

A Dual Functional Cardiomyocyte-based Hybrid-biosensor for the Detection of Diarrhetic Shellfish Poisoning and Paralytic Shellfish Poisoning Toxins

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Okadaic acid (OA) and saxitoxin (STX) are typical toxins of diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP), respectively, which are highly toxic marine toxins threatening human health and environmental safety. OA is a potent inhibitor of serine/threonine protein phosphatases that can cause cellular death, while STX is an inhibitor of sodium channel that can lead to neurological damage. In this work, a dual functional cardiomyocyte-based biosensor was proposed to detect DSP and PSP toxins by monitoring the viability and electrophysiology of cardiomyocytes. The results showed that the viability of cardiomyocytes was sensitive to the OA and STX, resulting in significant changes of the electrophysiological properties, including amplitude, firing rate and duration of the extracellular field potential (EFP). The detection limits of the hybrid-biosensor are as low as 7.16 ng/mL for OA and 5.19 ng/mL for STX. In summary, all of the results indicate that the dual functional cardiomyocyte-based hybrid-biosensor will be a promising and utility tool for shellfish toxin detection.

Keywords Dual functional cardiomyocyte-based biosensor, cell viability, electrophysiology, diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP)

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Introduction

With the increase of ocean pollutions, marine toxins, which are mainly produced by microalgae and enrich through the food chains, have become a worldwide threat to human health and environmental safety.^{1,2} Among all marine toxins, diarrhetic shellfish poisoning (DSP) toxins, commonly okadaic acid (OA) and its derivatives, may cause gastrointestinal illness like diarrhea, vomiting, and abdominal pain^{3,4} without neurologic manifestations,⁵ while paralytic shellfish poisoning (PSP) toxins are most widely-distributed⁶ and may cause neurological damage to mammals.⁴ In the past few decades, severe cases of marine toxins were reported all around the world resulting in huge human life and property loss.^{7,8}

Currently, mouse bioassays (MBAs), chemical analysis methods, immunoassays and cell-based assays are standard methods for the detection of marine toxins.^{9,10} The MBA method is easy to operate, and reflects the biotoxicity directly, making it the common and official recommended method in European Union, Japan and some other countries and regions since 1990.¹¹ Despite of this, the MBA method is limited due to

its low sensitivity, labor intensity and animal ethical problems. Relatively, liquid chromatography-mass spectrometry (LC-MS) offers a more convincing approach due to its high performance in qualitative and quantitative analysis in recent years,^{12,13} but high-cost materials and complicated operations limit its wide application in toxin detection. Immunoassay is another marine toxin measurement method, which offers highly specificity and sensitivity based on antigen-antibody reactions.¹⁴ However, the developments of specific monoclonal antibodies for emerging toxins are also time-consuming and complicated.¹⁵⁻¹⁷ Unlike chemical analytical methods and immunoassays, functional cell-based assays, in which cells act as sensitive elements, could present biological responses to active analytes rather than anticipated chemical structures, providing an alternative *in vitro* approach in recent decades,¹⁸ especially in the field of emerging toxins detection.

Traditional cell-based bioassays (*e.g.* MTT method¹⁹) are end-point methods and very complicated, which reduce the efficiency of detection, and may lose important details. To improve the cell-based bioassays for toxin analysis, cell-based biosensor technologies are developed to monitor different extracellular status *in vitro* in real-time and non-invasive ways,²⁰ including extracellular potentials (by microelectrode array (MEA)^{21,22}) and cellular morphology (by electrical cell-substrate impedance sensor (ECIS)^{23,24}). However, these signal-functional cell-based biosensors can only detect one type of marine toxin in our

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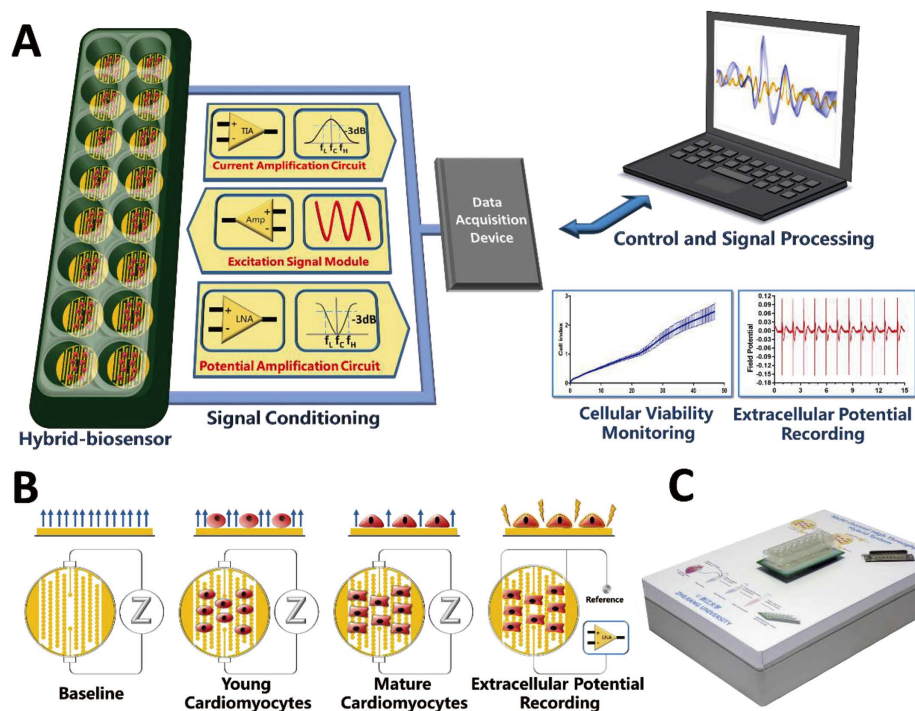


Fig. 1 (A) System diagram of a cardiomyocyte-based hybrid-biosensor. (B) Working principle of the impedance biosensor and potential biosensor. (C) Hybrid-biosensor and the hardware module of the cardiomyocyte-based hybrid-biosensor analyzer.

previous works.^{25–27} Consequently, a new cell-based biosensor that can detect both extracellular potentials changes and cellular morphology changes is demanded to detect multiple toxins.

