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A cardiomyocyte-based biosensor for antiarrhythmic drug evaluation by simultaneously monitoring cell growth and beating

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ABSTRACT

Drug-induced cardiotoxicity greatly endangers the human health and results in resource waste. Also, it is a leading attribution to drug withdrawal and late-stage attrition in pharmaceutical industry. In the study, a dual function cardiomyocyte-based biosensor was introduced for rapid drug evaluation with xCELLigence RTCA Cardio system. The cardiomyocyte-based biosensor can monitor the cardiomyocyte growth and beating status simultaneously under the drug effects. Two typical cardiovascular drug, verapamil and flecainide were selected as treatment agents to test the performance of this biosensor. The experiment results showed that the performance of cardiomyocyte-based biosensor verified the basic drug effects by beating status and also tested the drug cytotoxicity by the cell index curves of cardiomyocyte growth. Based on the advanced sensor detection technology and cell culture technology, this cardiomyocyte-based biosensor will be a utility platform for the drug preclinical assessment.

1. Introduction

Early and effective drug screening methods are demanded in the field of biotechnology and pharmaceutical industry for predicting drug-induced cardiotoxicity and reducing late-stage drug attrition (Kim et al., 2011; Natarajan et al., 2011; Xiao et al., 2010). Cardiotoxicity accounts for about one third of safety-based withdrawn pharmaceuticals (Lawrence et al., 2008). Earlier and broader screening is a validated approach to improve cardiovascular safety as demonstrated with human Ether-a-go-go-related gene (hERG) screening to reduce drug-induced arrhythmia (Braam et al., 2010; Giorgi et al., 2010; Moss and Kass, 2005). An urgent need was demanded for the novel *in vitro* assays to address other mechanism researches of cardiovascular function, including contractility, heart rate, toxicity, hypertrophy, and non hERG arrhythmia. Therefore, advanced *in vitro* systems for predicting drug-induced cardiotoxicity are meaningful in the pharmaceutical and biotechnology industries to decrease late-stage drug attrition.

Cell-based biosensor takes cells as the sensitive component to sense the physiological microenvironment, which is also an effective approach to achieve the drug preclinical assessing (Corcoran and Rechnitz, 1985; Pancrazio et al., 1999; Stenger et al., 2001; Ziegler, 2000). Compared to *in vivo* animal method, *in vitro* cell-based biosensor method can reflect the pharmacological effects sensitively in a shorter term. Electrical cell-substrate impedance sensing (ECIS) was common cell-based biosensor technology, which was first proposed by (Giaever and Keese, 1984). In the last two decades, ECIS had been successfully applied to a number of biological assays, such as cell growth, cell proliferation, cell migration and invasion, cytotoxicity (Keese et al., 2002; Luong et al., 2001; Opp et al., 2009; Xiao et al., 2002). Real time cell analysis (RTCA) is also the xCELLigence RTCA Cardio system developed by ACEA and Roche. The temporal resolution of RTCA was 12.9 ms for each well of 16-well sensor or 96-well sensor with the multiple A/D convertors. There are other similar ECIS detection system, such as Bionas 9600 adcon reader (Ceriotti et al., 2007; Thakur et al., 2012) and automated multi-well setup (Wegener et al., 2004), which can both detect the multi-well cell electrical impedance simultaneously. Bionas 9600 adcon reader has total scanning of the 96-well plate in less than 15 s. And the actual automated multi-well setup has a temporal resolution of 12 min in a 12-well sensor. The temporal resolution was most important for recording the relatively intact cardiomyocytes beating signals. However, both of them have a low temporal resolution that hampers to monitor the rapid changing impedance signals, such as cardiomyocyte beating signals. RTCA technology is an advanced ECIS technology that significantly explores the high sample rate and high throughput detection (Kammermann et al., 2011; Said and Aykut, 2011; Smout et al., 2009). Therefore, it can record the rapid changing impedance signals and facilitate to monitor the cardiomyocytes beating status. RTCA technology provides an efficient approach to establish a high performance cardiomyocyte-based biosensor.

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In the study, we employed the primary neonatal rats' cardiomyocytes to build a cardiomyocyte-based biosensor and applied the RTCA technology to monitoring the status of cardiomyocyte growth and beating simultaneously in the short term and in the long term. Cardiovascular drugs were used to test the performance of cardiomyocyte-based biosensor. The methods and experimental results are detailed in the following section.

2. Experimental and methods

2.1. Detection principle of a dual function cardiomyocyte-based biosensor

Cardiomyocyte-based biosensor applied the real time cell analysis (RTCA) technology to record the impedance of interdigitated electrodes (IDEs) to noninvasively quantify cardiomyocytes status in real time. A low voltage alternating current (AC) signal leads to the generation of an electric field between the IDEs, which interacts with the ionic environment of culture medium in the wells. Based on the IDEs, the electric field signals are differentially modulated by the number of cells covering the electrodes, the strength of cell attachment, and the morphology of cells, etc. (Fig. 1(a)) Beyond that, the biosensor can also monitor the cell morphology and adhesion that are induced by the rhythmic modulation of cardiomyocytes contraction and relaxation.(Fig. 1(b)). In these two stages, the cell morphology, cell–cell adhesion and cell-substrate adhesion are different, which induce the impedance change.

