mES Cell-derived Cardiomyocytes Characterization using the xCELLigence RTCA HT System

ABSTRACT (TRIF5)
Cardio toxicity, a drug-induced adverse effect, accounts for most drug recalls and delays in gaining regulatory approvals. The xCELLigence RTCA HT system has revolutionized this area by providing real-time measurements of compound effects on cell properties, allowing a new approach to toxicity testing. This article provides an introduction to the system and its use for cardiomyocyte characterization.

INTRODUCTION
Functional assay on cells could be very complicated due to the variations of compound concentrations and combinations, and it requires strict control of timing before and after compound addition. To analyze the characterization assay of ES cell-derived cardiomyocytes, we integrated the xCELLigence RTCA HT system on a Biomek FX workstation.

Biomek® FX Dual Arm System with Span-6 and Multi-Channel Pipetter

The Biomek FX Dual Arm System with Span-6 and Multi-Channel Pipette (Figure 1) is a dual-port automated liquid handling system with a Spain-6 configuration featuring independent well access as well as for horizontal operation, and a 6- and 96-well fiducialized reagent head which are easily stackable for the maximum flexibility and system expandability. The footprint built into the multidwell pipette provides fast and reliable labware movement around the deck, as well as the capability to do 24- and 96-well plates, routine manual multistep assembly and access integrated device.

RTCA HT System, integrated on Biomek FX®

Prior to seeding of 40,000 cells per well into the 384-E-Plates, 20 μl of media was depopulated, centrifuged and reseed on RTCA in background. The cells were allowed to attach and proliferation overnight. On the second day, a compound plate was generated with a serial dilution step. A concentrated sample step on shows the end values of signals to be automatically calculated based on the number of compounds and replicates, and updated in the Real-Time Volume to calculate target preparations (Figure 3). The serially diluted compounds were then simultaneously transferred using a 384-wide channel head to 384-E-Plates, where cells were attached after overnight incubation, and the remaining scheduled steps on RTCA software were then utilized for real-time monitoring of the cells' response to various concentrations of compounds.

RTCA HT setup

The background readings (media only) were set to zero at 1 mm intervals for 3 min before adding cells. The values at the time points right after adding the compounds were set as 1 and used to normalize the following dose responses which were read at 60 μm at 15 seconds intervals. Dose response curves were plotted using DRC software (6τ as time point vs. concentration) and significative Dose-Response function, and the IC50/IC30 were calculated and using the RTCA software as well.

ASSAY PROTOCOL & BIOMEK FX® WORKSTATION SETUP

Cardiomyocytes preparation

Mouse embryonic stem cells (from heterogeneous) were maintained on gelatin coated 11.5 mm diameter coverslips, pre-coated with fibronectin (10 μg/ml of albumin-coated, or all medium (DMEM/F12). Briefly, cells were cultured in media containing 17% FBS without I2F and placed into a 384-well round-bottom polystyrene plate in 40 μl, at a density of 500 cells per well for two days before being treated with or without compounds (10 μl of media). The embryos homozygous for five days after plating were grown harvested and resuspended to a plate-coated 96 wells plate. Two days later, a portion of the adherent cells were viable contractions. The different dilutions were then harvested and placed into a 384-well plate using a Biomek workstation of a density of 15,000 cells and a volume of 40 μl. After overnight incubation at 37°C, cells were treated with or without compound(s) at 1:7 dilution and signals were transferred and analyzed on RTCA HT analysis.

Clonal mouse cardiomyocytes (from C57BL/6J) were primary cultured cells derived from mouse neonatal heart. Cells were maintained and sub-cultured every 20 to 48 hours on mouse cardiomyocytes extracellular matrix before analysis. Cells were harvested and plated in a 384-well plate at 40,000 cells per 40 μl well for the day before compound treatment and results were recorded and analyzed using RTCA HT system. Data were compared with mouse ES cells derived cardiomyocytes.

Biomek® FX® Workstation setup and assay protocol

Cell seeding and the addition of serially diluted compound were automated on a Biomek FX® automation workstation with dual 48-well multidish pipetting head and a Spain-6 and 96-head. It is a step-down procedure with the 1st day of cell seeding and overnight incubation, and the 2nd day of compound additions and assay (Figure 2). The method provides options to run the two procedures separately or continuously with the option to start from any column on the plates with the desired number of compounds and replicates in the Used Linear step (Figure 3, left).

RESULTS

Data from cloning mCardiomyocytes

As shown in Figure 4, mouse cardiomyocytes were density stained from 50,000 to 60,000 cells per well and stimulated with 10 μl of dobutamine (10−6 compound) after overnight seeding. The wells containing 40,000 cells gave the highest rounding 1 (Figure 1). The same dilution stimulation curves were obtained and visualized after dobutamine stimulation (B). From this figure, 40,000 cells per well were used for the following assay for mouse cardiomyocytes.

Figure 4: Results of mCardiomyocytes density stained from 50,000 to 60,000 cells per well with 10 μl of dobutamine (10−6 compound) after overnight seeding. The wells containing 40,000 cells gave the highest rounding 1 (A). The same dilution stimulation curves were obtained and visualized after dobutamine stimulation (B).

Data from mES-Cell-derived cardiomyocytes

As shown in Figure 6, dobutamine induced dose-dependent responses in both ES-Cell-derived cardiomyocytes differentiated in the presence of stem cell, as well as in ES cells differentiated in the absence of stem cell; the differentiated cells in the control group. These results could indicate that increasing the number of cardiomyocytes in the acellular and conditioned medium is consistent with flow cytometry data of similar cells (refer to Michael Kowalski’s paper [97]). Differentiated ES cells for Figure 6. showed dose-dependent response to dobutamine.

Figure 6: Dose response curves (a) 1 μM-10 μM, (b) 0.1 μM-0.5 μM, (c) 0.01 μM-0.05 μM, (d) 0.001 μM-0.005 μM. (e) 0.0001 μM-0.0005 μM and (f) 0 μM. A stimulated 40,000 cells ctrl. and 50% ± 15% of IC50/IC30 were calculated (IC50/IC30 = mean fold induction above the control -1.00 = value at the mean fold induction above the control -1.00 ± 1.00). Differentiation ES cells for Figure 6. showed dose-dependent response to dobutamine.

SUMMARY

• The RTCA HT system was successfully integrated on Biomek FX Dual Arm System with Span-6 and Multi-Channel Pipette and functional assays on mouse cardiomyocytes were automated for cell treatments with various compounds at serially diluted concentrations.

• Cardiomyocytes characterization for both primary cultured mouse cardiomyocytes and mouse ES cells-derived cardiomyocytes were successfully performed on the integrated system. Comparison data were obtained and dose response curves were generated with RTCA HT software.

Note: *Method cited Not for Clinical purposes. ** All trademarks are property of their respective owners.