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A novel and functional assay for pharmacological effects of marine toxins, saxitoxin and tetrodotoxin by cardiomyocyte-based impedance biosensor

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ABSTRACT

Saxitoxin (STX) and tetrodotoxin (TTX) are highly toxic marine toxins targeting site 1 of α subunit of voltage-dependent sodium channels (VDSCs). Both of them disturb sodium channels' function by inhibition of ion current through the channels. And they are probably involved in some ion channel diseases, such as cardiovascular diseases and neurodegenerative diseases. The present work described a novel and functional method for detecting the pharmacological effects of STX and TTX using the cardiomyocyte-based biosensor. This biosensor was based on impedance technology through a label-free and real-time detection system which could monitor the cardiomyocyte growth and beating status simultaneously. The parameters of the cardiomyocyte-based biosensor, cell index, beating rate, and amplitude were analyzed to determine the biosensor performance under the treatment of toxins. The results showed that beating rate of this biosensor was a sensitive parameter to STX and TTX, and the detection limit of this biosensor was 0.087 and 89 ng/ml for STX and TTX, respectively. It could be concluded that the cardiomyocyte-based impedance biosensor would be a promising tool for quantitative analysis of the pharmacological effects of these two toxins.

1. Introduction

Marine toxins, mainly produced by microalgae, cause a serious threat to human health and environmental safety around the world. Both of saxitoxin (STX) and tetrodotoxin (TTX) are dangerous marine toxins. STX is the most typical paralytic shellfish poisoning (PSP) toxins in the research field of marine toxins, since it is discovered in 1957 [1]. STX is primarily produced by the eukaryotic dinoflagellates, belonging to the genera Alexandrium, Gymnodinium, and Pyrodinium [2–4]. Usually, STX is carried by filter-feeding bivalves such as butter clam, cherrystone clam, sea scallop, and mussel [5]. TTX is another typical neurotoxin which is believed to be expressed by several species of bacteria [6]. Transmitted through the food chain, TTX-producing bacteria can be found in the organs of a variety of marine and terrestrial animals such as fish, crab, octopus, frog, and newt [7,8]. Human obtain STX and TTX through food and water intake, and thus both of them seriously threaten public health. STX and TTX have shown similar properties of lethality, hypotensive activity, and cardiotoxicity in different animals [9]. They take the voltage-gated sodium channel (VDSC) protein as their sole molecular target and bind to specific sites on α subunit with high affinity [10–12]. VDSC is responsible for the sodium current and the depolarization phase of action potential in excitable cells, such as cardiomyocytes and neurons. STX and TTX exert their biological effects through interactions with site 1 of α subunit of the VDSC [13]. Both of them are potent and selective inhibitors of VDSCs and may affect cardiac action potential and cardiac beating rate.

The common method used to detect marine toxins is the mouse bioassay [14]. Although this method is reliable for regulatory purposes, it is labor-intensive, insensitive, and costly. Moreover, its major limitation is the controversial use of live animals, which is ethically problematic [15]. Several structure-based methods have emerged to overcome these limitations. For example, chromatography/mass spectroscopy (LC/MS) has high sensitivity and can be used to elucidate the chemical and structural properties

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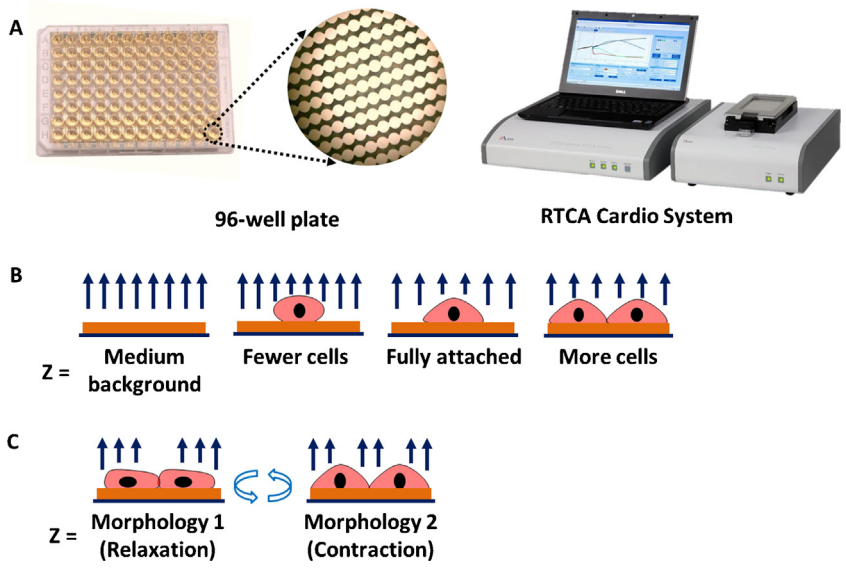

