SENSOR LETTERS
Vol. 12, 1–5, 2014

A Novel Cell-Based Biosensor for Bitter Taste Detection *In Vitro*

Liang Hu[1], Liping Du[1], Qi Dong[1], Beien Wang[1],
Liquan Huang[2], and Ping Wang[1,*]

[1] *Biosensor National Laboratory, Key Laboratory of Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, China*
[2] *Monell Chemical Senses Center, Philadelphia, PA, 19104, USA*

(Received: 30 June 2013. Accepted: 12 December 2013)

In natural environment, numerous bitter tasting compounds are toxic and noxious, which may cause danger for human when taken in mistakenly. This paper introduces a novel cell-based biosensor which could respond to bitter compounds sensitively and selectively. TRCs were utilized as sensing elements since they can interact with bitter compounds. 60-channel (microelectrode array) MEA chips were employed to record the extracellular potentials of TRCs. The responses of TRCs were analysed at the conditions of control, bitter stimulations and after-wash. The results showed that different bitter stimuli such as quinine, cycloheximide and denatonium significantly evoked specific responses respectively. Electrophysiological characteristics, such as firing rates and amplitudes, had visible increase with concentrations of bitterness. The result of principal component analysis (PCA) proved that this cell-based biosensor could discriminate quinine, cycloheximide and denatonium effectively. All these results demonstrate that this hybrid biosensor is reliable and effective in detection and discrimination of these bitter compounds. This study may provide a promising approach for detection of other bitter compounds.

Keywords: Cell-Based Biosensors, Bitter Taste, Taste Receptor Cell (TRC), Microelectrode Array (MEA).

RESEARCH ARTICLE

1. INTRODUCTION

Among five basic tastes, bitter perception functions as an important sensory input to warn against the ingestion of toxic and noxious compounds. Generally there are still many bitter tasting poisons unknown to us and it may not be possible for human to taste so many of these unknown bitter compounds because of their potential toxicity. Therefore an electronic tongue for these bitter tasting compounds can help to address this demanding problem. Electronic tongues have been widely applied in fields such as food safety, biomedicine, and environmental protection and anti-bioterrorism.[1–3] However, traditional electronic tongues for bitter detection usually employ electrochemical and optical sensors, which are not as elaborate as the biological material in performance of sensitivity and specificity.[4–6] And these instruments could not provide biological functional information about the detected chemicals. While natural biological gustatory components, such as taste receptor cells (TRCs), in taste system have the merit to provide the bitter and other taste information directly like animals and humans do. As a consequence, the well-evolved receptors in biological gustatory system have become a promising choice for bitter taste detection.

In the mammalian gustatory system, bitter taste sensation starts with interaction between taste modalities and various taste receptors in TRCs. Then the chemical signal is converted into action potential of TRCs by activating a family of G-protein-coupled receptors and then eliciting a series of intracellular interactions in signalling transduction pathway.[7,8] Different ligand-receptor interactions have distinctions in signalling pathways, which could result in unique firing patterns of taste cells.[9] Given the unmatched advantage of extraordinary sensitivity and specificity of receptor-ligand interaction, detection and discrimination of bitter compounds could be accomplished by analysing the electrophysiological characteristics of TRCs.[7,10] Therefore a cell-based biosensor in which TRCs are utilized as promising sensing element for bitter detection is put

*Corresponding author; E-mail: cnpwang@zju.edu.cn

forward. In our previous studies, taste epithelium was used to establish biosensor in bitter detection and showed good performance in sensitivity and specificity.[11] However, the TRCs on the surface of epithelium are easily impaired by anoxia or inappropriate manipulations. The density of TRCs on tongue epithelium cannot be controlled. To overcome these problems, we improved the biosensor by using TRCs as sensing elements, which could maintain active and pharmacologically responsive longer than epithelium. TRCs could be culture for over two month while lingual epithelium could not survive for such a long time.[12] Therefore the philological state of TRCs should be better than lingual epithelium. Besides, bitter TRCs could be preliminarily screened by taking the cells from circumvallate papillae. Because T2Rs (a large family of receptors that function as bitter taste receptors) are much abundant in TRCs in circumvallate papillae.[13] The density of isolated TRCs could also be controlled.

Conventional recording approaches for cellular electrophysiology include patch clamp and glass microelectrode technology. For both recording methods, the recording sites are limited and operation processes are usually complicated. Moreover, patch clamp cannot be used in long-term recording due to its invasion to cells. Consequently microelectrode arrays (MEAs) become desired choice with advantages of high throughput, non-invasion and convenient operation.

