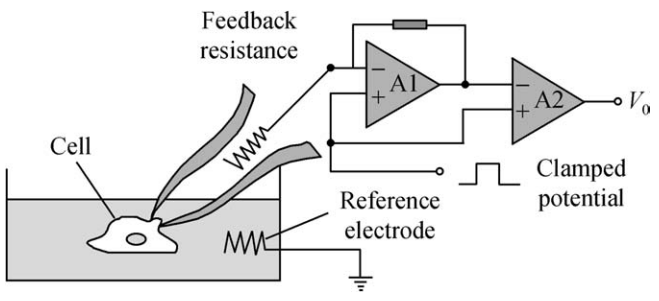

Fig. 1. The principle of patch clamp.

3. Development and application of planar patch clamp technology

Due to the problems mentioned above, conventional patch clamp technology is limited to research work in the laboratory. For its application in industry, scientists have made great efforts to improve conventional patch clamp technology by developing a new configuration of the conventional microelectrode and a new generation of microelectrode.

Improvement of the electrode configuration was firstly achieved by Sophion Bioscience. Afterwards, Neuropatch tried to replace the operator in conventional patch clamp technology with a computer visual control micro-operator-based robot to position the microelectrode on the cell automatically. Flyion put forward a technology called flip-tip [1] and produced a novel automatic patch clamp instrument, Flyscreen 8500 system, which inverted the interface between the cell and electrode. Cells were placed inside the microelectrode, so that the cell reached the tip of the pipette and formed a seal from the inside. However, as mentioned above, these systems were still based on a single microelectrode and could not be used in high throughput applications.

In the late 1990s, scientists set out to develop a patch clamp chip and raised the concept of guiding cells onto a micro-aperture, which replaced the glass microelectrode with a planar structure (Fig. 2(a)). A negative pressure or static electricity field was utilized to guide the cell onto the aperture. Then another negative pressure was applied to form a high seal resistance between the cell and chip. This operation was more convenient and rapid. A multi-electrode array chip could record multiple cells simultaneously as shown in Fig. 2(b).

So far, some materials, such as silicon, quartz crystal, glass and polymers have been utilized to fabricate patch clamp chips. The initial attempt for patch clamp chip fabrication was to use silicon, which was chosen as it is used as the standard semiconductor material, and micro-even nano-sized apertures can be readily processed on planar silicon substrates [3]. However, the use of silicon is prone to several problems, e.g. a high density of free charge carriers causes a transient parasitic current; silicon-based chips have an intensive photoelectric effect and have difficulty forming GΩ seal with cells. However, taking the advantage of the simple fabrication, silicon still has great potentials, particularly when it can be integrated with microfluidic technology [4]. In addition, when a certain

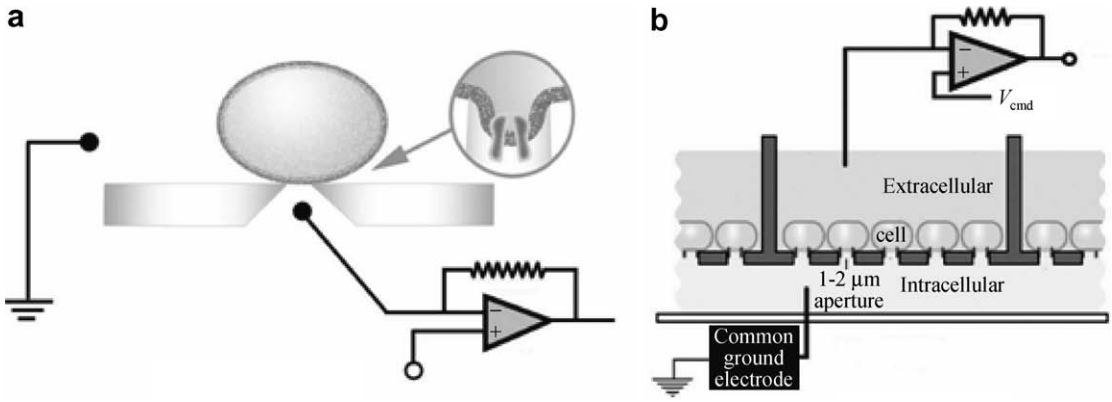

Fig. 2. Patch clamp chip. (a) Scheme of measurement principle; (b) patch clamp array [2].

voltage is applied to a silicon-based chip, a static electric field is elicited around the aperture, which can be used for guiding cells. Currently, this kind of chip is mainly used for studying the ionic channels in liposomes or artificial lipid bilayers.

