Biosensors and Bioelectronics 67 (2015) 458–464

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An improved sensitive assay for the detection of PSP toxins with neuroblastoma cell-based impedance biosensor

Ling Zou, Chunsheng Wu, Qin Wang, Jie Zhou, Kaiqi Su, Hongbo Li, Ning Hu *, Ping Wang *

Biosensor National Special Laboratory, Key Laboratory for Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form
23 August 2014
Accepted 2 September 2014
Available online 6 September 2014

Keywords:
Saxitoxins
Neuro-2a cell
Impedance detection
Cell-based impedance biosensor

ABSTRACT

Paralytic shellfish poisoning (PSP) toxins are well-known sodium channel-blocking marine toxins, which block the conduction of nerve impulses and lead to a series of neurological disorders symptoms. However, PSP toxins can inhibit the cytotoxicity effect of compounds (e.g., ouabain and veratridine). Under the treatment of ouabain and veratridine, neuroblastoma cell will swell and die gradually, since veratridine causes the persistent inflow of Na^+ and ouabain inhibits the activity of Na^+/K^+-ATPases. Therefore, PSP toxins with antagonism effect can raise the chance of cell survival by blocking inflow of Na^+. Based on the antagonism effect of PSP toxins, we designed an improved cell-based assay to detect PSP toxins using a neuroblastoma cell-based impedance biosensor. The results demonstrated that this biosensor showed high sensitivity and good specificity for saxitoxins detection. The detection limit of this biosensor was as low as 0.03 ng/ml, which was lower than previous reported cell-based assays and mouse bioassays. With the improvement of biosensor performance, the neuroblastoma cell-based impedance biosensor has great potential to be a universal PSP screening method.

1. Introduction

Marin toxins, produced by algae, are easily accumulated to a high level in filter-feeding fish or shellfish. People get poisoned commonly by eating the contaminated seafood. About 750–7500 people are killed by marine toxins all around the world every year, and there are 50,000 poisoned victims until 2013 (Cusick and Sayler, 2013). Among all of the marine toxins, paralytic shellfish poisoning (PSP) toxins are one type of the most widely-distributed toxins in the world, which do the most serious harm in public health and marine aquaculture industries. PSP toxins affect the propagation of action potential by blocking sodium channels, which will change the membrane potential on a cellular level and cause neurological symptoms (e.g. numbness and tingling) in human (Wiese et al., 2010; Chen et al., 2013). Therefore, the detection of marine toxins has great significance in human health and environmental safety.

To date, the official methods for PSP toxins detection are the mouse bioassay (MBA) and the pre-column oxidation liquid chromatography with fluorescence detection (ox-LC-FLD) (Turner et al., 2011; Ben-Gigirey et al., 2012; Meneely et al., 2013; Nicolas et al., 2014). The first analytical method permitted by the government in PSP toxins detection is ox-LC-FLD (Humpage et al., 2010). Although this method is sensitive and reliable, it is limited by the expensive reference material and chromophore which needs to produce fluorescent products (Humpage et al., 2010; Turner et al., 2011). Moreover, these PSP toxins can be converted from one structure to another in preparing samples of ox-LC-FLD. In addition, the non-analytical methods, which can only detect a small number of closely-related toxins, are insufficient to detect 30 structural variants of PSP toxins (Flanagan et al., 2001). As a consequence, the MBA method is still the "gold standard" in PSP toxins detection in many countries (Cusick and Sayler, 2013). As the most commonly used detection method, MBA can detect the PSP toxins by observing the symptoms and the time of death after injecting shellfish extracts. It can not only detect all of the PSP toxins, but also reflect the actual toxicity. However, a novel alternative biological method for the MBA method is still needed due to the low sensitivity and ethical problem of MBA. Cell-based bioassay was designed as a biological alternative method recently. Previous studies have identified that the cell-based assay could improve the detection limit markedly and reduce the number of animals used in experiments (Okaichi, 2004; Okumura et al., 2005; Hayashi et al., 2006). Both the primary cells and continuous cell lines can be used in the cell-based assays. The method based on continuous cell lines might be more promising than primary cells which still need to be derived from laboratory animals. What is more, the continuous cell lines

* Corresponding authors. Fax: +86 571 87952832.
E-mail addresses: huning@zju.edu.cn (N. Hu), cnpwang@zju.edu.cn (P. Wang).

http://dx.doi.org/10.1016/j.bios.2014.09.005
0956-5663/

could be applied to detect and screen marine toxins with high throughput. Cell-based assay using continuous cell lines has undergone several enhancements since it was first established in 1988 (Kogure et al., 1988). The presence of PSP toxins can be detected by measuring the cell viability co-treated with ouabain and veratridine, which is a widely accepted cell-based assay today (Manger et al., 2003; Okumura et al., 2005).

Cell-based biosensor is a new method in the cell-based bioassay, which can detect biological or chemical agents by monitoring the metabolism, impedance and excitability of cells in dynamic and real-time way (Yicong et al., 2001; Ahmad and Moore 2009; Liu et al., 2009; Hu et al., 2013a, 2013b; Wang et al., 2013). Cell-based impedance biosensor is an emerging technology which can realize real-time, noninvasive monitoring of the growth status of in vitro cultured cells (Tiruppathi et al., 1992; Keese et al., 2002). This biosensor has a wide range of applications, such as drug screening and toxicity assessment (Asphahani and Zhang, 2007; Arias et al., 2010; Zhou et al., 2013). Nevertheless, it is rarely used in marine toxins detection, hardly for PSP toxins. In present study, we developed a mouse neuroblastoma cell-based impedance biosensor (CIB) and used it to improve the existing cell based assay in PSP toxins detection successfully. Saxitoxin (STX, molecular weight=299), which is a standard representation of PSP toxins, was utilized to assess the feasibility of this method. All the details will be discussed in the following sections.

