Biosensors and Bioelectronics 72 (2015) 10–17

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An improved functional assay for rapid detection of marine toxins, saxitoxin and brevetoxin using a portable cardiomyocyte-based potential biosensor

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ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form
31 March 2015
Accepted 12 April 2015
Available online 13 April 2015

Keywords:
Saxitoxin (STX)
Brevetoxin (PbTX-2)
Cardiomyocyte-based potential biosensor
Multisite electrical activity of cardiomyocyte network
Extracelluar field potential (EFP) signals

ABSTRACT

Saxitoxin (STX) and brevetoxin (PbTX-2), which are produced by marine dinoflagellates, are highly-toxic marine toxins targeting separate sites of the α subunit of voltage-dependent sodium channels (VDSCs). In this work, a portable cardiomyocyte-based potential biosensor is designed for rapid detection of STX and PbTX-2. This potential biosensor is constructed by cardiomyocyte and microelectrode array (MEA) with a label-free and real-time wireless 8-channel recording system which can dynamically monitor the multisite electrical activity of cardiomyocyte network. The recording signal parameters, spike amplitude, firing rate and 50% of spike potential duration (SPD_{50}) extracted from extracelluar field potential (EFP) signals of the potential biosensor is analyzed to quantitatively evaluate toxicological risk of STX and PbTX-2. Firing rate of biosensor signals presents high sensitivity to STX with the detection limit of 0.35 ng/ml within 5 min. SPD_{50} shows high sensitivity to PbTX-2 with the detection limit of 1.55 ng/ml within 5 min. Based on the multi-parameter analysis, cardiomyocyte-based potential biosensor will be a promising tool for rapid detection of these two toxins.

1. Introduction

Marine toxins, mainly produced by microalgae, pose a serious threat to human health and environmental safety around the world. Numerous marine toxins, such as saxitoxin (STX) and brevetoxin (PbTX-2), have been demonstrated to disrupt sodium channel function, either by inhibition of sodium current through the channels, or by modifying the activation and inactivation gating processes (Al-Sabi et al., 2006; Gerssen et al., 2010). They are likely to be involved in some ion channel diseases, such as cardiovascular diseases and neurodegenerative diseases. STX and PbTX-2, which are produced by the marine dinoflagellates (Bottein Dechraoui and Ramsdell, 2003; Lefebvre et al., 2008) are highly toxic on the whole ecological system. Through food and water ingestion, human may experience exposure to these toxins and passively take them, resulting in fatal health problems (Assessment, 2005; Hinder et al., 2011; Vale et al., 2008). STX and PbTX-2 exert their biological effects through interactions with sites 1 and 5 of the α subunit of the voltage-dependent sodium channel (VDSC), respectively (Catterall and Gainer, 1985; Poli et al., 1986). VDSC is responsible for sodium current and depolarization phase of action potential in excitable cells, such as cardiomyocytes and neurons. STX is a potent and selective inhibitor of VDSC, which produces a blockade of action potentials (Lipkind and Fozzard, 1994), and may affect cardiac and neural electrophysiological activity. In contrast to STX, PbTX-2 is known as a VDSC activator, which triggers repetitive action potential discharges (Templeton et al., 1989), and leads action potential depression and complete blockade (Huang et al., 1984).

Mouse-bioassay (MBA) is a standard approach for detecting STX and PbTX-2. Despite this method is reliable for regulatory purposes and easy to operate, it is labor-intensive, insensitive and ethically problematic (Okumura et al., 2005). In order to overcome these limitations, alternative methods based on chemical (Oshima, 1995), immunological (Usleber et al., 2001) and cellular (Krishnan et al., 2001; Ruberu et al., 2003; Vilariño et al., 2010) methods have been employed. Among the commonly used analytical methods, liquid chromatography/mass spectroscopy (LC/MS) method has been more accepted in recent years due to its qualitative and

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http://dx.doi.org/10.1016/j.bios.2015.04.028
0956-5663/

quantitative analysis (Humpage et al., 2010). However, LC/MS needed expensive instrumentation and a skilled professional with detailed technical knowledge (Humpage et al., 2010). Immunoassays (Usleber et al., 2001), including radioimmunoassay and ELISA, have reasonably high specificity and sensitivity, but the specific monoclonal antibodies are relatively expensive and extremely difficult to obtain. Unlike analytical methods and immunoassays, functional cell-based assays, which respond to biologically active analytes rather than particular anticipated chemical structures, attract more and more attention recently. Many cell lines have been used to assess the toxic effects of STX and PbTX-2, such as N2A neuroblastoma cells expressing neuronal VDSCs (Manger et al., 1994) and human embryonic kidney cells (HEK cells) transfected with heart or skeletal muscle VDSCs (David et al., 2003; Fairey et al., 2001). These cell-based assays have used endpoints for cytotoxicity, which lose much dynamic and real-time information in toxin action.

Cell-based biosensor, as a new functional cell-based assay, can detect various environmental toxins by non-invasively monitoring physiological parameter changes of the cellular internal and external environment in dynamic and real-time way with high sensitivity and excellent selectivity (Gavello et al., 2012; Liu et al., 2007). Previous work in our group has established two type of cell-based impedance biosensors using cardiomyocyte and neuroblastoma cell for dynamically detection of STX with high sensitivity (Wang et al., 2015; Zou et al., 2015). However, both cell-based impedance biosensors for STX detection were time-consuming at least 24 h after toxin addition due to the detection cellular physiological parameters (cell growth or cardiomyocyte beating). Some details about both biosensors are described in Supplementary materials.