In this study, a dual functional cardiomyocyte-based biosensor was proposed to detect the PSP and DSP toxins by monitoring the viability and electrophysiology of cardiomyocytes. The dual functional cell-based biosensor consisted of interdigitated electrodes and potential electrodes. The performance of the cell-based biosensor was validated in marine toxins detection after optimizing the cardiomyocytes seeding density. The details will be discussed in the following sections.

Experimental and Methods

Reagents and materials

Hanks balanced salt solution (HBSS), Dulbecco's modified Eagle medium (DMEM), Fetal Bovine Serum (FBS), trypsin, collagenase II, Calcein-AM and propidium iodide (PI) were purchased from Invitrogen (USA). OA was purchased from Sigma Aldrich (USA) and saxitoxin (STX) was obtained from Calbiochem (USA). Toxin solutions were diluted by the culture medium 5 min before use.

Fabrication of the hybrid-biosensor

The hybrid-biosensor consisted of two kinds of sensors: interdigitated electrodes (IDEs) and potential sensors. The substrate material was 4-inch Pyrex Corning 7740 glass (500 μm) because of its biocompatibility and accessibility. Initially, a layer of Au (300 nm) was sputtered on the glass wafer as the metal layer and TiW (30 nm) was used to enhance the adhesion between Au and a glass wafer. The electrodes and lead patterns were etched based on the patterned photoresist using the wet etching technique. Each cell-based hybrid-

biosensor was composed of two potential electrodes centrally, two reference electrodes on both ends and interdigitated electrodes (IDEs) in the interval. For the cell culture, a custom sensor chamber was fixed on the cell-based hybrid-biosensor chip.

Design of a dual functional cardiomyocyte-based biosensor analyzer

The system diagram of a cardiomyocyte-based hybrid-biosensor is shown in Fig. 1(A). The analyzer consisted of a cardiomyocyte-based biosensor, a signal-conditioning module and a DAQ card (Model USB-6255 from National Instruments Inc.). A computer terminal was used for control and signal processing. The DAQ card was controlled by the computer terminal and acquired impedance and potential signals after applying reference or excitation signals on different sensor electrodes and corresponding signal conditioning. All of the data were analyzed by LabVIEW software or Prism 7 (GraphPad). A photo of the hybrid-biosensor and hardware module of the cardiomyocyte-based hybrid-biosensor analyzer is shown in Fig. 1(C).

For cell viability monitoring, IDEs were utilized to measure the impedance value dynamically and continuously, which is related to the number, attachment, and morphology of cardiomyocytes on the IDEs surface. Many researches have been focused on the simulation modeling of IDEs.^{28,29} Generally, the highly sensitive frequency ranges from 5 to 50 kHz. As shown in Fig. 1(B), a stable ion current was formed between the IDEs when a low sinusoidal signal was applied. Current signals were transferred into proper voltage signals by a transimpedance amplifier (TIA). The impedance Z_0 reflected the baseline impedance without cells on IDEs. After new cardiomyocytes were cultured on the surface, the ion current was impeded. When cardiomyocytes became larger, the ion current was

blocked more severely, and the impedance increased accordingly. The change of the impedance was measured as ΔZ and cell index (CI) value, defined as the ratio of ΔZ to Z_0 , was used for viability evaluation.

For electrophysiology monitoring, cardiomyocyte-based planar microelectrodes were utilized to measure the extracellular electrical activity of cardiomyocytes. Since the fundamental cells of heart muscle, cardiomyocytes are rhythmic, excitable and contractile, which is related to ions of sodium, potassium, and calcium. During the active phase, the transient transmembrane potential and the flow of ionic current polarize the electrodes by reestablishing the charge distribution at the electrode-electrolyte-cell interface. In this way, summed electric changes in the potential are amplified by a low-noise amplifier (LNA) and recorded as an extracellular field potential (EFP).

Cell culture

The chips were coated with 0.1% gelatin overnight in a 4°C refrigerator for preparation. Neonatal rat ventricular cardiomyocytes (NRVCs) were isolated from 2-day-old Sprague-Dawley rats. Ventricles obtained from rats after surgery were kept with 2 mL HBSS and minced with scissors into 1–2 mm³ pieces. After dissociated enzymatically by a trypsin/collagenase II mixture for 12 times and mechanical isolation, a cell suspension was obtained and centrifuged at 800 rpm for 5 min. The cell pellet was resuspended in 4 mL of DMEM supplemented with 10% FBS. After 45 min of differential attachment for 2 times, purified cardiomyocytes were derived and cultured onto the surface of hybrid-sensors and maintained at 37°C in an incubator with 5% CO₂. The medium was changed every 24 h.

Calcein-AM/propidium iodide staining assay for cell viability assessment

To determine the viability of cardiomyocytes, two fluorescent dyes, Calcein-AM and PI, which emit green and red fluorescence, were employed to label live and dead cells respectively. Cardiomyocytes on hybrid-biosensors with or without treatment were co-stained by Calcein-AM (0.3 mg/L) and PI (0.5 mg/L). Fluorescent images were taken by an inverted fluorescent microscope (NIB900, Nexcope, USA) after 20-min of staining at 37°C in the dark.