2. EXPERIMENTAL DETAILS

2.1. Fabrication of MEA

The fabrication of MEA chip was carried out based on the standard microelectronic process flow, which has been described detailed in our previous taste sensing works.[5] MEA was designed as 8×8 array pattern. The diameter of single electrode was 20 μm and the distances between each electrode were 150 μm. 60 electrodes were utilized for recording and 4 for grounding. In order to reduce the impedance of the electrodes and improve the signal-noise ratio (SNR), the MEAs were electroplated with platinum black with the deposition potential of 0.01 V for 40 s before experiments.

2.2. Isolation of Taste Receptor Cells

To isolate the taste cells from tongue, we adopted the method from Kinnamon.[14] Female Sprague Dawley rats (around 250 g) were anesthetized by intraperitoneal injection of urethane and the tongue was dissected. 1–1.5 ml tyrode's solution containing collagenase (2 mg/ml) and elastase (0.25 mg/ml) was injected under the epithelium of the dissected tongue. After incubation for 30 min in divalent-free tyrode's solution, lingual epithelium was peeled from the underlying connective tissue. Circumvallate TRCs from one rat were removed from epithelium with a glass pipette by a gentle suction. The cell density was about 10^3 per MEA. TRCs were cultured in tyrode's solution in incubator for about three hours in order to form relatively firm attachment to electrodes.

2.3. MEA System and the Detection of Extracellular Potentials

For extracellular recording of taste receptor cells, the MEA-1060 system (MCS, Germany) was applied in the experiment. 60 channels were used for synchronous recording. The measurement scheme was shown in Figure 2. The whole system was placed in the shielding box to avoid external electromagnetic interference. The software MC_Rack (MCS, Germany) was utilized for real-time display and analysis.

In our experiment, the concentrations for quinine (1 mM, 10 mM 100 mM), denatonium (1 μM, 10 μM, 100 μM) and cycloheximide (10 μM, 100 μM, 1 mM) were selected according to preliminary experiments. All the bitter solutions were prepared in PBS. Before bitter stimulation, the spontaneous electrophysiological activities of the taste cells were recorded for 150 s in phosphate buffered saline (PBS) as control condition. Then PBS was removed and bitter solutions were added into the MEAs chamber from low concentrations to higher ones (replaced one by one). The electrophysiological responses of TRCs were recorded for 150 s for each concentration gradient. The bitter stimuli were washed out by PBS. After-wash condition was also recorded for 150 s.

2.4. Mathematical Statistics

The data including normalized firing rates (NFR) and amplitudes (NA) were acquired from signals of different experiments respectively. For each bitter stimulus, three concentrations were normalized to three gradients according to the protocols (gradient 1 to 3 correspond to lowest concentration to highest ones respectively). Therefore the NFR and NA of three bitter responses could be evaluated at one time, which help to make better comparisons (Figs. 3(a) and (b)). For each series, data were normalized to the mean value at the highest response. The data were presented as mean $\pm$ SEM of n samples. Here n represents the number of electrodes. In the analysis of principal component analysis (PCA), ten signal segments at the third concentration gradient of each bitter stimulus were regarded as three input components of PC1, PC2 and PC3.

3. RESULTS AND DISCUSSION

3.1. Multi-Channel Recording of TRCs

The TRCs were visibly attached to the MEA shown in Figure 1(a). The extracelluar potentials of TRCs could be recorded simultaneously with this multi-channel MEA chip. Figure 1(c) showed the schematic diagram of the detecting process. In our experiments, 31 in 45 MEAs successufully detect the TRC extracellular potentials. This highly effectiveness for this detection benefits

