Talanta 211 (2020) 120731

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# A sperm-cell-based biosensor using a fluorescence probe for responsive signal readout toward bitter flavor detection

Yulan Tian[a], Ping Zhu[a], Yating Chen[a], Wei Chen[a], Liping Du[a], Chunsheng Wu[a,*], Ping Wang[b,**]

[a] Institute of Medical Engineering, Department of Biophysics, School of Basic Medical Sciences, Health Science Center, Xi'an Jiaotong University, Xi'an, 710061, PR China
[b] Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou, 310027, PR China

ARTICLE INFO

*Keywords:*
Bitter receptor
Biosensor
Sperm cells
Calcium detection
Fluorescence probe

ABSTRACT

Bitter flavor detection has attracted extensive attention in industry and basic research due to pharmacological and food safety issues. Opportunities exist to extend the conventional methods of bitter flavor evaluation in performance and operation. This study proposes a novel sperm-cell-based biosensor (SCB) that utilizes living mouse spermatids as the primary sensing element, employs Fluo 4-AM as a transducer and works in conjunction with flow cytometry to realize the rapid quantitative detection of bitter compounds. The preparation conditions of the SCB were optimized with different quinine concentrations, and quinine and two other bitter compounds were employed to verify the sensing properties. Furthermore, the responses of the SCB to five basic flavor types were characterized to evaluate the sensor specificity. The SCB enabled preliminary classification of three bitter substances by using principal component analysis (PCA). The results revealed that the SCB is convenient, inexpensive and easy to use and can respond to bitter compounds in a dose-dependent manner with high sensitivity, high specificity and a low limit of detection, providing a novel and efficient approach for comprehensive evaluation of bitter substances in many fields, such as the pharmaceutical and food industries and in biosafety.

## 1. Introduction

Bitter perception is one of the five basic tastes in mammals. Most poisons in the natural environment taste bitter; thus, bitter taste detection plays an important role in animal survival. Bitter substance detection and identification have wide applications in many fields, such as the pharmaceutical and food industries and in biosafety [1–3]. Various conventional methods and devices have been applied for bitter taste detection, including human tasting, conventional electronic tongues and high-performance liquid chromatography-mass spectrometry. However, these methods have some intrinsic shortcomings, such as large individual variation, single detection target and high cost [4–7]. For cell-based bioassays, calcium ion fluorescence imaging is usually employed to detect bitter compounds [8]. However, this method commonly relies on general taste cells (located mainly in the oral taste buds) [9–11], which express multiple sensitive taste receptors and cannot detect bitter flavors specifically [12]. In recent years, bitter taste receptor cells prepared by heterologous expression have been applied for bitter detection, but some deficiencies remain, such as a low efficiency, relatively long operation times, a relatively large number of operation steps, and an inability to detect multiple different bitter compounds [13]. In addition, the fluorescence imaging method is qualitative rather than quantitative. Recently, cell-based biosensors have received widespread attention because of their high sensitivity and specificity. Furthermore, studies have shown that there are a large number of bitter receptor genes in mouse testicular tissue [14–17]. Mouse sperm cells contain a large number of bitter receptors, which can be used as sensitive elements to sense bitter compounds [18,19]. In conjunction with calcium ion fluorescent probes [20], this method can realize the quantitative detection of bitter taste.

In this study, a sperm-cell-based biosensor (SCB) is proposed for quantitative bitter taste detection. The SCB integrates living sperm cells and calcium ion fluorescent probe technology and realizes bitter detection in conjunction with flow cytometry. To this end, spermatids as primary cells were isolated from mouse testicles. The SCB was prepared for optimal detection of bitter compounds, and five basic flavor types were employed to verify its sensing properties. Moreover, three bitter substances were adopted for classification with principal component analysis (PCA). As discussed in the following sections, this biosensing system provides convenient, low-cost, efficient, high-sensitivity, high-specificity, rapid and comprehensive evaluation of bitter taste.

* Corresponding author.
** Corresponding author.
*E-mail addresses:* wuchunsheng@xjtu.edu.cn (C. Wu), cnpwang@zju.edu.cn (P. Wang).

https://doi.org/10.1016/j.talanta.2020.120731
Received 29 November 2019; Received in revised form 6 January 2020; Accepted 8 January 2020
Available online 09 January 2020