Results and Discussion

Construction and optimization of a cardiomyocyte-based hybrid-biosensor

Cell-seeding density experiments were performed to establish an optimal cardiomyocyte-based biosensor model with high consistency. Cardiomyocytes at densities of 1.2×10^4 , 1.7×10^4 , 2.5×10^4 , and 3.3×10^4 cells/well were seeded onto sensor chips for viability and electrophysiology monitoring. The basic cardiomyocyte viability was monitored for 96 h at 30-min intervals, and the basic electrophysiological activities of cardiomyocytes were measured every 12 h. Curves of the cardiomyocyte viability at different culture densities were shown in Fig. 2(A). Each cardiomyocyte growth curve shows the average and standard deviation of the CI values ($n = 5$). During the first few hours after cell seeding, all cardiomyocyte growth curves presented a high increment speed. In this period, cardiomyocytes attached to the sensor surface, forming the electrode-cell-electrode interface and blocking the ion current directly. The CI values kept increasing and distributed in each seeding density before 48 h. After that, due to a lack of medium

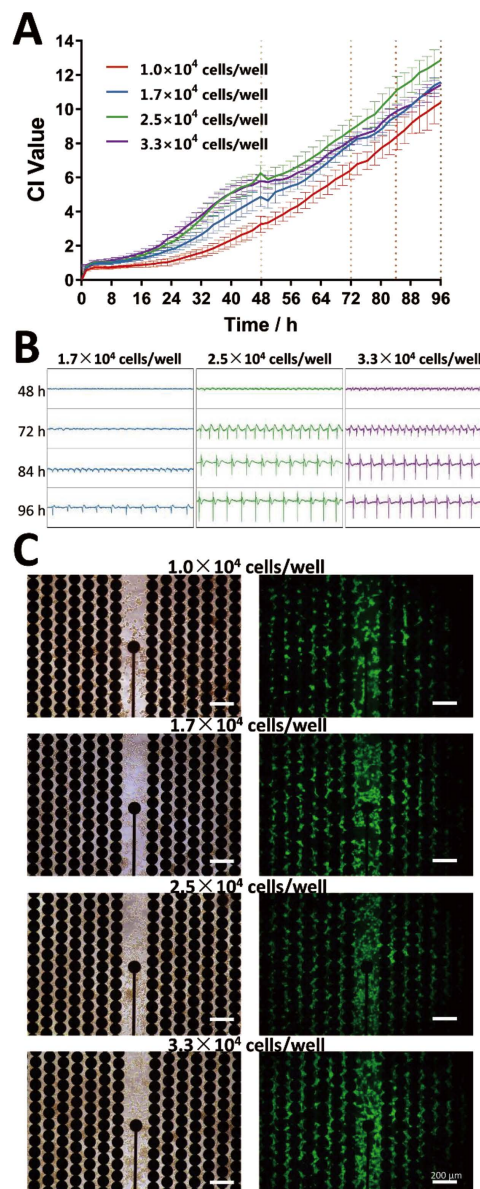


Fig. 2 (A) 96-h dynamic monitoring of cardiomyocyte viability at four different seeding densities. (B) Snapshots of representative EFP signals at seeding densities of 1.7×10^4 , 2.5×10^4 , and 3.3×10^4 cells/well. (C) Optical and fluorescent images of cardiomyocytes on the hybrid-biosensor surface at four different seeding densities.

and attached area, the CI values of 3.3×10^4 cells/well increased much slower than other curves. Correspondingly, Fig. 2(B) shows time-related cardiomyocyte EFP profiles (15 s) of the biosensors at densities of 1.7×10^4 , 2.5×10^4 , and 3.3×10^4 cells/well. Initially, no cardiomyocyte EFP signals were recorded from the cardiomyocyte-based hybrid-biosensor at an early time (24 h, data unshown). At approximately 48 h, faint periodic signals were recorded in channels at 2.5×10^4 and 3.3×10^4 cells/well. One day later, at 72 h, faint periodic signals turned more rhythmic, but the amplitude was still low. With the growth of cardiomyocytes, the cardiomyocyte electrical activity gradually became stable and rhythmic in channels at higher seeding densities. At 84 and 96 h, typical mature cardiomyocyte EFP signals with high amplitude and firing rate were recorded at a density of 2.5×10^4 and 3.3×10^4 cells/well. Optical and fluorescent images of cardiomyocytes coupled with