Quartz crystal has excellent insulation qualities. Adopting the standard planar process technology, micrometer- to sub-μm-sized apertures can be obtained. This kind of chip has excellent performance [5]. However, the as-formed aperture is always in the shape of a triangle, making it hard to form high seal resistance.

Glass and polymer, both are good insulating materials, and are transparent and convenient for observation, have also been chosen for patch clamp chip fabrication. In this respect, these materials are easy to combine with optical measurements, e.g. fluorescence. In addition, a nice seal can be formed. Glass also has good insulation and mechanical properties, and is hydrophilic in nature. However, an obvious disadvantage of glass is the lack of a standard fabrication method. Fertig et al. [6,7] utilized ion-track etching techniques to process a smooth aperture with a diameter of 1 μm or smaller. By comparison, the fabrication of a patch clamp chip based on poly-dimethylsiloxane (PDMS) is much simpler. Micro-molding is expected to be a good method to fabricate apertures at the micrometer level [8,9]. The hydrophobic nature of the surface can be modified by oxygen plasma, enabling the chip to form a high seal resistance with cells easily. Up to now, the smallest-sized aperture that can be achieved is 2 μm in diameter. The means to create smaller apertures and achieve mass production should be resolved immediately.

Molecular Devices, Axon Instrument, Sophion Bioscience, Cytocentrics and Nanion Technologies have already launched their planar patch clamp systems separately. These planar patch clamp systems have properties of a high throughput, the automatic formation of a high seal resistance, the involvement of voltage and current clamps, and capability of whole-cell and single channel recordings, drug perfusion and data analysis. Nowadays, the planar patch clamp systems are being developed toward miniaturization and portability, e.g. the Port-a-Patch® system by Nanion.

A patch clamp chip which achieves a higher resolution and comprises a range of useful features, is desirable, such as NPC©1 Port-a-Patch [10]. It combines with optical measurement, parallel and automatic recording, convenient exchange of intracellular solution, and easy operation (e.g. no need for a micro-operator, a microscope and a vibration isolation table) etc. A patch clamp chip based on glass can form a good seal with cells under software control, achieving good stability, low-noise and therefore long-term recording capability. The throughput reaches 50 data points per day (considered 8 h). The throughput of the multi-channel NPC©16s "sequential" system reaches up to 200 data points, while the NPC©16p "parallel" can get to 2000 data points, which will promote the work efficiency greatly. Using this system, rNav1.2a expressed in human embryonic kidney (HEK) cells when blocked with tetrodotoxin had IC50 of 14.9 ± 5.3 nM (12 nM in other literature), demonstrating its feasibility in fast-gated channels. The formation of a high seal resistance assured its application in single channel recordings [11].

Ionworks Quatro, a system made by Molecular Devices utilizes a multi-cellular recording technique [2] (Fig. 2(b)) for the rapid analysis of the structures and functions of massive mutant ionic channels. The 16-channel PatchXpress system made by Axon Instrument could also be applied in the research of ionic channels in a high throughput mode. The results recorded by this system were consistent with those of the conventional patch clamp. The throughput was improved by 4-fold, reaching 2000 data points. Tao et al. [12] studied the functions of the small molecules on the hERG potassium channels using the PatchXpress platform. Compared with the conventional patch clamp, it had equivalent reliability. Besides, the high throughput was satisfied with a drug test.

4. Taste cell electrophysiology and taste transduction

Taste buds are the taste sensing organisms, which are distributed in tiny papillae on the lingual epithelium, including fungiform, foliate and vallate papillae (Fig. 3(a)). Within a papilla, there are around one to hun-