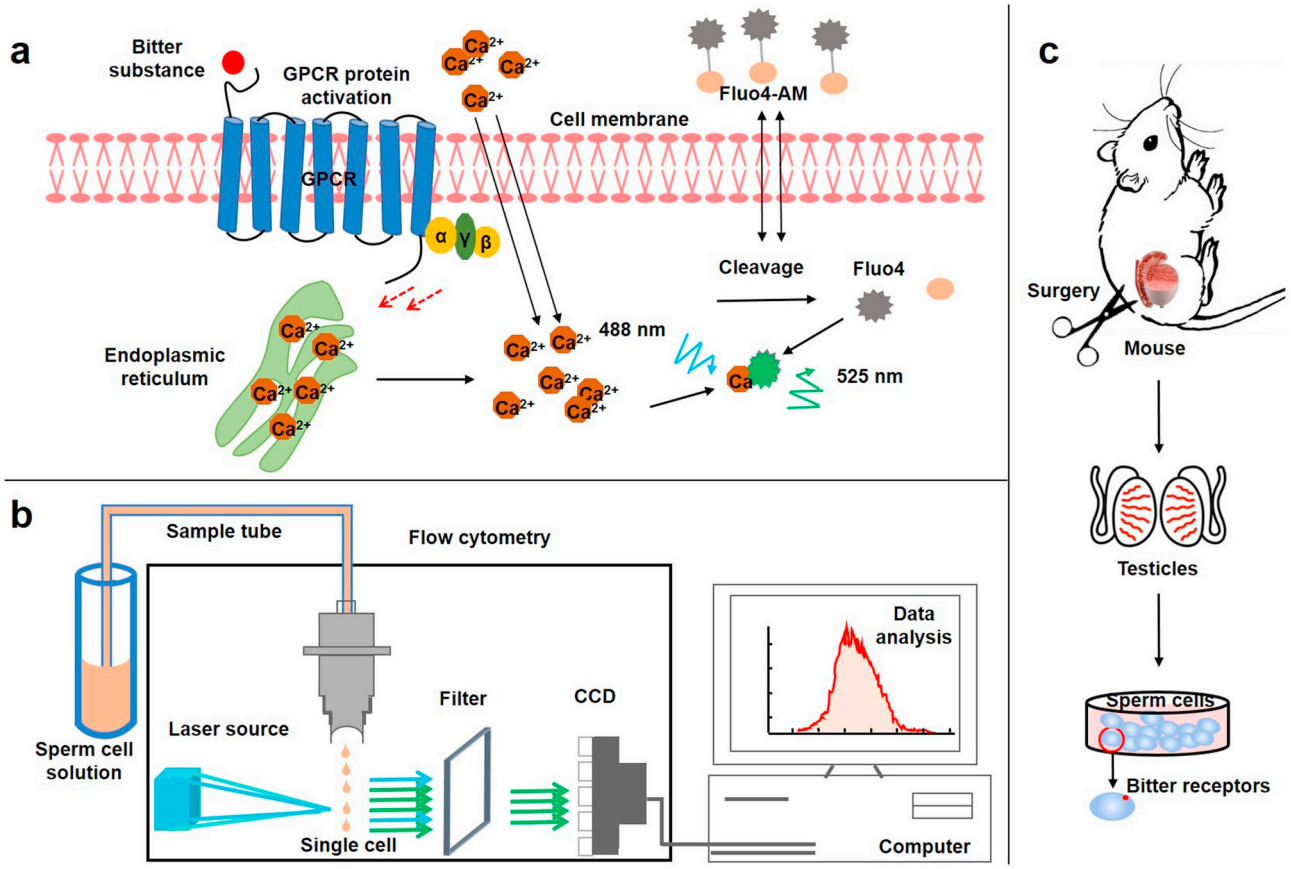


**Fig. 1.** (a) Schematic diagram of the detection principle of the SCB system; (b) Detection principle of the flow cytometry measurement system; (c) Process diagram for obtaining mouse sperm cells.

## 2. Working principle

The SCB uses spermatids as a sensing element to perceive the stimulus of a bitter substance, which alters the intracellular calcium flow. Then, a Fluo 4-AM-based probe acts as a transducer to convert the changes in calcium flow into a fluorescence signal, which can indicate the concentration of the bitter substance. Fig. 1a is a schematic diagram of the detection mechanism of the SCB. Fluo 4-AM is cleaved to form Fluo 4 after entering the cell. Fluo 4 is almost nonfluorescent when it exists in free form. The bitter receptor is a kind of G protein-coupled receptor (GPCR) [21,22]. When the GPCR is activated by bitter compounds, the calcium ion channel opens and promotes calcium influx [23,24]. Moreover, calcium ions bound to the endoplasmic reticulum are released into the cytoplasm. Both of these effects result in an increase in the intracellular calcium ion concentration. Intracellular Fluo 4 combines with calcium ions and then emits 525 nm green fluorescence under the irradiation of 488 nm excitation light [25]. Therefore, the green fluorescence intensity reflects the concentration of bitter compound.

A schematic diagram of the flow cytometry detection process is shown in Fig. 1b. The mixture is transferred into a sample tube, the individual cells are illuminated by light excitation while passing through the sample aperture, the emitted light is recorded by a CCD camera, and the fluorescence intensity data are transmitted to a computer for analysis.

