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Smartphone-based portable biosensing system using cell viability biosensor for okadaic acid detection

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ABSTRACT

Okadaic acid (OA), as a diarrheic shellfish poisoning toxin, had wide distribution and frequent occurrence. Therefore, low-cost, high-throughput, wide-range and portable detection of OA was in high demand for food safety and environmental monitoring. In this study, a novel and portable smartphone-based system using cell viability biosensor (CVBS) was developed for label-free, non-invasive and long-term monitoring of cell viability. The variation of cell viability reflected the changes of cell morphology, cell count and cell proliferation indirectly. And this system applied the combination of image analysis and cell counting kit-8 assay (CCK-8) to monitor the reflection. The biosensing system chose HepG2 cells as sensing elements to build CVBS and used it in OA detection. Results showed this system could synchronously detect OA in 96 channels. And this biosensor presented a good performance to various OA concentrations, with a wide linear detection range (10–800 μg/L). Moreover, the point-in-time having best detection performance could be located by the traversal algorithm in the monitoring duration. Thus, this cell-based biosensor system provided a convenient and efficient approach in seafood safety testing such as OA screening.

1. Introduction

OA, as a kind of diarrheic shellfish poisoning toxin, is produced by some unicellular algae from plankton and benthic microalgae and accumulates in the digestive glands of shellfish [1]. As one inhibitors of serine/threonine protein phosphatases type 1 (PP1) and 2A (PP2A) [2], OA can cause some diarrheic symptoms including diarrhea, nausea, vomiting and abdominal pain [3]. Moreover, OA has been identified as a tumor promoter and has been proved that it had mutagenic and immunotoxin effects [4]. Currently, there are some biochemical methods including mouse bioassay (MBA) [5], pre-column oxidation liquid chromatography with fluorescence detection [6], high performance liquid chromatography-mass spectrometry [7,8], enzyme linked immunosorbent assay [9,10] and cell-based biosensors [11,12] for the detection of marine shellfish toxins. Thereinto, the research using cell-based biosensors in shellfish toxin detection is beginning to attract the people's attention due to their abilities of observing the impact of toxin to cells and the quantitative analysis to toxin. However, shellfish toxins such as OA have wide distribution and frequent occurrence. Hence, the field of shellfish toxin detection requires a low-cost, high-throughput, wide-range and portable biosensing system for generalization, while the above cell-based biosensors can hardly meet these requirements.

How to establish a cell-based biosensing system, which can achieve low-cost, high-throughput, wide-range and portable detection? It is a great choice to combine image analysis with smartphone-based application. For biomedical research, the image analysis is utilized in the construction of low-cost cell-based biosensor [13,14]. On the other hand, many biochemical analysis platforms or methods combined with smartphone appear one after another in point-of-care test domain due to the high integration of smartphone to processor and diverse sensors [15,16]. These smartphone-based applications distribute in different fields including smartphone-based microscopy [17,18], fluorescent imaging [19], imaging cytometry [20], electrocardiography [21,22], lateral

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flow assays [23,24], surface plasmon resonance-based sensing [25], electrochemical sensing [26,27], immunoassays [28–30], and other applications [31–33]. Also, the accessories of smartphone-based system appear one after another to improve detection performance [34–36].

In this study, a novel and portable smartphone-based system called CVBS system was developed in accordance with the combination of image analysis and smartphone-based application. Cooperating with CCK-8 kit that had no cytotoxicity, this biosensing system could achieve label-free, non-invasive and long-term monitoring of cell viability. Besides, HepG2 cell lines were chosen as sensing elements to specifically detect OA. This smartphone-based system presented the features of low cost, high throughput, wide detection range and portability in OA detection. Also, the cell culture process used in this system was more common than that of other cell-based biosensors, which could promote the generalization of this system in the field of shellfish toxin measurement. All the details will be discussed in the following sections.

2. Materials and methods

2.1. Reagent and setup

The 96-well polystyrene plates are bought from Thermo Fisher Scientific, Germany. HepG2 cell line is bought from American Type Culture Collection and all of the reagents of cell culture are purchased from Gibico, USA. DMEM medium (Dulbecco's modified eagle medium) with high glucose, fetal calf serum (FBS), 0.25% Trypsin–EDTA, and phosphate buffered saline (PBS) are obtained from Gibico, USA. Gonyautoxin2&3 (GTX2&3) and brevetoxin-2 (PbTx-2) are purchased from National Research Council (Canada). OA (Sigma, USA) stock solution is prepared in DMSO and filtered with 0.22 μm membrane filter unit (Millipore, USA). OA is diluted by DMEM medium with high glucose when it is used to treat with cells. CCK-8 kit is obtained from Shanghai 7 sea biotech Co., LTD., China. The morphological changes of cells are observed by a stereo microscope (Sharp Inc., Japan).