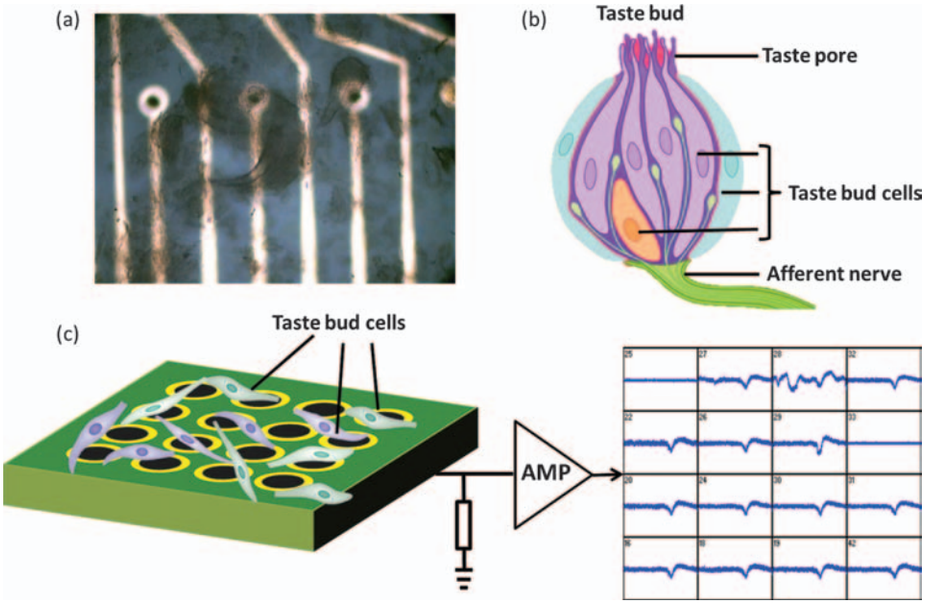

Fig. 1. (a) TRCs on the MEA prior to recording. Scale bar is 100 μm. White arrows indicate the TRCs. (b) Structure of taste bud. (c) Schematic of the TRC response recording by MEA system.

much from this MEA system, which provides a high-throughput (60-channel), high-sensitivity and long-term recording platform.

3.2. TRC Responses to Different Bitter Stimuli

The TRC responses to three different bitter compounds (cycloheximide, quinine and denatonium) were measured with different concentrations. And the electrophysiological characteristics of TRCs to different bitter compounds were investigated to evaluate the specificity and performance of this cell-based biosensor. Generally speaking, cycloheximide, quinine and denatonium all could increase firing activities of TRCs. The firing activities also presented a dose dependent manner in which the firing intensity has positive relations with the stimulus concentrations. However, there were still some distinctions in their firing properties.

Figure 2 showed the raw signals after wavelet filtering stimulated by cycloheximide, quinine and denatonium with different concentrations. The concentration ranges and gradients were selected based on preliminary experiments and previous results. The maximal amplitude of the field potentials could reach as high as 2.2 mV when TRCs were stimulated by 100 μM denatonium. And the SNR

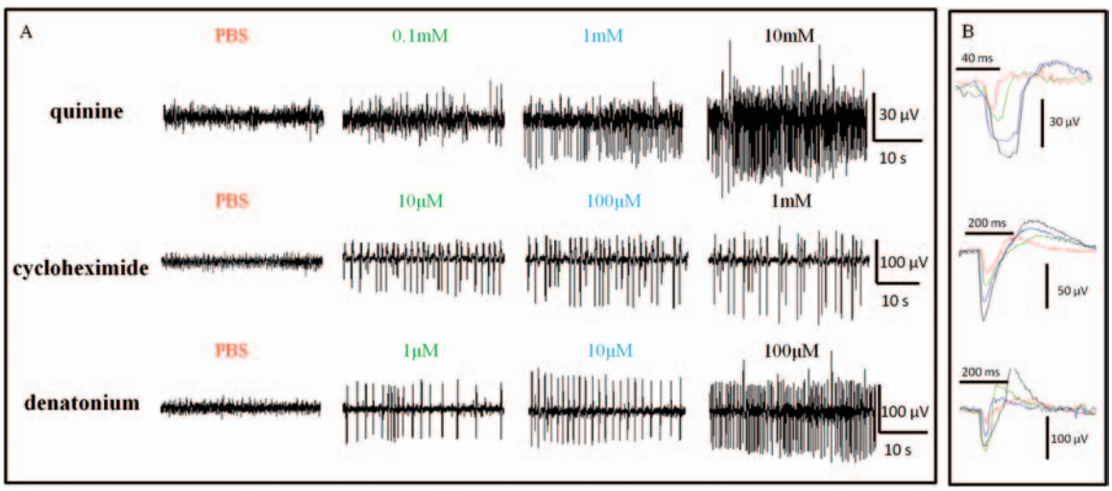

Fig. 2. (a) TRC response to quinine, cycloheximide and denatonium under three different concentrations. The signals were filtered by wavelet filter. (b) Waveforms of TRC responses to three stimuli. Red, green, blue, black represent three concentrations from low to high, consistent with the color-labeled concentration stamps in (a).