## 3. Materials and methods

### 3.1. Reagents and animals

Denatonium benzoate (Dena), N-phenylthiourea (PTC), quinine (Quin), citric acid (CA), sucrose (Suc), monosodium glutamate (MSG) and NaCl were purchased from Solarbio (Beijing, China). Urethane and bovine serum albumin (BSA) were purchased from Sinopharm Chemical Reagents Co. Ltd (Shanghai, China). Fluo 4-AM was purchased from Dojindo. Wild-type adult male mice were purchased from the Zhejiang Academy of Medical Sciences. Other reagents were purchased from Aladdin.

### 3.2. Mouse germ cell isolation

A wild-type adult male mouse (approximately 8 weeks old) was anesthetized by 25% (w/w) urethane. The two testes were immediately isolated; the fat pad, white membrane and blood vessels were removed; and the remaining tissue was transferred to a centrifuge tube with HS medium (mM: 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 30 HEPES, 10 glucose, 10 lactic acid and 1 pyruvid acid; pH adjusted to 7.4 with NaOH) [19]. For isolation of sperm cells, seminiferous tubules from mouse testicle were dissected out and cleaned twice in a 20-mm dish containing 3 ml of HS medium to remove blood cells and other impurities. The tissue was transferred into 1 ml HS medium containing 5 mg/ml BSA and finely minced. The mixture was filtered with a 100-mm nylon cell strainer, then the filtrates was centrifuged at 700 rpm for 5 min to remove the tissue clumps. The sperm cells were dissociated and collected from the upper portion of the solution by a pipette [26]. In this way, the germ cell suspension was obtained. The suspension was adjusted to 1,500,000 cells/ml density. The cells were maintained in a cell incubator at 37 °C and 5% $CO_2$ (Fig. 1c).

### 3.3. SCB construction

Before experiment, 5 μM Fluo 4-AM solution was freshly made with distilled HBSS from stock solutions (1 mM Fluo 4-AM stock solutions

(dissolved in DMSO), store at −20 °C, sealed away from light). The sperm cell suspension was separated into 1.5 ml centrifugal tubes (1 ml/tube). After 800 r/min centrifugation for 5 min and removal of the supernatant, 5 μM Fluo 4-AM was added to each tube, followed by incubation for 30 min at 37 °C in the dark [27] (hereafter referred to as the mixing time). The Fluo 4-AM staining solution was removed by centrifugation at 800 r/min for 5 min. The cells were washed twice with HBSS, followed each time by centrifugation of the supernatant at 800 r/min for 5 min. Then, the tubes were incubated for 20 min away from light to ensure that the Fluo 4-AM was completely cleaved to Fluo 4 in the cells (referred to hereafter as the cleavage time). Then, 500 μl of the bitter substance (Quin) was added to the cell suspension, which was then transferred to a flow tube, thus completing sensor construction.

### 3.4. Conditional optimization

For conditional optimization, the following comparative experiments were conducted: (1) 2000 μM Quin was used as the bitter substance with a fixed mixing time of 60 min and different cleavage times (20, 30, 40 min) of Fluo 4-AM in the cells; (2) a fixed cleavage time of 30 min was used with different mixing times (15, 30, 45, 60 min) of the Fluo 4-AM solution and cells; (3) different concentrations (5, 20, 50, 100, 250, 500, 1000, 2000, 4000 μM) of Quin were used to determine the best detection time after adding the bitter substance; and (4) the fluorescence response intensities at different detection times (3, 6, 10 min) were recorded.

### 3.5. Detection of bitter substances

Different concentrations (Dena or Quin: 5, 20, 50, 100, 250, 500, 1000, 2000, 4000 μM; or PTC: 10, 25, 50, 100, 200, 1000, 2000 μM) were prepared for administration to the germ cells. The complete solutions (bitter compound and sperm cells with Fluo 4) were analyzed by flow cytometry (FCM) in FITC mode to collect the signals.

### 3.6. Fluorescent image detection

The sensor's fluorescent imaging results were tested with Quin as the bitter substance. The obtained germ cell suspension (Section 3.2) was seeded in culture dishes precoated with poly-lysine (1 mg/ml). Next, the fluorescent probe fabrication steps were performed as described in Section 3.3. Different concentrations of Quin (20, 50, 100, 250, 500, 1000, 2000 μM) were added to the dishes, and the imaging results were observed at an emission wavelength of 488 nm.

### 3.7. Specificity analysis

Acidic (1 mM CA), salty (1 mM NaCl), sweet (1 mM Suc), umami (1 mM MSG) and bitter (0.1 mM Dena, Quin, PTC) substances were added to the prepared sperm sensor. FCM in FITC mode was used to test the performance of each mixed sample (the compound and sperm cells with Fluo 4).