2. Experimental and methods

2.1. Detection principle of cell-based impedance biosensor

CIB is an approach which can monitor cell activities noninvasively and dynamically. The detection system of CIB contains signal generator module, amplification and filter module, and data acquisition module. A sinusoidal voltage with amplitude of 30 mV and frequency of 10 kHz is generated by signal generator module and is applied on the interdigitated electrodes (IDEs), and then ion current is formed between the IDEs (Fig. S1). Then the current signals are converted to voltage signal by amplifier and filter module. Subsequently, the signals can be recorded by the data acquisition module. Finally, the digital signals are transferred to computer to calculate the impedance value by the signal processing. When the cells are located on the IDEs surface, the ion current is impeded, so the impedance of IDEs increases. And Impedance values are usually normalized by cell index (CI) values for comparison. CI is the ratio of the cell impedance change ΔZ to background impedance Z_0, which excludes the impacts of electrode–electrolyte combination and parasitic elements. Therefore, the CI can reflect the cell behavior (e.g., cell proliferation, cell morphology and cell–substrate adhesion) on the IDEs (Fig. 1(A)). Since impedance sensor detection technology is non-invasive, it can be used to monitor cell properties in real time. Fig. 1(B) and (C) shows the CIB detection system and fabricated sensor chip, respectively. As shown in Fig. 1(C), the cell-impedance sensor array has 16 IDEs with diameter of 5 mm, and center distance of two adjacent IDEs is 9 mm. Branch patterns of IDEs consist of many small circles, and the diameter of these small circles is 90 μm. Besides, the center distance of two adjacent electrodes is 120 μm, and the coverage of electrode is about 60%.

2.2. Cell culture

Neuroblastoma cells (Neuro-2a) (American Tissue Culture Collection, CCL131), were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium with 10% fetal calf serum (FCS; heat-inactivated) at 37 °C and 5.0% CO_2 in an incubator (Thermo Fisher Scientific, USA). Culture medium RPMI-1640 was supplemented with 1% sodium pyruvate solution (100 mM), 1% L-glutamine solution (200 mM), 1% nonessential amino acids (100 mM) and 0.5% antibiotic solution (10 mg/mL streptomycin and 1000 U/mL penicillin). The medium was changed every 1–2 days, and cells were detached from the culture flask using trypsin

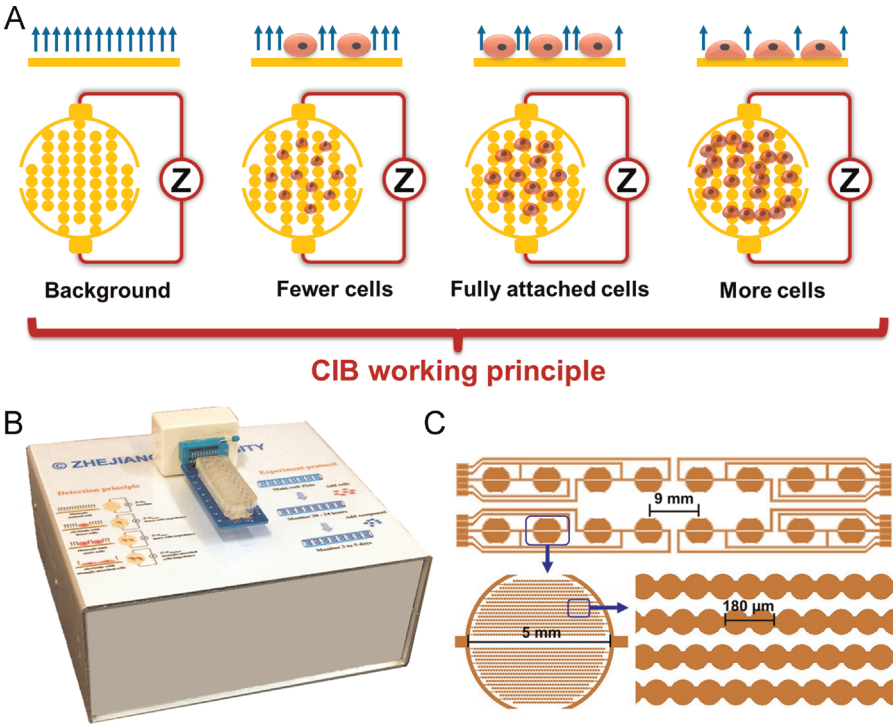

Fig. 1. (A) Detection principle of cell-based impedance biosensor (CIB). (B) CIB system with multichannel sensor unit. (C) Layout of the sensor chip which contains 16 wells with interdigitated gold electrodes.

solution after reached the confluence of 90%. All of the reagents needed in cell culture were purchased from Gibico, USA. Cell Counting Kit-8 (CCK8) was obtained from Dojindo Lab., Kumamoto, Japan.

2.3. Preparation of standard toxins and spiked shellfish extracts

Saxitoxin (STX) standard solution was obtained from the Institute for Marine Biosciences (Halifax, NS, Canada). Ouabain and veratridine were purchased from Sigma Aldrich (USA), and the stock solution of ouabain and veratridine were diluted in deionized water (pH 7, MilliQ) and DMSO, respectively. The working concentration of STX, ouabain and veratridine were prepared in cell culture medium before they were added into the sensor chip.