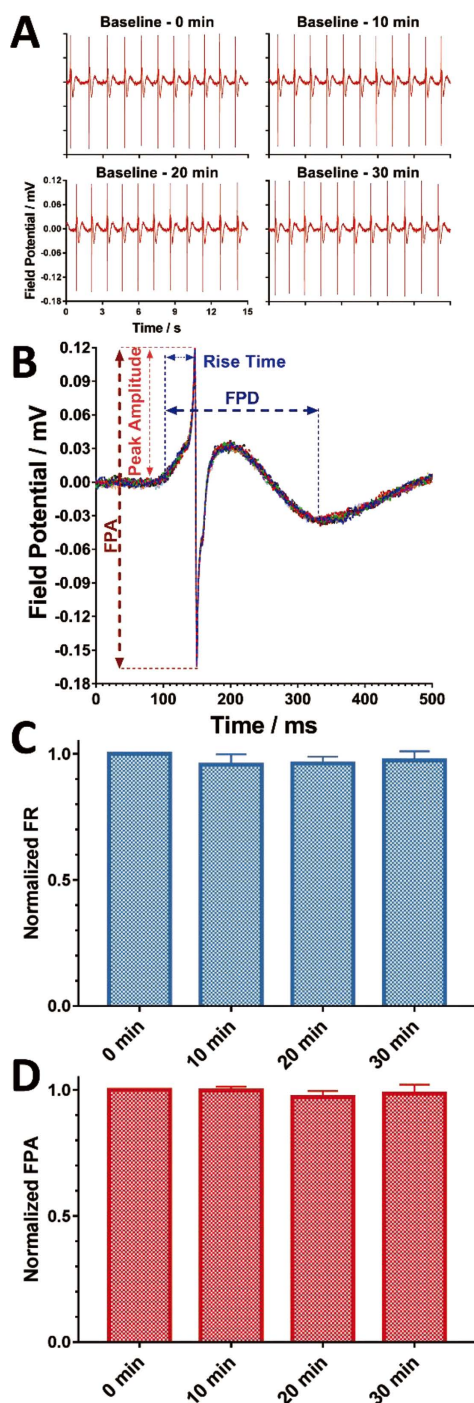


Fig. 3 Consistency and stability test of a cardiomyocyte-based hybrid-biosensor. (A) EFP profiles at baseline. (B) Superposition of EFP waveforms and demonstration of FPA, FPD, peak amplitude, and rise time. (C) and (D) Statistical results of normalized FPA and FR ($n = 4$).

electrodes at 24 h are shown in Fig. 2(C) to verify the cardiomyocyte-based hybrid-biosensor (scale bar 200 μm). The figure displays the viable cell numbers of cardiomyocytes in cultured channels at different seeding densities. More viable cardiomyocytes that are dyed green can be observed in channels with a higher seeding density. Based on the obtained results and analysis, a seeding cell density of 2.5×10^4 cells/well was chosen to establish cardiomyocyte-based hybrid-biosensors for further marine toxins experiments.

Stable and consistent cardiomyocyte status was important to establish a high-performance biosensor. The amplitude of mature EFPs should be larger than 100 μV , and the firing rate should be 30 – 100 min^{-1} . To ensure the consistency of the cardiomyocyte-based biosensor, EFPs especially, a baseline test of the cardiomyocyte electrical activity of cardiomyocytes at 2.5×10^4 cells/well was carried out by monitoring the cardiomyocyte electrophysiological activities every 10 min for 4 times before a compound treatment. As shown in Fig. 3(A), cardiomyocyte EFPs at the baseline presents a high stability in the amplitude and firing rate (FR). In Fig. 3(B), the superposition of every single waveform in one measurement was obtained based on the troughs of EFPs for filtering and further research. The cardiomyocyte extracellular field potential amplitude (FPA), field potential duration (FPD), peak amplitude, and rise time are demonstrated in the figure. The FPA and FR were normalized by the value at 0 min. Statistical results are shown in Figs. 3(C) and 3(D), in which the maximal variable coefficient of FPA was 2.86% and that of FR was 4.32%. These results validate that the electrical activity baseline of cardiomyocytes recorded by the biosensor is very stable and consistent at a density of 2.5×10^4 cells/well, which could be used for further toxins assessment.

Assessment of STX and OA using cardiomyocyte electrophysiology monitoring

After the cardiomyocytes were seeded on the hybrid-sensors for about 96 h, STX in the concentration range of 10 – 1000 ng/mL and OA in the concentration range of 10 – 800 ng/mL were added with cardiomyocyte electrophysiology monitoring. The changes of EFPs were analyzed *via* parameters including FPA, FR, and FPD to evaluate the electrophysiological effect of STX and OA.

Representative EFP signals before and after exposure to different concentrations (10, 50, 100, 200, 500, 1000 ng/mL) of STX for 10 min was shown in Fig. 4(A). Statistical data of FPA, FR, and FPD are shown in Figs. 4(B) – 4(D). The data were normalized by the FPA, FR, and FPD of cardiomyocytes before the compound treatment (normalized value of the control group is 1.00). The administration of STX resulted in a maximal decrease of 60.36% in FPA and 29.82% in FR. The average FPD had a maximal prolongation of 50.68% after 1000 ng/mL STX treatment. Moreover, the changes of FPA, FR and FPD presented a dose-dependent manner. The maximal changes were recorded after treatment of the highest concentration of STX. Similarly, the results of an OA effect on cardiomyocyte electrophysiology are shown in Fig. 5. The profiles of the EFP signals changed slightly after an OA treatment in different concentrations (10, 50, 100, 200, 400, 800 ng/mL) for 10 min (Fig. 5(A)). The injection of OA resulted in a maximal decrease of 25.28% at 800 ng/mL in FPA and 12.48% at 200 ng/mL in FR. The changes indicate no obvious dose-dependence. Moreover, the maximal change of FPD increased by 5.31% at 200 ng/mL, which also indicates no significant change in FPD.