### 3.8. Data process

Since the fluorescence intensity was different between each group of experiments, the results of each group needed to be processed. The relative fluorescence intensity was employed for analysis. The fluorescence of the control samples in each group was taken as the reference value $F_0$, and the fluorescence of the detection samples was designated $F_{ex}$. The relative fluorescence intensity was normalized by the following equation:

$$\boldsymbol{F}_r = (\boldsymbol{F}_{ex} - \boldsymbol{F}_0)/\boldsymbol{F}_0$$

### 3.9. Bitter flavors classification

Five parameters (Gate mean FITC fluorescence means fluorescence intensity of the gating FITC-H-Count histogram. Total mean FITC fluorescence means fluorescence intensity of all cells FITC-H-Count histogram. Gate maximum FITC fluorescence is fluorescence intensity at maximum cell count peak of gating FITC-H-Count histogram. Gate maximum FITC fluorescence cell number represents count value at maximum cell count peak of gating FITC-H-Count histogram. 1/2 gate FITC fluorescence difference is width value between 1/2 of the mean fluorescence intensity of gating FITC-H-Count histogram) were extracted from the detection results of Dena, Quin, and PTC. Then, the five eigenvalues were analyzed by a PCA algorithm to discriminate the features of different bitter flavors.

## 4. Results and discussion

### 4.1. Conditional optimization results

It is necessary to explore the relationship between the fluorescence intensity and the mixing, cleavage and detection times for detection performance optimization. Since the detected calcium ion concentration depended on the intracellular Fluo 4 concentration, the mixing time of the Fluo 4-AM and cells and the cleavage time of the intracellular Fluo 4-AM were explored to ensure a sufficient concentration of Fluo 4 in the cells without excessive time consumption. Fig. 2a shows the fluorescence intensity for different incubation times of 20, 30, and 40 min and a fixed mixing time of 60 min. The fluorescence intensity at 30 min is much higher than that at 20 min and almost the same as that at 40 min, suggesting that the probe had completely entered the cell at 30 min. Similarly, the fluorescence intensities at 15, 30, 45, and 60 min were measured, as shown in Fig. 2b. The Fluo 4-AM was completely cleaved within 30 min, so 30 min was selected for each of the mixing and incubation times.

Fig. 2c shows the relationship between the fluorescence intensity and detection time as characterized by flow cytometry. The fluorescence intensity values at 3 min are much lower than those at 6 min, indicating that response had not yet reached the maximum value at 3 min. The results at 10 min were lower than those at 6 min, possibly caused by a decrease in the calcium ion concentration after reaction completion and fluorescence quenching. Therefore, 6 min was employed for the detection time after adding the bitter compound.

### 4.2. Fluorescence response of mouse male germ cells to bitter compounds

Three common bitter compounds with diverse chemical structures, namely, PTC, Quin and Dena, were selected as bitter stimuli to test the performance of the biosensor.

Dena is a bitter compound commonly used for sensor performance testing. With this stimulus, the fluorescence intensity increased with increasing concentration over the range from 5 to 4000 μM. The Dena linear detection range was 50–4000 μM; as shown in Fig. 3a, a correlation coefficient of 0.96 and a LOD of 15.6 μM were obtained. The LOD was calculated by the 3δ/slope method.

Quin is an alkaloid isolated from the bark of the cinchona tree. A linear relationship was identified from 50 to 2000 μM with a correlation coefficient of 0.91 (Fig. 3b). The LOD was 5.7 μM.

PTC tastes very bitter or virtually tasteless to an individual, depending on the genetic makeup of the taster. Therefore, this substance is commonly used to define taste genetic variations in humans. Fig. 3c shows that PTC stimulation induced a dose-dependent increase in fluorescence intensity. A linear relationship between the fluorescence intensity and PTC concentration was found over a range of PTC concentrations from 25 to 1000 μM with a correlation coefficient of 0.95. The detection limit was 25.1 μM.

These results indicated that this biosensor had good linear characteristics for all three bitter substances within the detection range.