Fig. 1. Construction of the cardiomyocyte-based biosensor. (A) The 96-well sensor plate and the sensor detection system. The sensor plate is integrated with gold microelectrodes in the bottom of each well. (B) The impedance detection principle of cell growth. (C) The impedance detection principle of cardiomyocyte beating status. Beating signal is based on the rhythmic changes in cell attachment and morphology due to contraction and relaxation of cardiomyocytes, which modulates the impedance signal accordingly.

of new toxins [16]. However, LC/MS systems are expensive and require a skilled professional with detailed technical knowledge [16]. In recent years, function-based methods are being developed as alternatives to the mouse bioassay [17–19], which are based on the action mechanism of toxin in biological processes [17]. For example, conventional cell-based assays used endpoint cytotoxicity evaluation to achieve detection of PSP toxins [15,20]. Several cell lines have been employed as the sensitive elements, such as N2A

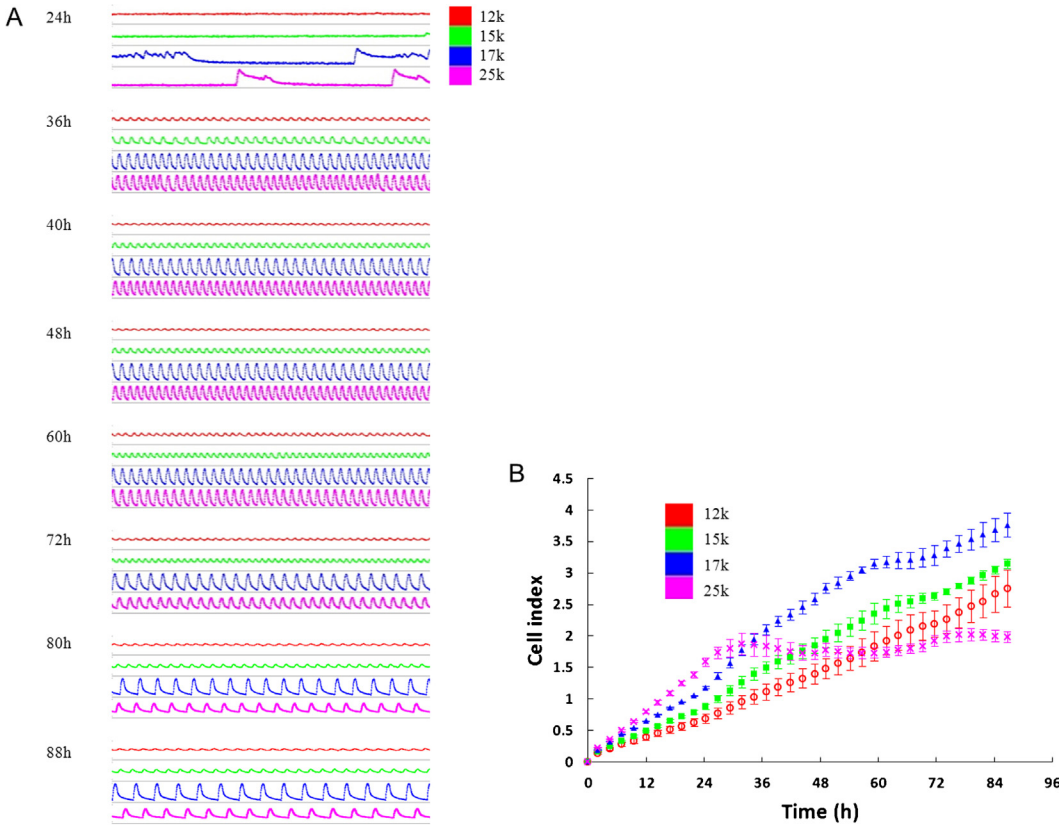

Fig. 2. Dynamic monitoring of attachment, growth, and beating activity of rat cardiomyocytes using the cell-based impedance biosensor. (A) Snapshots of representative beating profiles at selected time points under four different cell seeding densities. Each snapshot spanned duration of 20 s. (B) Cell index of 4-day continuous monitoring on cardiomyocytes of four different seeding densities.