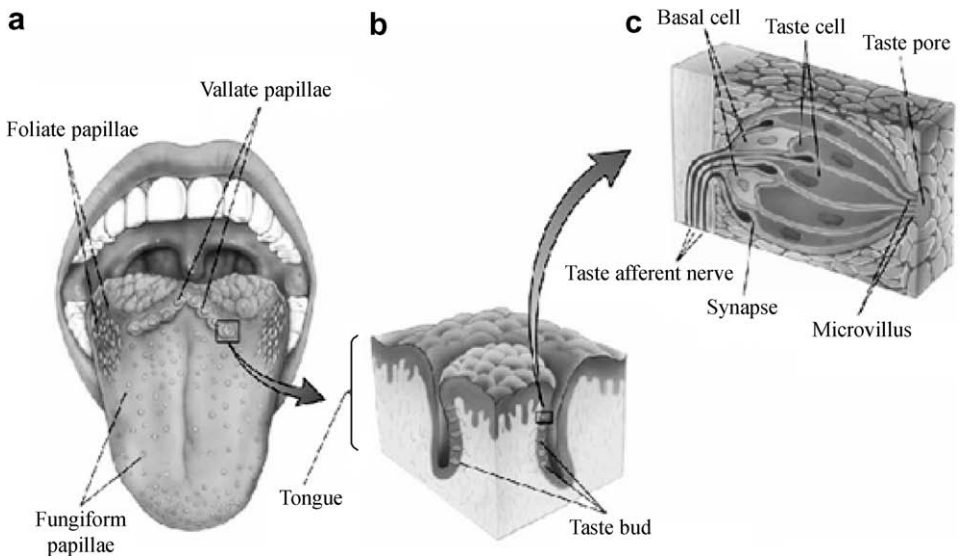

Fig. 3. Papillae and taste buds in the lingual epithelium [13]. (a) The distribution of papillae; (b) taste buds in a vallate papillae; (c) the scheme of the structure of a taste bud.

dreds of taste buds which host taste cells and basal cells (Fig. 3(b)). In a single taste bud, there are about 50–100 taste cells of around 50 μm in length and 5 μm in width, with a lifespan of 10 days. Taste cells are classified into type I, II and III based on their ultrastructural feature. At the apex of the taste receptor cells (TRCs), small microvilli are collectively exposed to external world via taste pores. These microvilli are sensitive to various tastants dissolved in saliva. The basal cells are located at the bottom of the taste buds and they finally differentiate into the other types of taste cells. Type III cells form a synaptic connection with the taste afferent nerve fibers (Fig. 3(c)).

There are five basic taste modalities: sour, sweet, bitter, salty and umami, each of which may utilize one or more transduction mechanisms. Taste molecules may (1) directly enter through the ionic channels on the taste receptor cell membrane (saltiness and sourness), (2) bind to and block ionic channels (sourness), (3) bind to and open ionic channels (some amino acid), or (4) bind to the G-protein coupled receptor (GPCR) and activate the second messenger, open or close ionic channels (sweetness, bitterness and umami), thus causing the membrane potential change and transmitting chemical signals [13].

There are gap junctions among the taste cells. Type II cells are sensitive to taste stimuli, which trigger the release of the neural transmitter (e.g. ATP) to the type III cells [14]. The stimuli information is transmitted via the afferent fibers, including the chorda tympani, glossopharyngeal nerve and vagus, to the brainstem, where it is then projected to the gustatory cortex through the thalamus, and finally to the brain.

Taste receptor cells are epithelium cells, which inherit the features of neurons with various kinds of ionic channels. Voltage-gated ionic channels mainly comprise Na^+ [15], L-type and T-type Ca^{2+} [15–17], outward delayed rectifier K^+ [18], transient outward K^+ [18], K^+-activated Ca^{2+}, inward rectifier K^+ [19] and Cl^- channels [20]. Additionally, some other types of ionic channels also play an important role in taste sensation and transduction, such as epithelium Na^+ channel (ENaC), acid sensitive ionic channel (ASIC) [21] and Na^+–H^+ exchange channel (NHE-1) [22] in saltiness and sourness transduction. Non-selective cation channels include the quinine-gated ionic channel, hyperpolarization-activated cyclic nucleotide-gated (HCN) channel and the amino acid-gated channel [23], etc.

Taste cells can also be classified into three types by their anatomic and morphological features, with different electrophysiological properties, or by the Na^+, K^+, and Ca^{2+} currents and resting potentials [16,24,25]. The action potentials have three types as well [16]. The relationships among these categories are not well understood as yet. According to patch clamp recordings on type I, II, and III taste cells in mice, Medler [26] found that even the same type of taste cells had different electrophysiological features.

Different receptors are only expressed in different subsets of taste cells. The molecular and functional studies show that taste receptor cells of different types have different properties. Moreover, activation of one subset of TRCs is able to encode taste information [27,28].

5. Prospective application of the planar patch clamp technology in taste

5.1. Research status of cell-based chip

Cell-based chips, including Light Addressable Potentiometric Sensor (LAPS), Field Effect Transistor (FET),

Micro Electrode Arrays (MEA), can detect the signals of a single neuron and neural network.