Venerupis variegate were purchased from the market, and the acidic extracts of shellfish were processed according to the previous research (Louzao et al., 2003). Then the extracts were filtered with 0.22 μm filters before used in the cell-based assay. It is relatively difficult to obtain the saxitoxin (STX) contaminated shellfish extracts. Therefore, the non-toxic shellfish extracts which have been validated by cell-based assay and HPLC–MS were selected to spike with STX standard solution to obtain different concentrations of STX in shellfish extracts.

2.4. Real-time monitoring of cellular response to marine toxins

In the cell experiment, 100 μL of cell cuture medium was added to each well of sensor chip which was coated with 0.1% gelatin to measure the baseline of impedance Z_0. Then, 200 μL of neuro-2a cell suspensions with different cell seeding densities were added. After cell seeding, the sensor chip was placed in incubator during the whole process. Before exposure to toxins, the cells cultured on the interdigitated electrodes (IDEs) were monitored for 24 h by the CIB system. Then the medium of treated groups was removed and replaced by 290 μL fresh medium with 0.5 mM ouabain and 0.05 mM veratridine, and then 10 μL toxin standard solutions or sample extracts were added. The OV group was treated with 300 μL medium containing 0.5 mM ouabain and 0.05 mM veratridine without addition of toxins. While medium of control group was removed and replaced by 300 μL fresh medium without OV or toxin. Finally, the sensor chip was placed back in the incubator after the compounds were added.

2.5. CCK8 assay for cell viability assessment

Assessment of cell viability was implemented using CCK8 assay which was a modified method of MTT. Briefly, Neuro-2a cells were seeded in the sensor chip at different seeding densities. After cell cultured for 24 h, 30 μL CCK8 solution was added to each well of the sensor chip, and then cells were incubated for 0.5 h at 37 °C. Finally, 100 μL of medium was collected and read at 450 nm wavelength.

2.6. Scanning electron microscopy

The neuro-2a cells which treated with different concentrations of saxitoxin (STX) and OV for 24 h along with the control group were pre-fixed in 2.5% glutaraldehyde in 0.01 M phosphate buffer

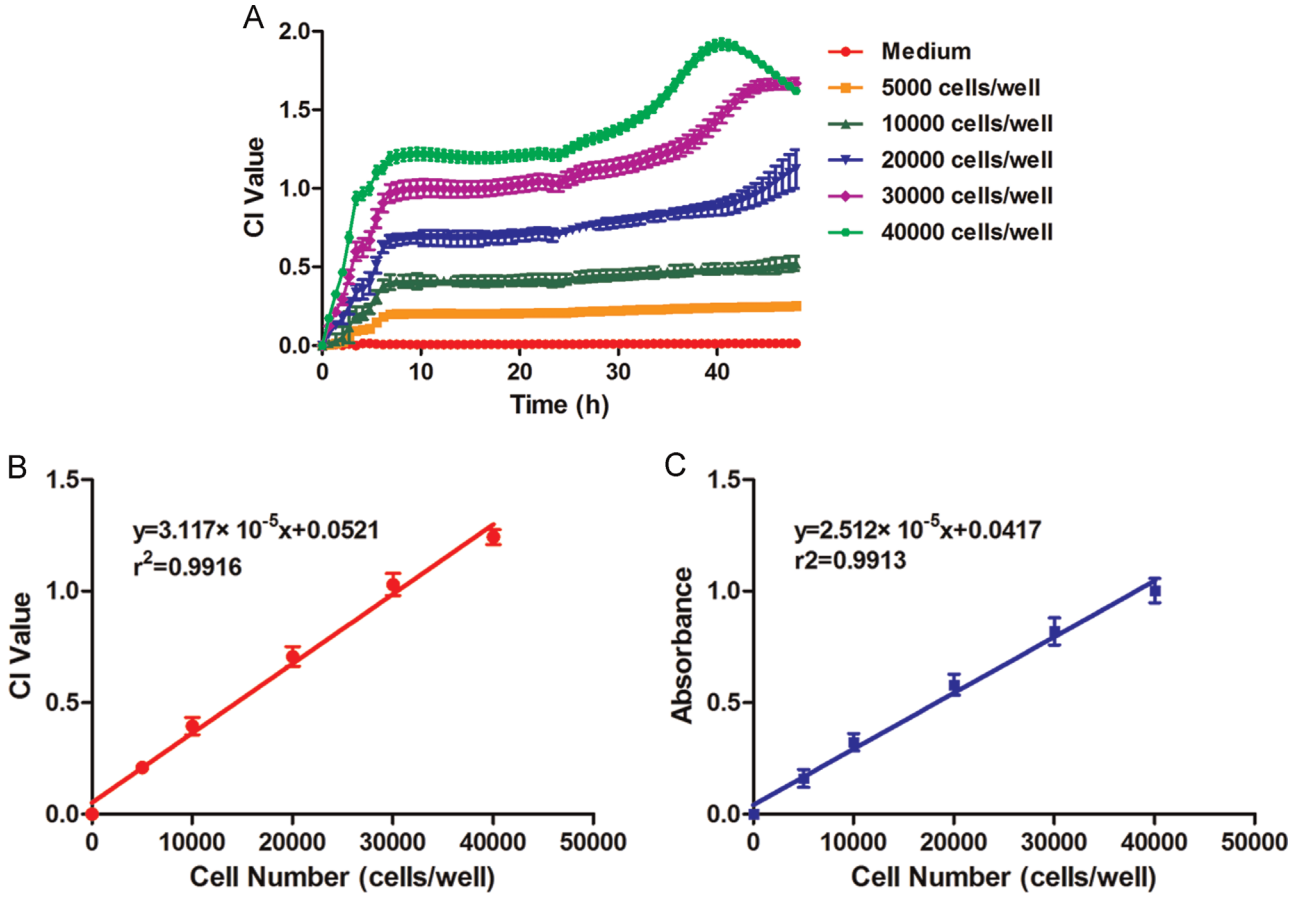

Fig. 2. (A) A group of representative cell growth curves of neuro-2a cells by CIB system at different cell numbers ranging from 5000 to 40,000 cells/well. (B) The linear relationship between the cell number and the CI value measured by CIB system at 24 h after cells were seeded. (C) The linear relationship between the cell number and the absorbance measured by CCK8 at 24 h after the cell were seeded.