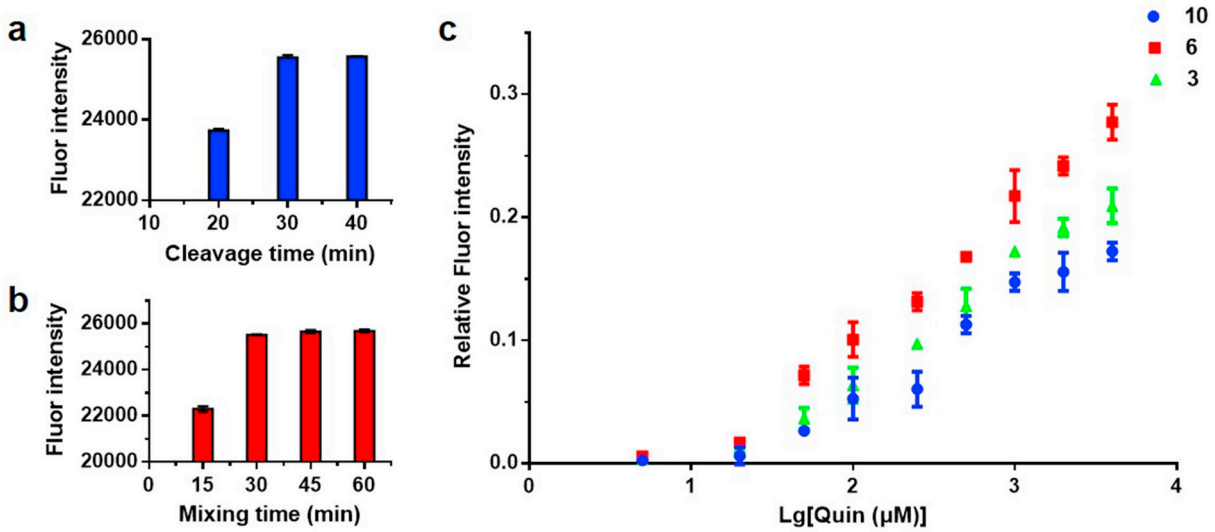


**Fig. 2.** (a) Relationship between the fluorescence intensity and the cleavage time (mixing time: 60 min); (b) Relationship between the fluorescence intensity and the mixing time (cleavage time: 30 min); (c) Relationship between the relative fluorescence intensity and the detection time by flow cytometry. All the data in (a), (b) and (c) were obtained in 3 repeated tests, and the error bars represent the standard deviation (SD).

### 4.3. Fluorescence detection results

Corresponding to Quin detection as described in Section 3.2, fluorescence response images of different concentrations of Quin were recorded to verify that this biosensor could be used for bitter detection. Fig. 4 shows the Fluorescence response maps of Quin at different concentrations. There is almost no fluorescence at 0 μM Quin, with increasing Quin concentration, the increase in the fluorescence response. For comparison, the fluorescence response of 0 μM was set to 1, the response of 0 μM, 20 μM, 50 μM, 100 μM, 250 μM, 500 μM, 1000 μM and 2000 μM were about 1,8,20, 40, 50, 55,65 and 75, respectively. The fluorescence result consistent with the earlier Quin results, proving the approach of bitter detection with fluorescence response is feasible.