In 1991, Fromherz et al. [29] used an integrated transistor as the sensing component to replace the metal electrode. Silicon was used for the substrate and the resulting chip was fabricated using standard micromachining technology. They developed a FET-based cell chip (Fig. 4(a)) and cultured a single neuron on the non-metal gate of a p-channel FET. The measurement principle is shown in Fig. 4(b). The cell activities were reflected by the changes in the I–V curve of the FET. The current stimuli could be applied through stimulation points on the FET. Moreover, FET arrays have been applied in the research of cell communications in neural networks as well. Two synaptic-connected neurons are shown in Fig. 4(c). A capacitor was used to stimulate the presynaptic neuron, then the postsynaptic potential was elicited and could be recorded using a transistor [30]. Fig. 4(d) shows the snail neural network cultured on the FET-based cell chip [31]. The neural excitability and the transmission of the action potentials between the synaptic-connected neurons could be recorded.

Another important application is the research into the effects of drugs. Yeung et al. [32] cultured rat cardiac cells on the FET array and found that the auto-rhythmicity changed under the application of excitatory drugs like norepinephrine, or using an inhibitory drug such as verapamil or carbachol. The drug effects could be reflected through the frequency and amplitude of extracellular potentials.

In the late 1970s, MEA was used as the substrate to measure the electric activities of single cells and neural networks. Currently, MEA has been mostly applied in the research of signal transduction and drug screening.

LAPS, which was first invented in the late 1980s, has also been proposed as a LAPS-based taste cell chip. This chip is able to record the extracellular signals of cells by addressable scanning any desired spot, the principle of which is shown in Fig. 5(a). Essentially, a modulated light is focused on the surface of the taste receptor cell cultured on LAPS ($\varphi \approx 10$ μm). An extracellular potential coupled onto the surface of the LAPS chip elicits a photocurrent, which is then recorded by the LAPS system (Fig. 5(b)). Taste buds and taste cells were cultured on the LAPS chip, as shown in Fig. 5(c and d). The extracellular signals of the taste cells elicited by tastants could be recorded with an amplitude of around 10–20 μV [33]. The firing frequency increased in the presence of taste stimuli (Fig. 5(e and f)).

5.2. Application of patch clamp chip in taste research

Based on PDMS, we developed a novel patch clamp chip, which integrates with the microfluidic channels and bielectrophoresis, and used it to guide cells onto the