solution (PBS, PH=7.4) for 2 h at 4 °C. After that, the treated cells were post-fixed with 1% of osmium tetraoxide for 1 h at 4 °C after washed with PBS. Then, the cells were dehydrated with a graded series of ethanol solutions (30%, 50%, 70%, 80%, 90%, 95% and 100%, 15 min each grade). Finally, the fixed cells were dried. Scanning electron microscopy (SEM) analyzes were performed on KYKY-EM3200 (KYKY, China) microscopy.

3. Result and discussion

3.1. Dynamically monitoring of cell proliferation, attachment and detachment

To verify our system can be directly utilized to detect the growth and proliferation of cells, Neuro-2a cells were detached from the culture flask and inoculated onto sensor chip at 5000, 10,000, 20,000, 30,000, and 40,000 cells/well. Cell growth curves of each well were measured by long-term impedance working mode of CIB system during the whole experiment. Fig. 2(A) shows the cell growth curves of neuro-2a cells measured by CIB system with different cell numbers ranging from 5000 to 40,000 cells/well. Cell growth curves show the mean ± SD of CI values in the three measurements. At the time of zero, the CI values under different seeding densities were all zero, since no cell was attached on the electrodes. The CI value increased during the incubation period. At each time point, IDEs with the same cell numbers have similar CI values, and the higher cell number resulted in the higher CI value. The CI values of the medium groups without any cells were steady at zero in the whole measurement process. In order to determine whether the CI value measured by the CIB system was quantitatively correlated with the cell number, a standard method CCK8 was employed. The CI values and absorbance for each cell number were measured by three replicate of different cell numbers to evaluate the precision in both assays. As shown in Fig. 2(B) and (C), the CI values measured by the CIB system were linear with cell numbers over the range between 5000 and 40,000 with high linearity (r^2=0.9916). The linearity of CCK8 assay was 0.9913. The results indicated that the CI values measured by CIB system were consistent with the absorbance obtained from CCK8 assay. Therefore, the viable cell number could also be quantified by the CIB system.

As demonstrated by Fig. 2(A), the number of initial seeding cells shows obvious influences on the output of sensor signals, which suggested that it was important to optimize the number of initial seeding cells in order to establish a sensitive cell-based biosensor. As shown in Fig. 2(A), the CI values measured at low cell number (5000 and 10,000 cells/well) were small and increased slowly, which led to low sensor output signals and low sensitivity. On the contrary, the CI values were larger and increased more rapidly with the number of seeding cells increases. However, the CI values of initial cell number of 40,000 cells/well decreased after 40 h culture. The main reason is that the cell in logarithmic growth phase grew rapidly and well, whereas the cell growth will plateau due to exhaustion of the medium or contact inhibition (van Meerloo et al., 2011). Therefore, the cell-based assay should not be conducted after the log phase of cell growth. Generally, the cell-based assay for toxins detection requires 48 h or more. In addition, the neuro-2a cells still in logarithmic growth phase after culture for 48 h when the number of seeding cells is 30,000. Based on the obtained results and analysis, 30,000 was the suitable seeding number to obtain a better cell-based biosensor.

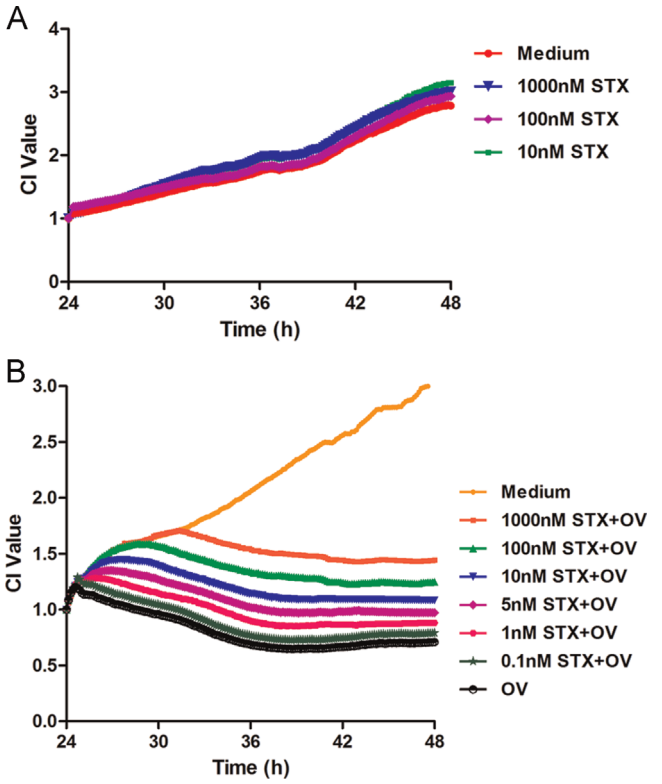

Fig. 3. (A) A group of normalized cell growth curves of neuro-2a cells responding to different concentrations of STX without OV. (B) A group of normalized cell growth curves of neuro-2a cells responding to STX in the presence of OV.