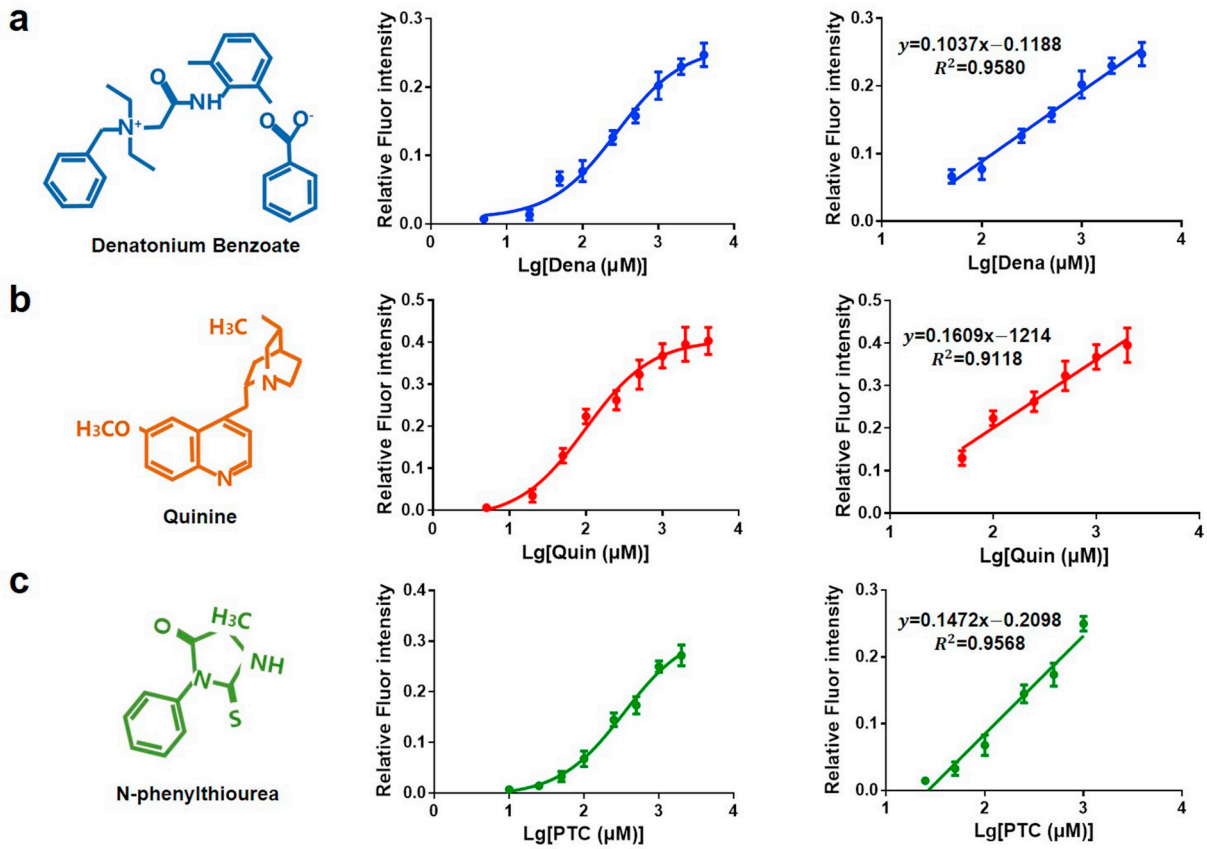


**Fig. 3.** (a) Dena structure and fluorescence response standard curve; (b) Quin structure and fluorescence response standard curve; (c) PTC structure and fluorescence response standard curve. All the data in (a), (b) and (c) were obtained in 3 repeated tests, and the error bars represent the SD.

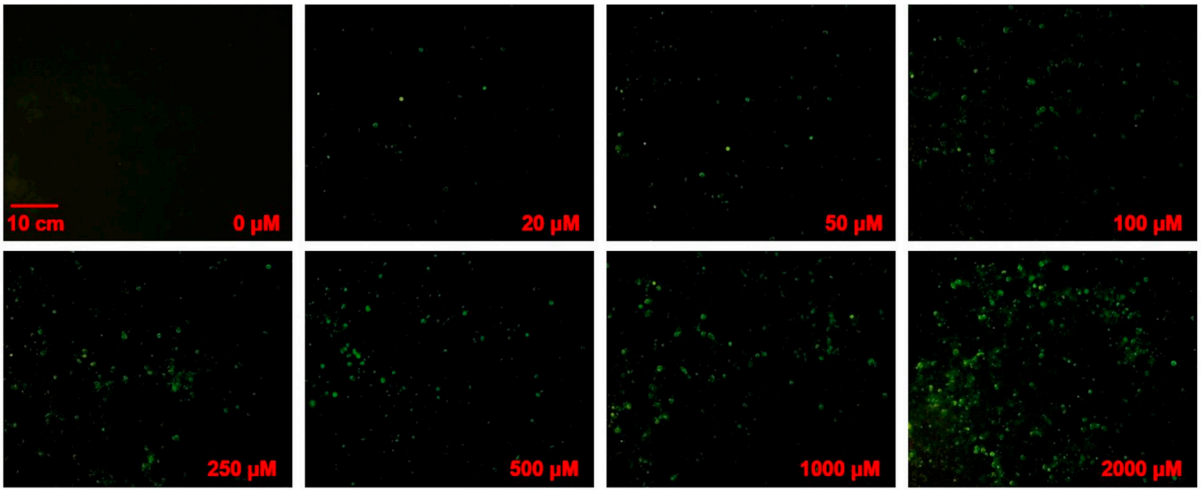


**Fig. 4.** Fluorescence response maps of Quin at different concentrations.

### 4.4. Germ cells respond to bitter compounds with high sensitivity and specificity

Five kinds of taste compounds were selected to verify the specificity of the SCB. Fig. 5 shows the biosensor response to the following substances: CA, NaCl, Suc and MSG (1000 μM) and the three bitter flavors Dena, Quin and PTC (100 μM). The response of the SCB to NaCl ($0.0249 \pm 0.0031$) was the strongest of all the nonbitter flavor responses. However, the responses to Dena ($0.1006 \pm 0.00577$), Quin ($0.2233 \pm 0.0100$) and PTC ($0.0845 \pm 0.0058$) were significantly higher ($p < 0.001$, $N = 3$) than the response induced by NaCl. The reason might be attributed to the fact that sperm cells express only bitter receptors and no other taste receptors. When different types of taste substances were added to sperm-based biosensor, only bitter compounds could activate corresponding bitter receptors, thus only bitter compounds could result in intracellular calcium ion concentration increasing sharply and then fluorescence emitted. The results show that the biosensor response is specific to bitter compounds.

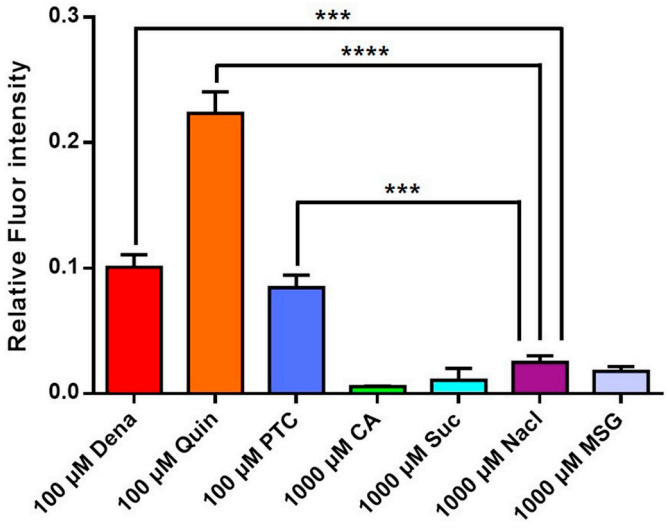


**Fig. 5.** Specificity of the detection system. All the data were obtained in 3 repeated tests, and the error bars represent the SD. Levels of significance: ***$p < 0.001$; ****$p < 0.0001$.