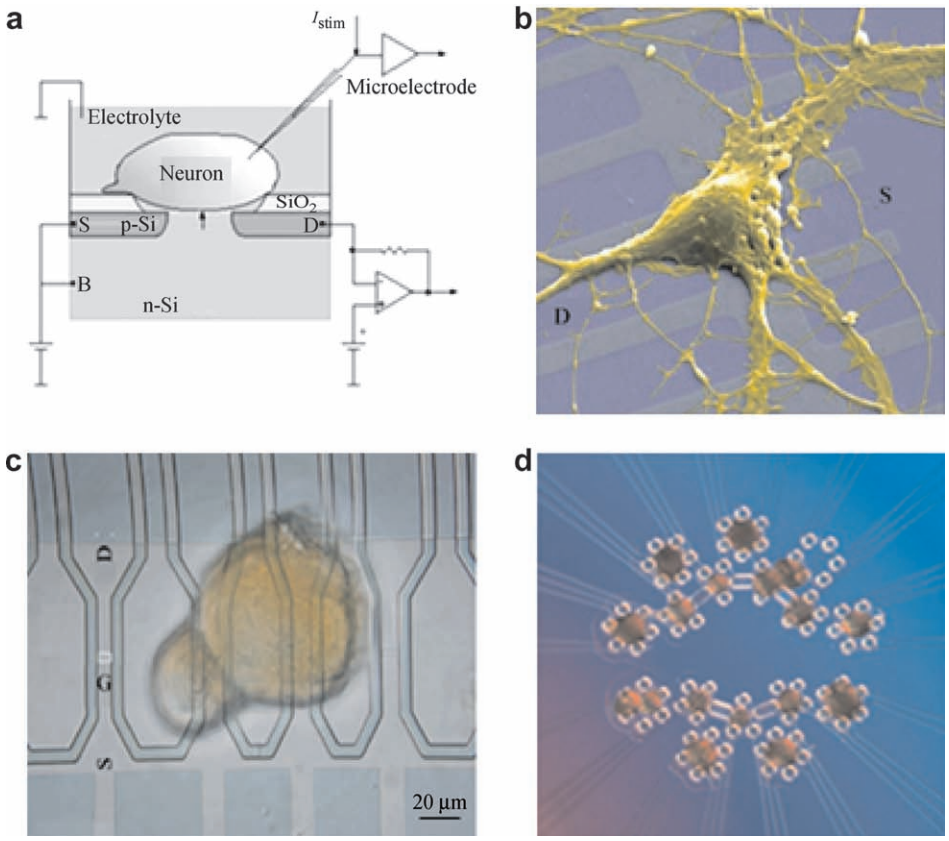

Fig. 4. FET cell-based chip. (a) The principle of measurement; (b) neuron grown on the chip [29]; (c) two synaptic-connected neurons on the chip [30]; (d) snail neural network cultured on FET [31].

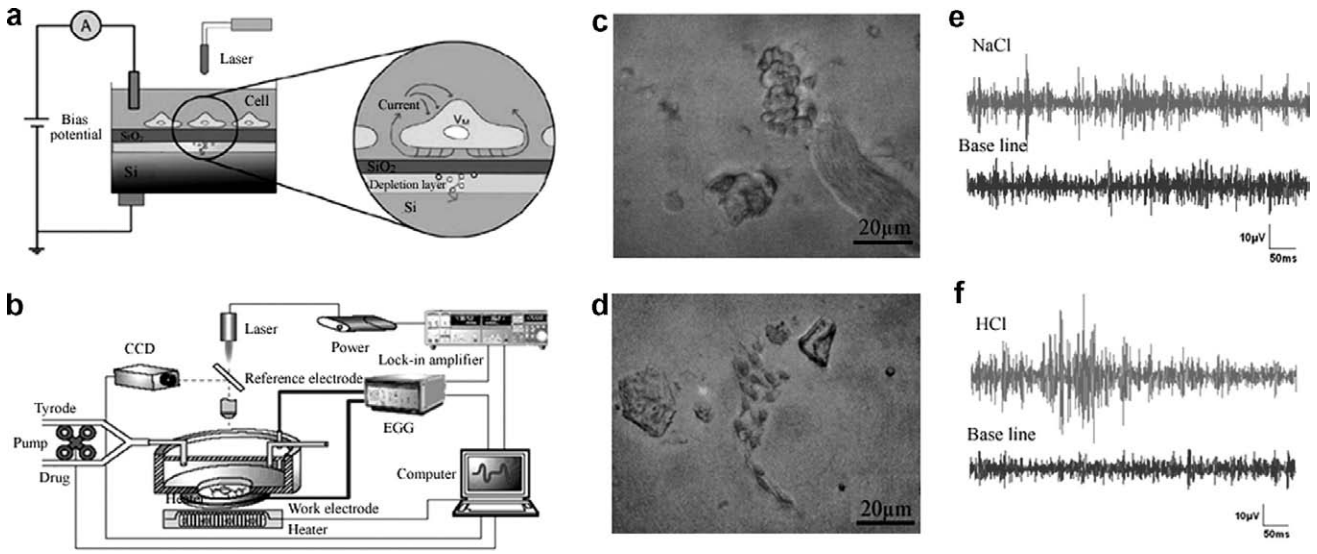

Fig. 5. LAPS taste cell-based chip. (a) The measurement principle of a taste cell-based LAPS chip; (b) LAPS measurement system; (c) a taste bud cultured on a LAPS chip; (d) taste cells cultured on a LAPS chip; (e) taste cell response to NaCl recorded by LAPS; (f) taste cell responses to HCl [33].

micro-aperture to form a giga-Ohm seal by negative pressure. The soft lithography fabrication technique was applied to construct the 3D PDMS-silicon structure and a 2 μm diameter aperture was obtained by using a micro-mold (Fig. 6).

Patch clamp chips based on glass and PDMS are transparent and as such, they can be combined with an optical measurement capability to perform both electrophysiology recording and cell-type identification simultaneously. Although the three types of taste cells are barely distinguished under a microscope, different types express distinct antigens [26]. With the corresponding antibody, we can acquire both biological and electrophysiological information, which is useful in the research of the taste transduction mechanism at the periphery.