The results illustrate the specific electrophysiological effect of STX on cardiomyocytes. The specificity of the hybrid biosensor was based on the principle differences between STX and OA. STX is a typical sodium channel inhibitor, which impacts the action potential process of cardiomyocytes, while OA presents long-term cytotoxicity. STX, a heterocyclic guanidine-like tetrodotoxin, may interact with the carboxyl in the voltage-regulated sodium channels.^{2,30} The strong bonding between STX and carboxyl group leads to a blockage of voltage-regulated sodium channels, and finally depression of the cardiomyocyte action potentials.³¹ Further, the parameters of FPD were selected to evaluate the performance of the cardiomyocyte electrophysiology

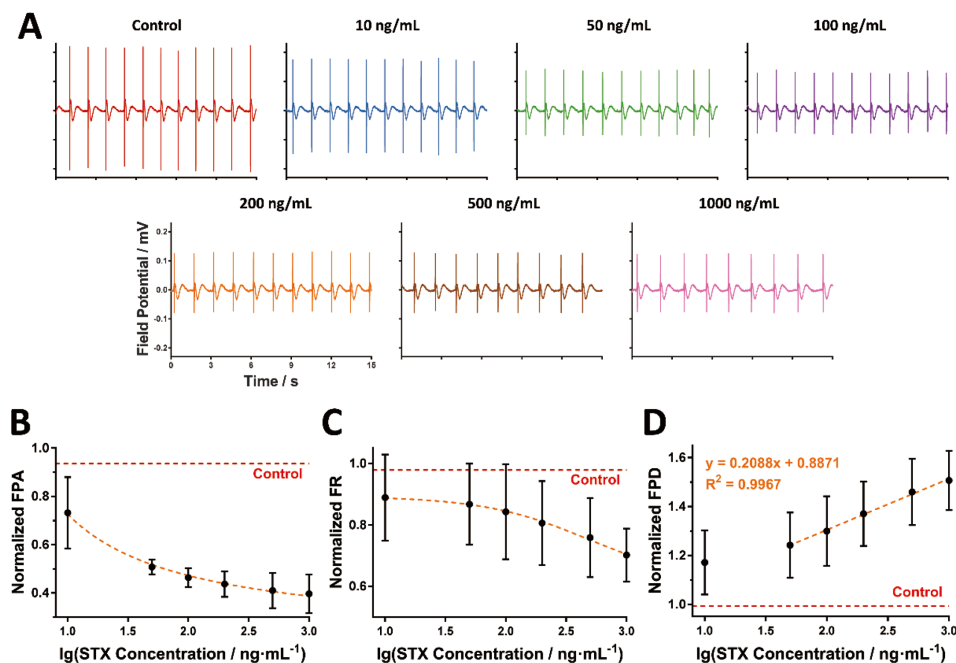


Fig. 4 Cardiomyocyte electrophysiology response to a STX treatment. (A) EFP profiles after a STX treatment of control and at 10, 50, 100, 200, 500, 1000 ng/mL. (B) – (D) Statistical results of normalized FPA, FR and FPD after a STX treatment ($n = 3$).

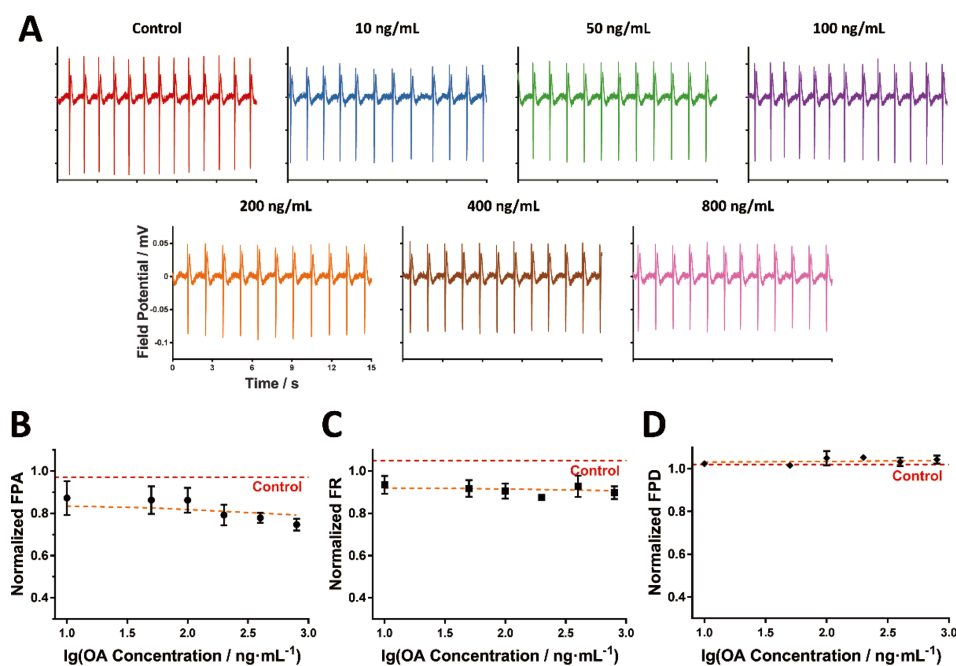


Fig. 5 Cardiomyocyte electrophysiology response to an OA treatment. (A) EFP profiles after an OA treatment of control and at 10, 50, 100, 200, 400, 800 ng/mL. (B) – (D) Statistical results of normalized FPA, FR and FPD after an OA treatment ($n = 3$).

responses to STX (Fig. 4(D)). The limit of detection (LOD) was defined as three-times the standard deviation of the background noise. The LOD of FPD responding to STX was 5.19 ng/mL in the linear range from 50 to 1000 ng/mL (sensitivity of $0.2088/\lg(\text{ng/mL})$, R^2 is 0.9967). Moreover, to investigate the sodium channel-related changes, single representative waveforms of EFPs in different concentrations,

which were obtained from the superposition of many waveforms, are shown in Fig. 6(A). The inset shows more clearly about changes of the peak amplitude and the rise time of the rising edge of EFPs. The peak amplitude dropped from 0.2577 ± 0.0634 mV (Control, $n = 3$) to 0.0998 ± 0.0233 mV with the dose-dependence (Fig. 6(B)). The rise time was prolonged from 4.03 ± 1.15 ms (Control, $n = 3$) to 41.17 ± 5.31 ms, which is