3.2. Real-time monitoring of saxitoxin (STX) inhibiting the apoptosis induced by ouabain and veratridine

Fig. 3(A) and (B) shows a group of normalized cell growth curves of neuro-2a cells responding to STX treatment in the absence and the presence of 0.5 mM ouabain and 0.05 mM veratridine (OV), respectively (Fig. S4). From Fig. 3(A), it can be seen that there was neglected effect of STX on cell growth after 24 h of treatment because there were no significant differences in CI values between the high concentration STX and control group, indicating that treatment with STX have no effect on the growth and multiplication of cells which was consistent with previous research (Cañete and Diogène, 2008). As shown in Fig. 3(B), each CI value curve provided detailed kinetic information of cells in response to OV and STX with various concentrations, which was ignored in conventional endpoint assay. CI values measured by the CIB system decreased after OV treatment due to the continuous increasing of the intracellular Na^+. And STX inhibited the OV-induced cell death by blocking Na^+ inward current. For example, CI value began to decrease within 1 h after OV treatment, whereas the group of 1000 nM and 100 nM STX started to decrease at approximately 8 h and 5 h after STX addition, respectively. Cells co-treated with OV and 1–1000 nM STX presents higher CI values than that of only OV treated. This result indicated that OV co-treated with higher concentration of STX need longer time to induce neuro-2a cells apoptosis, while lower concentration of STX co-treated with OV induced cell apoptosis quickly. It was suggested that this CIB method can monitor STX inhibits neuro-2a cells apoptosis induced by OV dynamically and noninvasively, and provided real-time and dynamic information of cells response to different concentration compounds throughout the experiments.

Fig. 4(A) presents cell relative viability curves at a given time point (6 h, 12 h, 18 h and 24 h) for different concentrations of STX with 0.5 mM ouabain and 0.05 mM veratridine (OV) treatment

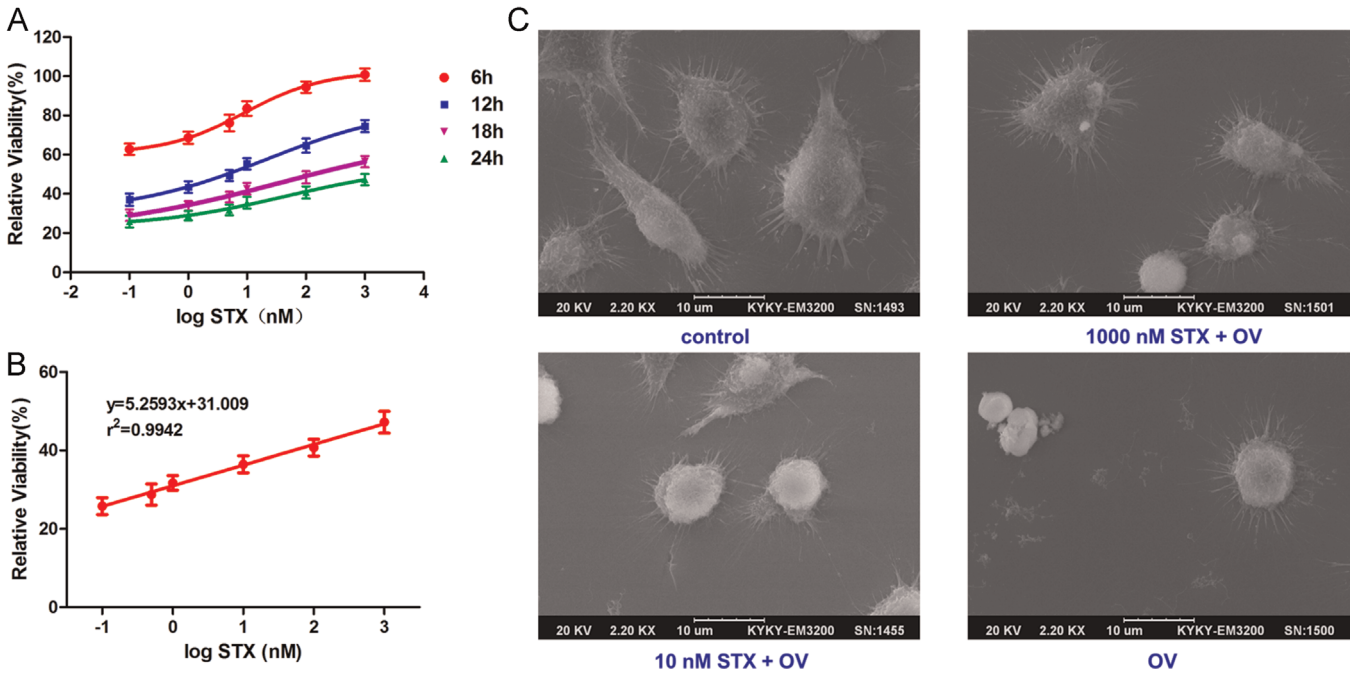

Fig. 4. (A) Dose–response curve of neuro-2a cells exposed at 6, 12, 18 and 24 h to STX with OV treatment. (B) Relationship between cell viability and log concentration of STX standard solutions in the presence of OV for 24 h. (C) Morphological changes of neuro-2a cells observed by SEM at 24 h after treatment with several doses of STX and OV treated groups and the control group.

derived from the cell growth results after toxin addition in Fig. 3 (B). These curves were the cell relative viability *versus* the log concentration of STX, and the cell relative viability was calculated by the following equation: Relative viability (%) = $CI_{y\_t}/CI_{x\_t} \times 100\%$, where CI_{y_t} represents the CI value co-treated with different concentrations of STX and OV or OV alone at given time point *t*, and CI_{x_t} means the CI value in the control well at the same time point. At each point, the change in cell viability induced by OV and different concentrations of STX treatment was reduced with the increasing treatment duration. In all the experiments, the dose–response relationship between cell relative viability and STX can be observed. In this study, the CIB system was able to provide the real-time and dynamic information of cellular responses. As a result, the STX inhibition of neuro-2a cell death can be detected as early as 6 h after treatment. There was a linear relationship between cell viability and the log concentration of standard STX solutions in the presence of OV at 24 h (Fig. 4(B)).