The easy exchange of intracellular and extracellular solutions can make various kinds of voltage-gated currents (e.g. Na^+, K^+, Ca^{2+} and Cl^- currents) of the same cell and multiple cell recordings simultaneously possible. Meanwhile, the type of taste cells can be identified by immunohistochemistry and statistics can be calculated for every kind of taste cell. It is considered that the construction of a taste computational model and the corresponding simulation studies can be more accurate and significant using massive macroscopic currents.

Bitter, sweet and umami receptors and α-gustducin, which play a role in both the sweet and bitter sensations and transduction, are specifically expressed in type II taste cells. These type II cells lack voltage-gated Ca^{2+} channels and the classic synapse with afferent nerves. However, recent studies have shown that type II cells communicate by indirect mechanisms, including electrical and chemical pathways. The former is via gap junctions, while the latter is via neural transmitters, e.g. 5-serotonin (5-HT) [34], epinephrine [23], acetylcholine [35], glutamate [36], γ-aminobutyric acid (GABA), adenosine triphosphate (ATP) [37], cholecystokinin (CCK) [38], etc. Although these transmitters and corresponding receptors were demonstrated to express in taste cells using molecular technologies, their functions are still not well understood. The research of communication among taste cells will potentially be promoted by the emergence of patch clamp chip technology.

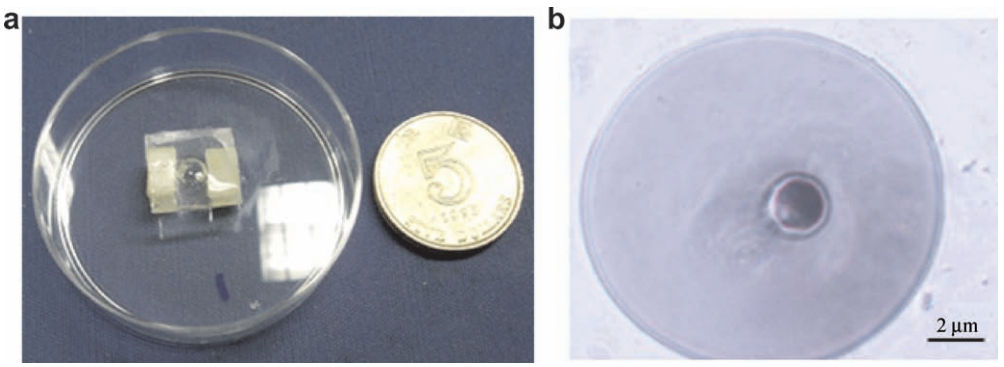

Fig. 6. Patch clamp chip based on PDMS. (a) PDMS substrate with micro-sized aperture; (b) micro-molded aperture with a diameter of about 2 μm.

As mentioned above, cell-based chips have been applied to the research of cell communications in a network, with the advantage of long-term, non-invasive recordings. However, the cells and chip could not always be coupled well enough and as a result, the amplitude of the recorded extracellular signals was small, typically of the order of micro- to milli-volts. Till now, the relationship between the extracellular and the intracellular signals has not been well explained. Therefore the investigation of the taste transduction mechanism using, patch clamp chips will present a unique direction.

In this case, a high requirement is needed for the preparation of taste cells. At present, taste cells are obtained via one of two methods: (i) excising papillae using papain [19]; (ii) extracting taste buds from the peeled epithelium using a glass pipette with a diameter of about 50 to 100 μm [35]. Recent research has shown that culturing tissues of the lingual epithelium could be an alternative way to obtain taste cells [39]. However, these methods also yielded other kinds of cells that were not needed, e.g. epithelial cells and basal cells. Moreover, taste cells *in vitro* are likely to become spherical some 2 h after being excised. Then, how to produce a large amount of taste cells with a clean surface and healthy condition, and how to make taste cells form a network *in vitro* should be resolved first.

6. Conclusion

Emergence of the planar patch clamp technology makes the highly parallel and automatic electrophysiology recording of ionic channels possible. This novel chip can record many cells simultaneously and can be combined with multiple measurement methods easily. Taste cells are epithelial cells which have the features of neurons. They can transform taste information into electrical signals, which can then be transmitted to the brain through nerves. The results from cellular and molecular biology combined with patch clamp electrophysiology have demonstrated that cellular communications exist among taste cell networks. Planar patch clamp technology will be a potential and effective approach in the study of taste transduction mechanisms. Meanwhile, the novel chip technology will promote the rapid development of taste prosthesis and artificial taste technology.

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