### 4.5. Classification of bitter flavors by the SCB

To further explore the feasibility of the SCB for bitter flavor classification, five eigenvalues were extracted from the detection results of Dena, Quin, and PTC. Bitter classification was performed with a PCA algorithm. Fig. 6 is three-dimensional pattern clustering result of signals produced by different bitter compounds based on PCA. As illustrated in Fig. 6, three aggregation areas are in the picture, corresponding to the results of three bitter substances, indicating that the three bitter flavors could be accurately classified. The reason might be that different bitter substances activated different bitter receptors on the sperm cells, leading to different response patterns of sperm cells and that the responses of the SCB were correspondingly different. The results indicate that the SCB can be employed to classify bitter substances.

## 5. Conclusions

The sperm cells used in this study were primary cells with bitter receptors; their acquisition was relatively simple, and their availability is high. The mixing time and degreasing time were 30 min, meaning that it took approximately only 1 h to complete the sensor construction. In addition, the detection time was merely 6 min; hence, the test period was quite rapid. The SCB was used to conduct a comprehensive

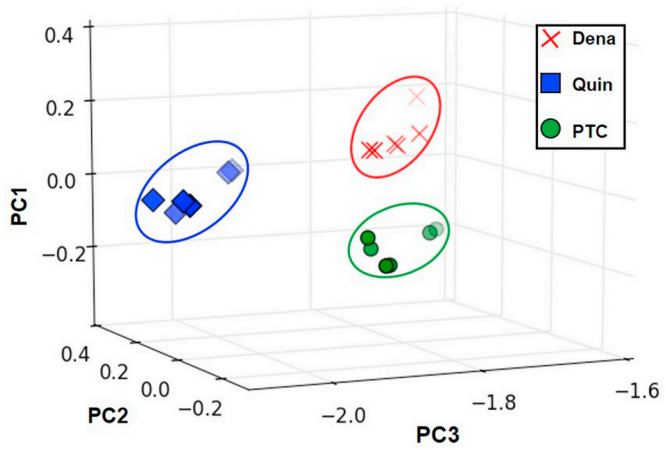


**Fig. 6.** Three-dimensional pattern clustering result of signals treated by different bitter compounds based on PCA.

evaluation of bitter substances due to the multiple bitter receptors on sperm cells. Notably, the SCB can be used with calcium ion fluorescent probes to improve the sensitivity and reduce the LOD. The SCB detected three bitter compounds: Dena, Quin and PTC, with LOD values of 15.6 μM, 5.7 μM and 25.1 μM, respectively. The responses caused by other flavors were significantly lower than those induced by the bitter substances, illustrating that the SCB offers good specificity for bitterness. A PCA analysis indicated that the SCB can provide preliminary bitter substance classification. In addition, flow cytometry enabled quantitative detection with a straightforward detection process.

In summary, a novel biosensor that incorporates sperm cells is proposed for quantitative bitter flavor detection. The SCB integrates living sperm cells and calcium ion fluorescent probe technology and realizes bitter evaluation and classification in conjunction with flow cytometry. The results verify that this sensor is convenient, low cost and easy to use, with high sensitivity, high specificity, and a low LOD for bitter detection. This biosensor has great potential in detecting bitterness in the fields of the pharmaceutical and food industries and in biosafety.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China [grant numbers 51861145307, 31700859, 31661143030 and 31470956] and the Fundamental Research Funds of Xi'an Jiaotong University [grant number xzy022019047].

The authors would like to thank AiMi(www.aimieditor.com) for providing linguistic assistance.