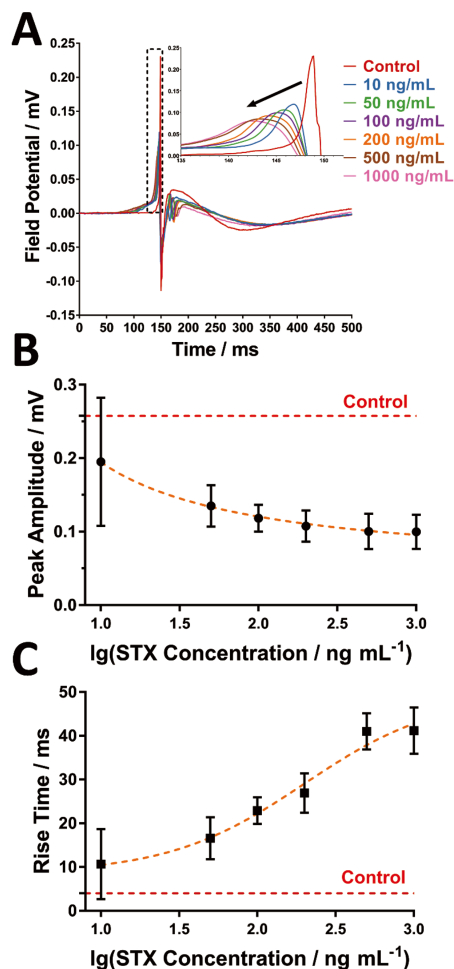


Fig. 6 Changes of the rising edges in response to the STX treatment. (A) Representative single waveforms of EFPs of control and at 10, 50, 100, 200, 500, 1000 ng/mL. (B) and (C) Statistical results of peak amplitude and rise time after STX treatment ($n = 3$).

ten-times of the control group at 1000 ng/mL (Fig. 6(C)). The rising edge of EFP is related to the depolarization of the cardiomyocyte action potential, in which phase the sodium channels are opened for sodium ions.²² The blockage of sodium channels by STX eventually results in a decrease of peak amplitude and a prolongation of the rise time. Therefore, cardiomyocyte electrophysiology monitoring acts as an effective and sensitive approach for STX detection.

Assessment of OA and STX by cardiomyocyte viability monitoring

Cardiomyocyte viability experiments were carried out after the treatment of OA in the concentration range of 10 – 800 ng/mL and STX in the concentration range of 10 – 1000 ng/mL. Figures 7(A) and 7(B) present two groups of normalized CI value curves of the cardiomyocyte viability response to OA and STX for 24 h, respectively. Each cell growth curve shows the mean $\pm$ SD of CI values ($n = 3$). The CI values were normalized by the CI value at 0 h of each curve before treatment of the compound. Normalized CI values of OA-treated hybrid-biosensors are smaller than that of the control group, indicating the dose-dependent OA-induced cardiomyocyte cytotoxicity. The CI values significantly drop to a low level under a high-dose OA. On the other hand, compared to the control group, the normalized CI values of STX-treated hybrid-biosensors decrease slightly and present no dose-dependence. Moreover, the

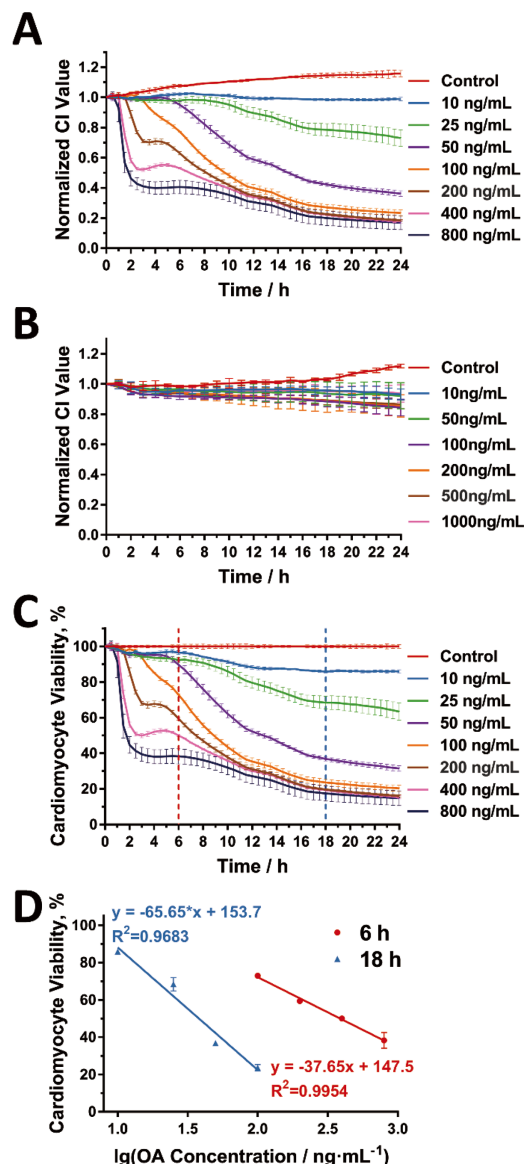


Fig. 7 (A) Normalized CI values before and after OA treatment at 10, 25, 50, 100, 200, 400, 800 ng/mL. (B) Normalized CI values before and after STX treatment at 10, 50, 100, 200, 500, 1000 ng/mL. (C) Cardiomyocyte viability response to OA treatment at 10, 25, 50, 100, 200, 400, 800 ng/mL. (D) Dose-response curves obtained from cardiomyocyte viability curves at 6 and 18 h after OA treatment.

cardiomyocyte viability curves of OA can be calculated based on the ratio of the normalized CI values of the treatment groups to that of the control group (Fig. 7(C)).