Figs. 4(C) shows the typical SEM results obtained at 24 h after treatment, which displayed the distinct morphological changes on the neuro-2a cells. The cells in control group were big and spindle-shaped or polygonal. Cells became round and shrinking in size at 24 h after OV and STX exposure, and the size became smaller.

3.3. Specificity test of cell-based biosensor

In order to evaluate the specificity of this improved cell-based assay in PSP toxins detection, the changes of CI value induced by other marine toxins and shellfish extracts co-treatment with 0.5 mM ouabain and 0.05 mM veratridine (OV) at 24 h were recorded by CIB system. Okadaic acid (OA) is the main constituent of diarrhetic shellfish poisoning (DSP) toxins, which inhibits the activity of phosphatase but had no effect on the sodium channel (Bialojan and Takai, 1988; Rajesh et al., 1999). Brevetoxin (PbTx-2) is a kind of neurologic shellfish poisoning (NSP) toxins which induces the enhancement of sodium inflow into the cell. However, the effects are different for the binding sites of PbTx-2 and saxitoxin (STX) on the sodium channel (Campas et al., 2007). The shellfish extracts did not contain PSP toxins which have been validated by HPLC and mouse bioassay (Fig. S5). The concentration of STX, OA and PbTx-2 applied in this specificity assay was 10 nM. From the specificity result shown in Fig. 5(A) and (B), it can be found that the CI value of STX was significantly higher than that of other group. And the CI values of other groups were barely higher than that of OV group. It was confirmed that the antagonism effect of OV can be only found in STX, which induced to higher CI value. And the result of shellfish extract group demonstrated the cytotoxicity effect of shellfish extracts can be negligible. Therefore, it can be concluded that the developed impedance biosensor presented a good specificity for PSP toxins detection.

3.4. Detection of saxitoxin (STX) in spiked mussel extracts

To demonstrate the practicality and accuracy of this improved method using biosensor, nontoxic shellfish extracts were spiked with known amounts of STX and analyzed at every toxic level in triplicate. Fig. 5(D) showed the relationship between the cell viability and toxins at different concentrations in spiked shellfish extracts, which was derived from Fig. 5(C). The equation of the calculation was $y = 5.53x + 28.63$, where y stands for relative viability, and x stands for log concentration of STX. The equation of calculation were obtained by the linear fitting of experimental data from -1 to -3 which was considered as the linear range of relative viability and log STX (nM) concentration. The detection limit was 0.09 nM (0.03 ng/ml), which was a theoretic value. It was calculated by the intersection point of the extrapolated linear region of the calibration curve with abscissa at the point of OV without STX (when $y = 22.83$). The detection limit of cell-based biosensor was about 0.03 ng/ml which was far below the regulatory limit of 800 ng/ml in shellfish. It is more sensitive than the MBA which is regarded as the official regulatory method in most of the world with the detection limit of 200 ng/ml (40 μg STX/100 g shellfish) (Botana, 2008; Etheridge, 2010; Cusick and Sayler, 2013). And the LOD of the AOAC HPLC official method for STX is about 1.2 ng/ml was also higher than that of cell-based biosensor (Rodrigues et al., 2012). The information of different cell-based assays was listed in Table 1.