## References

[1] A. Drewnowski, C. Gomez-Carneros, Bitter taste, phytonutrients, and the consumer: a review, Am. J. Clin. Nutr. 72 (2000) 1424–1435.
[2] L. Lemieux, R. Simard, Bitter flavour in dairy products. I. A review of the factors likely to influence its development, mainly in cheese manufacture, Lait 71 (1991) 599–636.
[3] E. Kinnaird, C. Stewart, K. Tchanturia, Taste sensitivity in anorexia nervosa: a systematic review, Int. J. Eat. Disord. 51 (2018) 771–784.
[4] A.M. Mustafa, G. Caprioli, M. Ricciutelli, F. Maggi, R. Marín, S. Vittori, et al., Comparative HPLC/ESI-MS and HPLC/DAD study of different populations of cultivated, wild and commercial Gentiana lutea L, Food Chem. 174 (2015) 426–433.
[5] F. Gutierrez, M. Albi, R. Palma, J. Rios, J. Olias, Bitter taste of virgin olive oil: correlation of sensory evaluation and instrumental HPLC analysis, J. Food Sci. 54 (1989) 68–70.
[6] M. Wesoły, M. Zabadaj, A. Amelian, K. Winnicka, W. Wróblewski, P. Ciosek, Tasting cetirizine-based microspheres with an electronic tongue, Sens. Actuators B Chem. 238 (2017) 1190–1198.
[7] P. Wiriyawattana, S. Suwonsichon, T. Suwonsichon, Effects of aging on taste thresholds: a case of Asian people, J. Sens. Stud. 33 (2018) e12436.
[8] X. Xu, M. Soutto, Q. Xie, S. Servick, C. Subramanian, A.G. von Arnim, et al., Imaging protein interactions with bioluminescence resonance energy transfer (BRET) in plant and mammalian cells and tissues, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 10264–10269.
[9] J. Chandrashekar, T2Rs function as bitter taste receptors, Cell 100 (2000) 703–711.
[10] E. Adler, M.A. Hoon, K.L. Mueller, J. Chandrashekar, N.J. Ryba, C.S. Zuker, A novel family of mammalian taste receptors, Cell 100 (2000) 693–702.
[11] G.T. Wong, K.S. Gannon, R.F. Margolskee, Transduction of bitter and sweet taste by gustducin, Nature 381 (1996) 796.
[12] X. Cetó, N.H. Voelcker, B. Prietosimón, Bioelectronic tongues: new trends and applications in water and food analysis, Biosens. Bioelectron. 79 (2016) 608–626.
[13] L. Hu, L. Zou, Z. Qin, J. Fang, L. Huang, P. Wang, A novel label-free bioengineered cell-based biosensor for salicin detection, Sens. Actuators B Chem. 238 (2017) 1151–1158.
[14] D. Höfer, E. Asan, D. Drenckhahn, Chemosensory perception in the gut, News Physiol. Sci. 14 (1999) 18–23.
[15] S.V. Wu, N. Rozengurt, M. Yang, S.H. Young, E. Rozengurt, Expression of bitter taste receptors of the T2R family in gastrointestinal tract and enteroendocrine STC-1 cells, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 2392–2397.
[16] J. Xu, J. Cao, N. Iguchi, D. Riethmacher, L. Huang, Functional characterization of bitter-taste receptors expressed in mammalian testis, Mol. Hum. Reprod. 19 (2013) 17–28.
[17] F. Li, M. Zhou, Depletion of bitter taste transduction leads to massive spermatid loss in transgenic mice, Mol. Hum. Reprod. 18 (2012) 289–297.
[18] L. Hu, J. Xu, Z. Qin, N. Hu, M. Zhou, L. Huang, et al., Detection of bitterness in vitro by a novel male mouse germ cell-based biosensor, Sens. Actuators B Chem. 223 (2016) 461–469.
[19] J. Xu, J. Cao, N. Iguchi, D. Riethmacher, L. Huang, Functional characterization of bitter-taste receptors expressed in mammalian testis, MHR: Basic science of reproductive medicine 19 (2012) 17–28.
[20] G. Chen, Z. Zhou, H. Feng, C. Zhang, Y. Wang, Z. Qian, et al., An aggregation-induced phosphorescence probe for calcium ion-specific detection and live-cell imaging in Arabidopsis thaliana, Chem. Commun. 55 (2019) 4841–4844.
[21] Y. Zhang, M.A. Hoon, J. Chandrashekar, K.L. Mueller, B. Cook, D. Wu, et al., Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways, Cell 112 (2003) 293–301.
[22] J.-P. Montmayeur, H. Matsunami, Receptors for bitter and sweet taste, Curr. Opin. Neurobiol. 12 (2002) 366–371.
[23] T. Ogura, R.F. Margolskee, S.C. Kinnamon, Taste receptor cell responses to the bitter stimulus denatonium involve Ca2+ influx via store-operated channels, J. Neurophysiol. 87 (2002) 3152–3155.
[24] C.A. Pérez, R.F. Margolskee, S.C. Kinnamon, T. Ogura, Making sense with TRP channels: store-operated calcium entry and the ion channel Trpm5 in taste receptor cells, Cell Calcium 33 (2003) 541–549.
[25] S. Santra, H. Yang, D. Dutta, J.T. Stanley, P.H. Holloway, W. Tan, et al., TAT conjugated, FITC doped silica nanoparticles for bioimaging applications, Chem. Commun. (2004) 2810–2811.
[26] I. Dorval-Coiffec, J.-G. Delcros, H. Hakovirta, J. Toppari, B. Jégou, C. Piquet-Pellorce, Identification of the leukemia inhibitory factor cell targets within the rat testis, Biol. Reprod. 72 (2005) 602–611.
[27] K.R. Gee, K. Brown, W.U. Chen, J. Bishop-Stewart, D. Gray, I. Johnson, Chemical and physiological characterization of fluo-4 Ca2+-indicator dyes, Cell Calcium 27 (2000) 97–106.