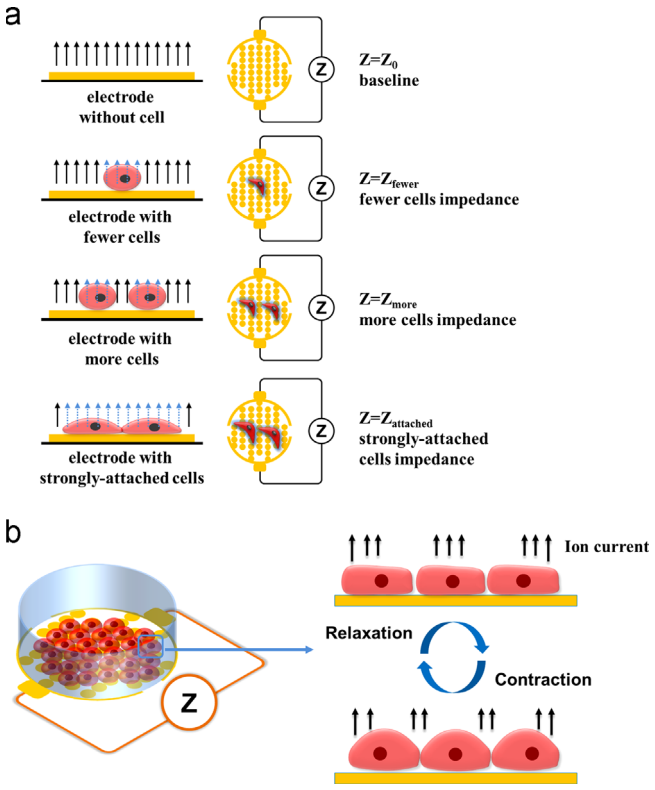

Fig. 1. Detection principle of a dual function cardiomyocyte-based biosensor. (a) The impedance signal Z is generated by the application of AC signal, which creates an ion current between the electrodes. The interaction of cells with the electrodes blocks the current and generates the impedance signal Z, which is proportional to the number of cells covering the electrode and the morphologic and adhesive characteristics of cells. The impedance signal represents in the form of cell index, which is a ratio of the change in impedance to background impedance. (b) The cardiomyocytes beating signal is based on the rhythmic changes of cell attachment and morphology due to contraction and relaxation of cardiomyocytes, which induces the fluctuation of impedance signal. (Reprinted with permission from website of ACEA Bioscience Inc.).

The instrument we used was the xCELLigence RTCA Cardio system, and it was developed by ACEA and Roche. The biosensor plate contains microtiter wells, which are integrated with gold microelectrodes in the bottom (Fig. 2). An AC sinusoidal signal (20 mV RMS @10 kHz) is applied onto the sensors, and the ion current signals were amplified and filtered before the A/D conversion. Finally, impedance signal Z value can be calculated by the original data. An AC voltage of 20 mV RMS is applied onto the each well including the lead rather than IDEs, so the IDEs lead impedance Rs on the sensor chip cannot be ignored, typically, 30Ω–80 Ω, and the well impedance Rw was 15 Ω. The 3–6 mV RMS is applied onto the IDEs. This voltage range was also tested for determining the non-invasive measurement. The AC voltage was applied onto some wells in the whole experiment, while AC voltage was applied onto other wells once every 1 h. The cardiomyocytes growth and beating was similar between the two groups. Therefore, the AC voltage range is available for non-invasive measurements. In the xCELLigence RTCA Cardio system, MCU can operate system simultaneously and continuously to measure and calculate the impedance signals. RTCA system measures impedance signal, processes and calculates the data by converting impedance value into a cell index (CI) value. CI is an arbitrary unit which is a ratio $(Z_X–Z_0)/Z_0$ of the well impedance change $Z_X–Z_0$ to well background impedance Z_0. CI value is influenced by a complex mixture factors such as cell growth, proliferation, cell–cell contact and cell-substrate adhesion. Therefore, it can be used to reflect cell viability, number, morphology, and adhesion degree in the cell-based impedance assay. The RTCA system has a multi-well fast data acquisition rate, which allows high temporal resolution for recording cardiomyocytes rhythmic beating. Therefore, impedance sensor detection technology can also be applied for monitoring cardiomyocytes beating status. Since impedance measurement is non-invasive, the millisecond data acquisition rate can be combined with longer-term monitoring to study both the short-term and long-term compound effects on cardiomyocytes growth and beating.

2.2. Cell culture procedure

The sensor plate was coated with 0.1% gelatin overnight in 4 °C refrigerator. Rats are sterilized by 75% alcohol. The rat chest wall was cut and heart was isolate by scissors. Hearts from neonatal rats should be rapidly excised, rinsed in ice-cold Dulbecco's modified Eagle medium (DMEM), and washed to remove blood and debris. Atriums were removed from the isolated hearts. Ventricles are left

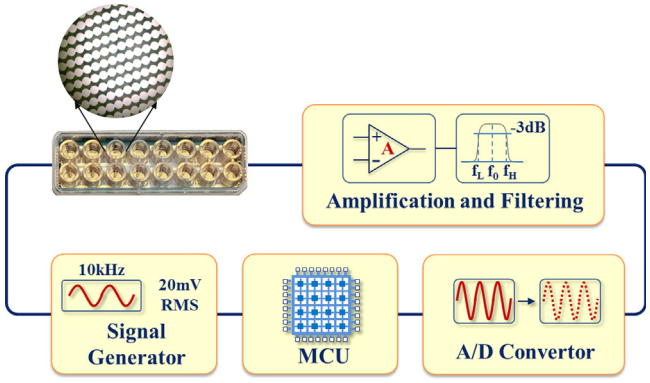

Fig. 2. The schematic of RTCA detection system including signal generator, amplification and filtering, MCU, and AD Convertor module. Due to fast data acquisition rate of the RTCA system, the cardiomyocytes beating can be monitored with high temporal resolution.