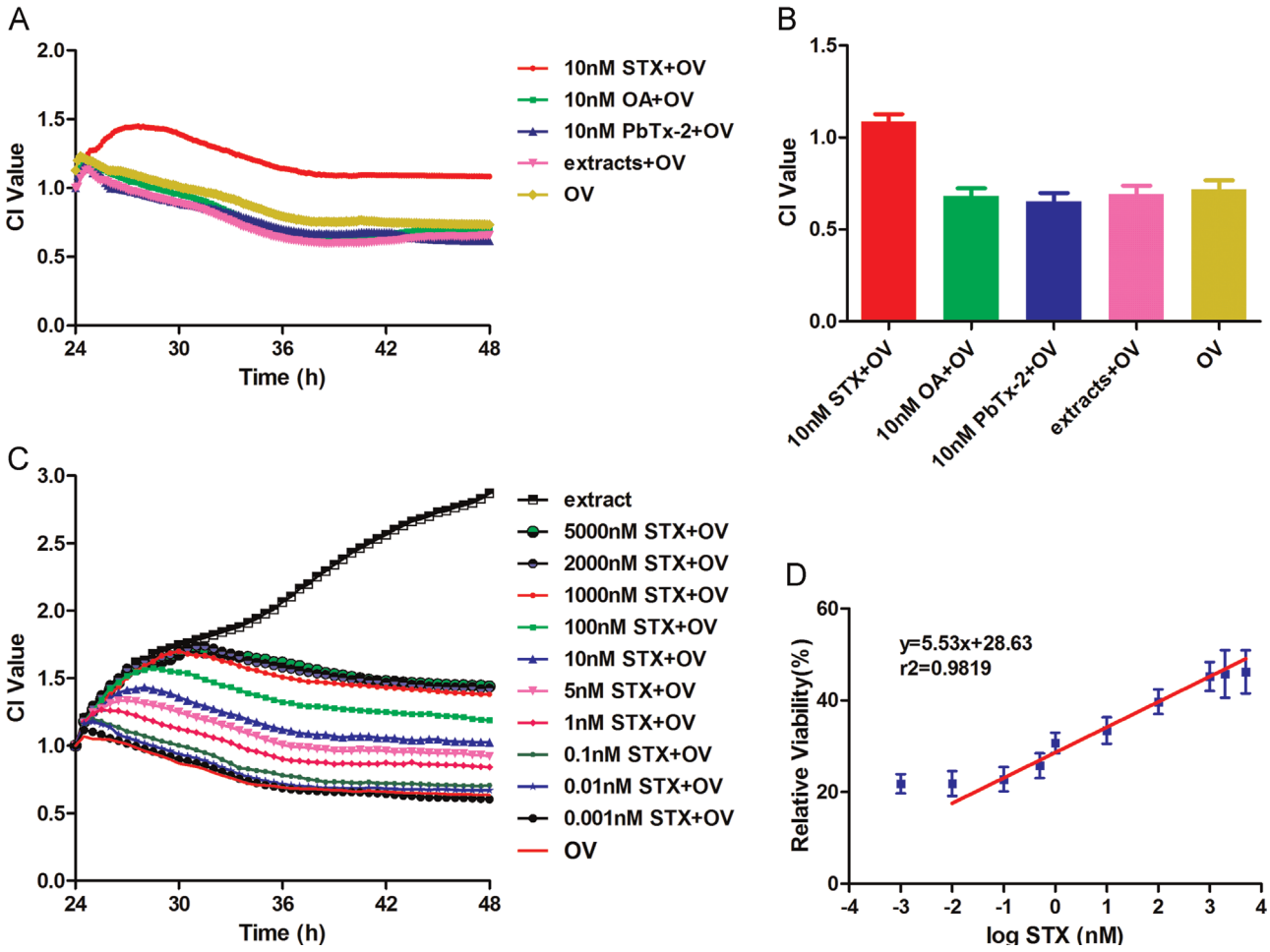

Fig. 5. (A) A group of normalized cell growth curves of neuro-2a cells responding to 10 nM STX, 10 nM OA, 10 nM PbTx-2 and shellfish extracts in the presence of 0.5 mM ouabain and 0.05 mM veratridine (OV) for 24 h. (B) Comparison of the cell viability measured by CIB system after treated with 10 nM STX, 10 nM OA, 10 nM PbTx-2 and shellfish extracts in the presence of OV for 24 h. (C) A group of normalized cell growth curves of neuro-2a cells responding to STX in spiked extracts co-treated with OV for 24 h. (D) Relationship between cell relative viability and log concentration of STX in spiked extracts after co-treated with OV for 24 h.

Table 1
Comparison of different cell-based assays for the detection of PSP toxins.

Detection mode	Detection reagent	Information	Protocol	Cost	Detection limit (ng/ml)	Reference
Fluorescence	Bis-oxonol	Endpoint	Complicated	High	< 0.1	Louzao et al. (2003)
Fluorescence	Rhodamine	Endpoint	Complicated	High	0.5	Nicholson et al. (2002)
Colorimetry	WST, MTT	Endpoint	Complicated	Low	0.14	Okumura et al. (2005), Cañete and Diogène (2008)
Electrophysiology	No	Real-time and dynamic	Simple	High	0.21	Kulagina et al. (2004)
Electrical impedance	No	Real-time and dynamic	Simple	Low	0.03l	This work

In this study, we focused on establish a new biosensor platform with a high sensitivity for marine toxin detection, and 30,000 seeding number can satisfy experiment requirement, so we selected this seeding number. 30,000 was not the optimal quantity, but from the results of toxins detection, cell-based biosensor with 30,000 seeding number was suitable for STX detection with detection limit of 0.03 ng/ml, which was similar with the conventional cell-based assay (Nicholson et al., 2002; Louzao et al., 2003; Okumura et al., 2005). Seeding number between 30,000 and 40,000 will be explored to find an optimal quantity and build a cell-based biosensor with better performance in the following study.

4. Conclusions

In this study, we proposed a developed neuroblastoma cell-based impedance system for the detection of PSP toxins based on antagonism of voltage-gated sodium channel. This improved method has obvious advantages in PSP toxins primary screening by combining cell-based assay and real-time, noninvasive monitoring impedance technologies. Compared with previous cell-based methods, it not only simplified the procedure but also improved the sensitivity. Moreover, the real time performance of this method enables monitoring of cell viability before toxins detection, which guarantees the experimental accuracy. In addition, the application of this method reduces the number of animals required and obtains a higher sensitivity than the MBA method. Even though the analytical methods have recently been recommended as alternative methods to the MBA, there is still a lot of potential of functional assays in marine toxins detection, especially in the emerging toxins. Therefore, this neuro-2a cell-based impedance biosensor holds a promising application in PSP toxins detection.

Acknowledgements

This work was supported by Major International Cooperation Project of Natural Science Foundation of China (No. 61320106002), Marine Public Welfare Project of China (No. 201305010), and National Natural Science Foundation of China (No. 31228008).

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.09.005.