2.2. Action principle of CCK-8 kit

For label-free, non-invasive and long-term monitoring of cell viability, CCK-8 kit is chosen as the developer of system. Comparing with other detection kits of cell proliferation and cytotoxicity such as MTT, XTT and WST-1, CCK-8 has excellent stability, a wider detection range, higher sensitivity and is easy to use. More importantly, it has no cytotoxicity. Fig. 1A shows the action principle of CCK-8 kit. CCK-8 kit is based on water-soluble tetrazolium salt-8 (WST-8) [37]. And WST-8 can be reduced by dehydrogenases in cells to give a water-soluble formazan dye (WST-8 formazan) in the presence of electron mediator (1-Methoxy PMS). The WST-8 formazan is an orange colored product and reflects the living cell status. Hence, this system monitors the cell viability by the variation of orange intensity over time.

2.3. The portable smartphone-based biosensing system

This system consists of CVBS, illumination provider and smartphone. The CVBS includes living cells, microtiter plate (MTP) and CCK-8 kit (Fig. 1B). The basic structure design of illumination provider has been described elsewhere [38] and the contour structure has been upgraded according to industrial design in this study (Fig. 1C). The smartphone installed with homemade iOS APP – iPlate Monitor (designed by Swift 1.0 and Object-C in Xcode 7) undertakes the image acquisition, image analysis, data storage and transmission (Fig. 1D). The iPlate Monitor introduces cell viability index (CVI) and normalized cell viability index (NCVI) to evaluate cell viability monitoring and analyze the response of CVBS. CVI and NCVI are calculated by the equ1 and equ2, respectively.

$$CVI = B_{Blank} - B_{Test} \quad (1)$$

Where CVI, B_{Blank} and B_{Test} stand for cell viability index, blue channel value of blank MTP and blue channel value of CVBS, respectively.

$$NCVI = \frac{CVI_t}{CVI_0} \quad (2)$$

Where CVI_t and CVI_0 are the CVI at any point-in-time and the CVI at 0 h, respectively. To confirm the best detection point-in-time, iPlate Monitor applies the traversal method to perform linear fitting at all time-points of monitoring process. Afterwards, the point-in-time of fitting curve having best performance, which is the best detection point-in-time, is determined by the fitting goodness and sensitivity. The supplementary material (Fig. S1) shows the workflow of this APP and some actual smartphone screen picture during OA detection experiments.

2.4. Cell culture

HepG2 cell line is cultured in DMEM medium which includes 10% heat inactivated FBS and 0.5% antibiotic solution (10 mg/mL streptomycin and 1000 U/mL penicillin). Then it is incubated at 37 °C in humidified air with 5% CO_2 in an incubator (Thermo, USA). When the confluent cells reach 80%, 0.25%, Trypsin-EDTA is used to dislodge cells from the flask to 96-well plates.

2.5. Shellfish extracts preparation

Mytilus edulis are selected for actual sample testing and the non-toxin samples are purchased from the market. The preparation of shellfish extracts are carried out according to the protocol offered by Ledreux et al. [39].

2.6. Experiment setup

2.6.1. Cell viability monitoring of smartphone-based system

HepG2 cells are detached from the culture flask and 100 μL cell suspensions with different cell seeding densities are prepared. Then HepG2 are inoculated in the 96-well MTP at different seeding densities. After cell inoculation, HepG2 are cultured for 4 h to adhere. Then 10 μL of CCK-8 solution is added to each well of the MTP and the MTP with smartphone-based system are placed inside a humidified incubator (at 37 °C, 5% CO_2). Meanwhile, the detection system starts to monitor the cell viability curves.

2.6.2. Real-time monitoring of CVBS's response to OA

100 μL of HepG2 cell suspensions with the same cell seeding density are added onto the MTP to build HepG2-CVBS. Before exposure to toxins, the cells are cultured for 24 h to achieve cell adherence and cell status stability. Then the medium of treated groups is removed and replaced by 100 μL fresh medium and 10 μL different concentrations of OA. Subsequently, 10 μL of CCK-8 solution and the CVBS with the smartphone-based system are placed in the incubator (at 37 °C, 5% CO_2). Meanwhile, the detection system starts to monitor the responses of HepG2-CVBS to OA.

3. Results and discussion

3.1. Stability of image monitoring using the portable smartphone-based system

Stability was the core of real-time monitoring and pixel intensity was the initial output of system. The system captured image and calculated pixel intensity once each 2 min. As shown in Fig. 2,