RESEARCH ARTICLE

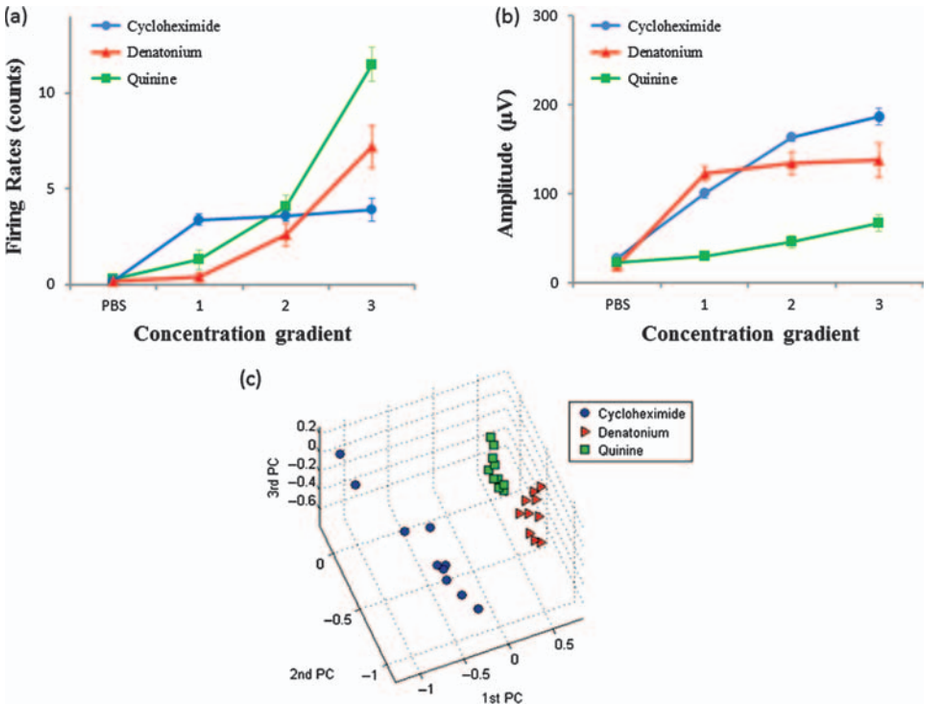

Fig. 3. (a) Firing rates analysis for TRC response to three bitter compounds with 3 concentration gradients. The concentration gradients were divided based on the three concentrations used for each bitter stimulus in Figure 2. (Gradient 1 represents the lowest concentrations for three bitter stimuli. Gradient 2 represents the middle and gradient 3 represents the highest.) Data were given as mean ± SEM ($n \geq 10$). (b) Signal amplitude analysis for TRC response to three bitter compounds with 3 concentration gradients. Data were given as mean ± SEM ($n \geq 10$). (c) PCA results of three bitterness compounds. 10 samples were selected for each bitter stimulus in PCA analysis.

RESEARCH ARTICLE

was about 5.4, which demonstrated the high sensitivity of this TRC-based biosensor. The thresholds of detection limit were 0.1 mM for quinine, 10 μM for cycloheximide and 1 μM for denatonium. The detection thresholds have been lowered by an order of concentrations for all these three bitter compounds compared with the results from our colleagues.[5] In order to further improve the sensitivity of cell-based biosensor, surface modification will be applied to improve cell adhesion and immobilization. We have used SAM (self-assembled monolayer) technology to immobilized rats' cardiomyocytes in design of cell-based biosensor.[15] In future study, we will apply this technology in TRCs' adhesion in our bitter TRC-based biosensor design.

In the temporal domain, firing rates and potential amplitudes were analyzed. All the concentrations were normalized to 3 gradients referred to protocols in order to evaluate all three bitter stimuli at one time. The firing rates and amplitudes of population spikes increased visibly with the concentrations of three bitter compounds. Figures 3(a) and (b) showed the firing rates and amplitudes under different bitter stimuli. Generally, the response of TRCs had a dose dependent manner with bitterness concentrations, consistent with the consequences of genetic, behavioural and physiological investigations about bitter taste.[7, 16, 17] There were also some distinctions for their firing patterns. For quinine, both firing rates and amplitude increased with increasing concentrations. For cycloheximide, amplitude had a dose dependent manner. However, there was no significant difference in firing rates under different concentrations. For denatonium, the firing rates had a remarkable increase with concentrations. Yet it seemed to have little effect on amplitudes. These different firing patterns may be the encoding information for bitter discrimination in central system.[18, 19] This part remains to be studied.

Figure 3(b) showed typical magnified waveforms of TRC extracellular potentials stimulated by quinine, cycloheximide and denatonium with different concentrations. In each series, red, green, blue and black represented the conditions including PBS and three concentrations from low to high respectively. For all three sets of waveforms responding to each bitter stimulus, both the amplitudes and the durations became larger with the increasing concentrations. For each bitter response, the comparison of waveforms with different concentrations proved a dose dependent manner of TRC responses, consistent with the amplitude analysis. Besides, for different bitter stimuli, the distinctions in the shape of waveforms also revealed the specificity of TRC responses to different bitter compounds.