References

Ahmad, A., Moore, E.J., 2009. Anal. Lett. 42 (1), 1–28.
Arias, L.R., Perry, C.A., Yang, L., 2010. Biosens. Bioelectron. 25 (10), 2225–2231.
Asphahani, F., Zhang, M., 2007. Analyst 132 (9), 835–841.
Ben-Gigirey, B., Rodriguez-Velasco, M.L., Gago-Martinez, A., 2012. J. AOAC Int. 95 (1), 111–121.
Bialojan, C., Takai, A., 1988. J. Biochem. 256, 283–290.
Botana, L.M., 2008. Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection. CRC Press, Boca Raton, Florida, United States.
Cañete, E., Diogène, J., 2008. Toxicon 52 (4), 541–550.
Campas, M., Prieto-Simón, B., Marty, J.-L., 2007. Talanta 72 (3), 884–895.
Chen, J.-H., Yu, R.-C., Gao, Y., Kong, F.-Z., Wang, Y.-F., Zhang, Q.-C., Kang, Z.-J., Yan, T., Zhou, M.-J., 2013. Food Addit. Contam. A 30 (11), 1933–1945.
Cusick, K., Sayler, G.S., 2013. Mar. Drugs 11 (4), 991–1018.
Etheridge, S.M., 2010. Toxicon 56 (2), 108–122.
Flanagan, A., Callanan, K., Donlon, J., Palmer, R., Forde, A., Kane, M., 2001. Toxicon 39 (7), 1021–1027.
Hayashi, R., Saito, H., Okumura, M., Kondo, F., 2006. J. Agric. Food. Chem. 54 (2), 269–273.
Hu, N., Wang, T., Cao, J., Su, K., Zhou, J., Wu, J., Wang, P., 2013a. Sens. Actuators B: Chem. 185, 238–244.
Hu, N., Wu, C., Ha, D., Wang, T., Liu, Q., Wang, P., 2013b. Biosen. Bioelectron. 40 (1), 167–173.
Humpage, A., Magalhaes, V., Froscio, S., 2010. Anal. Bioanal. Chem. 397 (5), 1655–1671.
Keese, C.R., Bhawe, K., Wegener, J., Giaever, I., 2002. Biotechniques 33 (4), 842–851.
Kulagina, N.V., O'Shaughnessy, T.J., Ma, W., Ramsdell, J.S., Pancrazio, J.J., 2004. Pharmacological effects of the marine toxins, brevetoxin and saxitoxin, on murine frontal cortex neuronal networks. Toxicon 44 (6), 669–676.
Kogure, K., Tamplin, M.L., Simidu, U., Colwell, R.R., 1988. Toxicon 26 (2), 191–197.
Liu, Q., Yu, J., Xiao, L., Tang, J.C.O., Zhang, Y., Wang, P., Yang, M., 2009. Biosens. Bioelectron. 24 (5), 1305–1310.
Louzao, M.C., Vieytes, M.R., Cabado, A.G., de Sousa, Vieites Baptista, Botana, L.M., J. M., 2003. Chem. Res. Toxicol. 16 (4), 433–438.
Manger, R.L., Leja, L.S., Lee, S.Y., Hungerford, J.M., Kirkpatrick, M.A., Yasumoto, T., Wekell, M.M., 2003. J. AOAC Int. 86 (3), 540–543.
Meneely, J.P., Campbell, K., Greef, C., Lochhead, M.J., Elliott, C.T., 2013. Biosens. Bioelectron. 41, 691–697.
Nicholson, R.A., Li, G.H., Buenaventura, E., Graham, D., 2002. Toxicon 40 (6), 831–838.
Nicolas, J., Hendriksen, P.J., Gerssen, A., Bovee, T.F., Rietjens, I.M., 2014. Mol. Nutr. Food Res. 58 (1), 87–100.
Okaichi, T., 2004. Red Tides. Springer, Tokyo, Japan.
Okumura, M., Tsuzuki, H., Tomita, B.-I., 2005. Toxicon 46 (1), 93–98.
Rajesh, D., Schell, K., Verma, A., 1999. Mol. Pharmacol. 56 (3), 515–525.
Rodrigues, S.M., de Carvalho, M., Mestre, T., Ferreira, J.J., Coelho, M., Peralta, R., Vale, P., 2012. Toxicon 59 (5), 558–566.
Tiruppathi, C., Malik, A.B., Del Vecchio, P.J., Keese, C.R., Giaever, I., 1992. Proc. Natl. Acad. Sci. USA 89 (17), 7919–7923.
Turner, A.D., Hatfield, R.G., Rapkova, M., Higman, W., Algoet, M., Suarez-Isla, B.A., Cordova, M., Caceres, C., van de Riet, J., Gibbs, R., 2011. Anal. Bioanal. Chem. 399 (3), 1257–1270.
van Meerloo, J., Kaspers, G.J., Cloos, J., 2011. Cancer Cell Culture. Humana Press, New York City, United States, pp. 237–245.
Wang, T., Hu, N., Cao, J., Wu, J., Su, K., Wang, P., 2013. Biosens. Bioelectron. 49, 9–13.
Wiese, M., D'agostino, P.M., Mihali, T.K., Moffitt, M.C., Neilan, B.A., 2010. Mar. Drugs 8 (7), 2185–2211.
Yicong, W., Ping, W., Xuesong, Y., Gaoyan, Z., Huiqi, H., Weimin, Y., Xiaoxiang, Z., Jinghong, H., Dafu, C., 2001. Sens. Actuators B 80 (3), 215–221.
Zhou, J., Wu, C., Tu, J., Ling, Y., Hu, N., Zhang, Y., Su, K., Wang, P., 2013. Sens. Actuators A 199, 156–164.