The results illustrate the specific cytotoxicity of OA on cardiomyocytes. Previous studies have shown the OA is a potent inhibitor of serine/threonine protein phosphatases (mainly PP1 and PP2A),^{32,33} which are essentials in cell structure, regulation and function during cell growth. On the other hand, the toxic effects of STX are mainly on voltage-regulated sodium channels in excitable cells. Further, according to the toxicity extension, shown in Fig. 7(D), the cardiomyocyte viability curve at 6 h, obtained from Fig. 7(C), is selected for the detection of OA in a higher concentration range from 100 to 800 ng/mL (sensitivity of $-37.65\%/lg$ (ng/mL), R^2 is 0.9954) while the cardiomyocyte viability curve at 18 h is selected for the detection of OA in a lower concentration range from 10 to

Table 1 Comparison of different methods for the detection of STX and OA

Method	Marine toxin	Analysis principle	Information	LOD/ng mL ⁻¹	Quantification	Ref.
ELISA	STX	Immunoassay	Endpoint	<1	Yes	37, 38
	OA			<1		
LC-MS	STX	Mass spectrometry	Endpoint	2	Yes	14, 40, 41
	OA			<1		
MBA	STX	<i>In vivo</i> animal	Endpoint	400	No	35, 36
	OA	bioassay		200		
Hybrid-biosensor	STX	<i>In vitro</i> cell-based	Real-time and	5.19	Yes	This work
	OA	bioassay	dynamic	7.16		

100 ng/mL (sensitivity of $-65.65\%/Ig$ (ng/mL), R^2 is 0.9683). The LOD of cardiomyocyte viability responding to OA was 7.16 ng/mL. From the results, the cardiomyocyte viability showed a high sensitivity to OA, and provided an appropriate method for OA detection.

Discussion of STX and OA detection using cardiomyocyte-based hybrid-biosensor

Recently, owing to their extreme sensitivity to stimulation or disturbances in physiological microenvironment, living cells were utilized as sensing elements to detect environmental toxic compounds. Up to date, a number of previous cell-based methods were applied for marine toxins detection. Compared to conventional end-point and complicated cell-based assays (e.g. MTT method),¹⁹ cell-based biosensor methods have intrinsic properties, such as non-invasiveness, real-time, label-free, easy operation, and relatively low-cost. Previous works in our group have established two types of cell-based biosensors for detection of STX²⁵ and OA,²⁶ separately. However, the function of either EFP detection or cell viability detection is isolated. In this study, cardiomyocyte is coupled with the newly developed hybrid-biosensor, providing a comprehensive cardiomyocyte model *via* short-term electrophysiological recording and long-term viability monitoring in the same culture. The cardiomyocyte-based hybrid-biosensor analyzer managed to realize the dual functions of detections of STX and OA, respectively. The hybrid-biosensor is more efficient and provides a more comprehensive view for compound evaluation by making full use of the characteristics of cardiomyocytes.

The comparison of toxin detection methods using different analysis principles is shown in Table 1. The LODs of the hybrid-biosensor are as low as 5.19 ng/mL for STX and 7.16 ng/mL for OA which are far below the regulatory limit of 800 ng/mL for STX and 160 ng/mL for OA in Europe Union.^{10,34} The hybrid-biosensor is also more sensitive than the MBA, which is regarded as the official regulatory method in most countries with the detection limits of 400 ng/mL for STX³⁵ and 200 ng/mL for OA,³⁶ serving as a promising alternative bioassay for the MBA. Though LODs of the hybrid-biosensor are not as low as that of ELISA (<1 ng/mL for both STX and OA^{37,38}) with high sensitivity and high selectivity, the hybrid-biosensor could dynamically evaluate the biotoxic effects of determinands in real time. The cardiomyocyte-based hybrid-biosensor is a biological test system that can be used to directly demonstrate the presence of unknown toxins, while the developments of monoclonal antibodies for emerging toxins³⁹ could be time-consuming and difficult. Future developments foreseen in the analysis of marine toxins will be multiplex-based analysis, and the hybrid-biosensor method could serve as a complementary functional bioanalytical method for marine toxins detection.

Conclusion

In this study, a novel cardiomyocyte-based hybrid-biosensor was proposed for the dual functions of detections of DSP and PSP toxins using cardiomyocyte viability and electrophysiology monitoring. This hybrid-biosensor shows good linearity in DSP detection using cardiomyocyte viability monitoring, and provides an effective and sensitive way for studies on neurotoxic effects of PSP. The LODs of the hybrid-biosensor are as low as 5.19 ng/mL for STX and 7.16 ng/mL for OA. Compared with previous detection methods, it provides a highly efficient and effective way for real-time and non-invasive marine toxins detection by dual functions of cardiomyocyte status monitoring, making it a promising detection approach in the future.