In this study, a portable cardiomyocyte-based potential biosensor is designed with a wearable wireless 8-channel system, which was established by cardiomyocytes and microelectrode array (MEA). Cardiomyocytes grown on MEA have emerged as potential biosensors for rapid detection of ion channel compounds (Cerignoli et al., 2012; Jahnke et al., 2013; Yeung et al., 2009). Theoretically, cardiomyocyte-based potential biosensor can also rapidly detect the toxins that affect the Na^+ channels, such as STX and PbTX-2. Furthermore, the regular extracellular field potential (EFP) signals of cardiomyocyte-based potential biosensors can be also used to quantitatively analyze toxicity. Detailed work will be described in the following sections.

2. Experimental and methods

2.1. Construction of cardiomyocyte-based potential biosensor

To establish the portable cardiomyocyte-based potential biosensors, cardiomyocytes and nano-platinum electroplated MEA are employed (Fig. 1(A)), and the portable wireless 8-channel MEA system is used for noninvasively monitoring electrical activity of cardiomyocytes. Fig. 1(B) illustrates the schematic of cardiomyocyte-based potential biosensor. Cardiomyocytes are cultured on the surface of MEA which is modified with self-assembled monolayer (SAM). When cell–electrode coupling forms, there still exists a nanoscale gap between them filled with trace amount of electrolyte. For electrogenic cells in excitation phase, transient transmembrane potential and ionic current generate and polarize the electrodes by reestablishing the charge distribution at electrode–electrolyte–cell interface. In that way, the electrodes record changed voltage as EFP. Generally, their components originate from the derivative of transmembrane potential and ionic flows including Na^+, Ca^{2+} and K^+ in different phases of action potential (Meyer et al., 2004).

EFP recording is performed by self-developed portable wireless 8-channel MEA system. The recording system is packaged into a 13 cm long, 10 cm wide, and 4 cm high plastic case which weighs about 400 g with battery packs installed (Fig. 1(D)). The recording system consists of three main parts: an 8-channel signal conditioning circuit that amplifiers and filters EFP signals from MEA chip, an embedded Wi-Fi module which integrates a Cortex-M3 microcontroller STM32F205 and a user interface to handle the data captured by STM32F205. Wi-Fi module acts as an access point, and PC or iPad device can wirelessly connect to the module

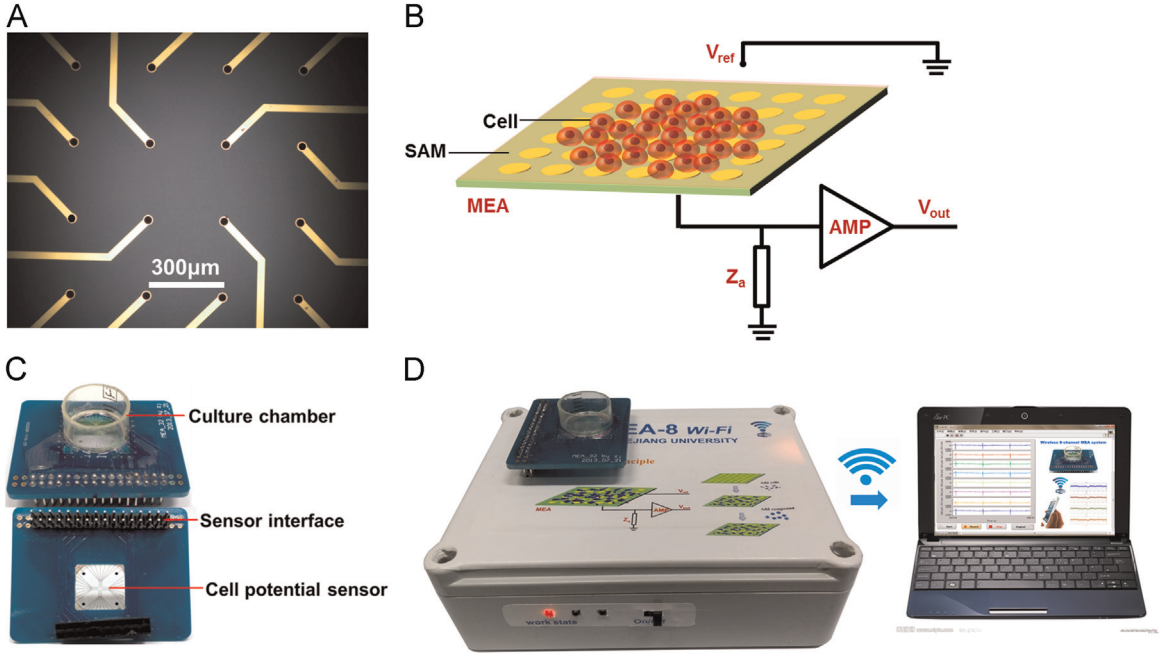

Fig. 1. Construction of the cardiomyocyte-based potential biosensor. (A) The MEA pattern with 32 channels. Electrode diameter is 30 μm and inter electrode distance is 300 μm. (B) The schematic of the cardiomyocyte-based potential biosensor. (C) The MEA sensor chip with a single culture chamber (inner diameter of 19 mm). (D) The wearable wireless 8-channel MEA system.