Based on the firing characteristics in temporal domain, PCA was applied to differentiated responses to different

bitter compounds. 10 filtered segments (0.5 s) of typical waveforms under each bitter stimulus at the third concentration gradients (10 mM for quinine, 100 μM for cycloheximide and 0.1 mM for denatonium) were regarded as input to calculate three components of PC1, PC2 and PC3. The PCA result shown in Figure 3(c) displayed that responses to three bitter compounds were scattered into three clusters in a three-dimensional space. The PCA result demonstrated that this cell-based biosensor could respond specifically to quinine, denatonium and cycloheximide. And the responses could be distinguished in three-dimensional space.

4. CONCLUSION

This paper introduced the establishment of a hybrid TRC-based biosensor and has applied it in detection of three bitter compounds. By taking advantages of MEA technology, this hybrid biosensor could monitor and detect the TRC responses for a long term in a convenient and effective way. Besides, the results also confirmed the electrophysiological responses of TRCs to bitter stimuli have a dose dependent manner. Moreover, the signal analysing results proved this TRC-based biosensor could discriminate these compounds effectively. This study may provide a novel approach for bitter detection and is promising for various applications such as drug screening.

Acknowledgments: This work has been supported by grants from the National Basic Research Program of China (973 program). Grant No. 2009CB320303.

References and Notes

1. H. Men, S. Zou, Y. Li, Y. Wang, X. Ye, and P. Wang, *Sens. Actuator, B* 110, 350 (**2005**).
2. P. Wang, G. Xu, L. Qin, Y. Xu, Y. Li, and R. Li, *Sens. Actuator, B* 108, 576 (**2005**).
3. J. Gallardo, S. Alegret, and M. del Valle, *Talanta* 66, 1303 (**2005**).
4. N. Whittle, H. Eldridge, J. Bartley, and G. Organ, *J. Inst. Brew.* 105, 89 (**1999**).
5. A. Prelle, D. Spadaro, A. Garibaldi, and M. L. Gullino, *Food Chem.* (**2013**).
6. L. Escuder-Gilabert and M. Peris, *Anal. Chim. Acta* 665, 15 (**2010**).
7. K. L. Mueller, M. A. Hoon, I. Erlenbach, J. Chandrashekar, C. S. Zuker, and N. J. Ryba, *Nature* 434, 225 (**2005**).
8. A. Taruno, V. Vingtdeux, M. Ohmoto, Z. Ma, G. Dvoryanchikov, A. Li, L. Adrien, H. Zhao, S. Leung, and M. Abernethy, *Nature* 495, 223 (**2013**).
9. J. I. Glendinning and T. T. Hills, *J. Neurophysiol.* 78, 734 (**1997**).
10. M. Sugita, *Cellular and Molecular Life Sciences CMLS* 63, 2000 (**2006**).
11. Q. Liu, D. Zhang, F. Zhang, Y. Zhao, K. J. Hsia, and P. Wang, *Sensor Actuator, B: Chemical* 176, 504 (**2013**).
12. H. Ozdener, K. K. Yee, J. Cao, J. G. Brand, J. H. Teeter, and N. E. Rawson, *Chemical Senses* 31, 279 (**2006**).
13. E. Adler, M. A. Hoon, K. L. Mueller, J. Chandrashekar, N. J. Ryba, and C. S. Zuker, *Cell* 100, 693 (**2000**).
14. S. C. Kinnamon, V. E. Dionne, and K. G. Beam, *Proc. Natl. Acad. Sci.* 85, 7023 (**1988**).
15. W. Zhang, L. Hu, L. Xiao, Z. Hu, and P. Wang, *J. Zhejiang Uni. (Eng. Sci.)* 2, 28 (**2012**).
16. J. Chandrashekar, K. L. Mueller, M. A. Hoon, E. Adler, L. Feng, W. Guo, C. S. Zuker, and N. J. Ryba, *Cell* 100, 703 (**2000**).
17. S. Damak, M. Rong, K. Yasumatsu, Z. Kokrashvili, C. A. Pérez, N. Shigemura, R. Yoshida, B. Mosinger, J. I. Glendinning, and Y. Ninomiya, *Chemical Senses* 31, 253 (**2006**).
18. S. M. Tomchik, S. Berg, J. W. Kim, N. Chaudhari, and S. D. Roper, *J. Neurosci.* 27, 10840 (**2007**).
19. S. S. Schiffman, *Physiol. Behav.* 69, 147 (**2000**).