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References

1. K. D. Cusick and G. S. Sayler, *Mar. Drugs*, **2013**, *11*, 991.
2. W. M. Haschek, C. G. Rousseaux, M. A. Wallig, B. Bolon, and R. Ochoa, "Haschek and Rousseaux's Handbook of Toxicologic Pathology", **2013**, Academic Press.
3. E. EFSA, *The EFSA J.*, **2008**, *589*, 1.
4. M. Campas, B. Prieto-Simón, and J.-L. Marty, *Talanta*, **2007**, *72*, 884.
5. S. Svensson, *Aquaculture*, **2003**, *218*, 277.
6. K. Whittle and S. Gallacher, *Br. Med. Bull.*, **2000**, *56*, 236.
7. A. Li, J. Ma, J. Cao, and P. McCarron, *Toxicol.*, **2012**, *60*, 420.
8. V. L. Trainer, L. Moore, B. D. Bill, N. G. Adams, N. Harrington, J. Borchert, D. A. Da Silva, and B.-T. L. Eberhart, *Mar. Drugs*, **2013**, *11*, 1815.
9. A. Sassolas, A. Hayat, G. Catanante, and J.-L. Marty, *Talanta*, **2013**, *105*, 306.
10. K. Campbell, N. Vilarino, L. M. Botana, and C. T. Elliott, *TrAC Trends Anal. Chem.*, **2011**, *30*, 239.
11. A. Gerssen, I. E. Pol-Hofstad, M. Poelman, P. P. Mulder, H. J. Van den Top, and J. De Boer, *Toxins*, **2010**, *2*, 878.
12. J. P. Meneely, K. Campbell, C. Greef, M. J. Lochhead, and C. T. Elliott, *Biosens. Bioelectron.*, **2013**, *41*, 691.
13. J. Nicolas, P. J. Hendriksen, A. Gerssen, T. F. Bovee, and I. M. Rietjens, *Mol. Nutr. Food Res.*, **2014**, *58*, 87.
14. A. Humpage, V. Magalhaes, and S. Frosio, *Anal. Bioanal.*

- Chem.*, **2010**, 397, 1655.
15. T. Mouratidou, I. Kaniou-Grigoriadou, C. Samara, and T. Kouimtzis, *Sci. Total Environ.*, **2006**, 366, 894.
 16. M. Campàs and J.-L. Marty, *Anal. Chim. Acta*, **2007**, 605, 87.
 17. R. Wirtz, F. Zavala, Y. Charoenvit, G. Campbell, T. Burkot, I. Schneider, K. Esser, R. L. Beaudoin, and R. Andre, *Bull. World Health Org.*, **1987**, 65, 39.
 18. W. F. An and N. Tolliday, *Mol. Biotechnol.*, **2010**, 45, 180.
 19. J. van Meerloo, G. J. Kaspers, and J. Cloos, in "Cancer Cell Culture", **2011**, Springer, 237.
 20. Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, and P. Wang, *Chem. Rev.*, **2014**, 114, 6423.
 21. J. Pine, *Advances in Network Electrophysiology*, **2006**, 3.
 22. M. Halbach, U. Egert, J. Hescheler, and K. Banach, *Cell. Physiol. Biochem.*, **2003**, 13, 271.
 23. I. Giaever and C. R. Keese, *Proceedings of the National Academy of Sciences*, **1984**, 81, 3761.
 24. I. Giaever and C. R. Keese, *Nature*, **1993**, 366, 591.
 25. Q. Wang, J. Fang, D. Cao, H. Li, K. Su, N. Hu, and P. Wang, *Biosens. Bioelectron.*, **2015**, 72, 10.
 26. L. Zou, Q. Wang, M. Tong, H. Li, J. Wang, N. Hu, and P. Wang, *Sens. Actuators, B*, **2016**, 222, 205.
 27. N. Hu, J. Fang, L. Zou, H. Wan, Y. Pan, K. Su, X. Zhang, and P. Wang, *Biomed. Microdevices*, **2016**, 18, 94.
 28. J. Paredes, S. Becerro, F. Arizti, A. Aguinaga, J. Del Pozo, and S. Arana, *Biosens. Bioelectron.*, **2012**, 38, 226.
 29. L. Wang, H. Wang, K. Mitchelson, Z. Yu, and J. Cheng, *Biosens. Bioelectron.*, **2008**, 24, 14.
 30. W. A. Catterall, *Ann. Rev. Pharmacol. Toxicol.*, **1980**, 20, 15.
 31. G. M. Lipkind and H. A. Fozzard, *Biophys. J.*, **1994**, 66, 1.
 32. C. Bialojan and A. Takai, *Biochem. J.*, **1988**, 256, 283.
 33. R. E. Honkkanen, B. A. Codispoti, K. Tse, and A. L. Boynton, *Toxicol.*, **1994**, 32, 339.
 34. L. Zou, C. Wu, Q. Wang, J. Zhou, K. Su, H. Li, N. Hu, and P. Wang, *Biosens. Bioelectron.*, **2015**, 67, 458.
 35. M. C. Louzao, M. R. Vieytes, A. G. Cabado, J. M. Vieites Baptista de Sousa, and L. M. Botana, *Chem. Res. Toxicol.*, **2003**, 16, 433.
 36. D. O. Mountfort, G. Kennedy, I. Garthwaite, M. Quilliam, P. Truman, and D. J. Hannah, *Toxicol.*, **1999**, 37, 909.
 37. M. P. Kreuzer, C. K. O'Sullivan, and G. G. Guilbault, *Anal. Chem.*, **1999**, 71, 4198.
 38. K. Campbell, A.-C. Huet, C. Charlier, C. Higgins, P. Delahaut, and C. T. Elliott, *J. Chromatogr. B*, **2009**, 877, 4079.
 39. L. Reverté, L. Soliño, O. Carnicer, J. Diogène, and M. Campàs, *Mar. Drugs*, **2014**, 12, 5719.
 40. C. Dell'Aversano, P. Hess, and M. A. Quilliam, *J. Chromatogr. A*, **2005**, 1081, 190.
 41. B. Ben-Gigirey, M. L. Rodríguez-Velasco, and A. Gago-Martínez, *J. AOAC Int.*, **2012**, 95, 111.
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