High-Throughput Fluorescence Measurements of Ca²⁺ Transients in Human iPSC-derived Cardiomyocytes: Detection of Beating-Rate Dependence through Electric Field Stimulation

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Introduction

High-throughput measurement of Ca²⁺ transients in human iPSC-derived cardiomyocytes is expected to be a novel useful method to evaluate drug compound-induced cardiotoxicity in vitro. FDSS/iCELL (Hamamatsu) is an imaging microplate reader for cell-based kinetic assays that measures fluorescence/luminescence signals of all 968944 wells in a microplate simultaneously with up to 9 ms time interval and has been widely used for measurements of intracellular Ca²⁺ concentration changes in drug discovery fields. Recently, we developed a 96-channel electrode array that is mounted on the FDSS/iCELL, which can add electric field stimulation (EFS) to all 96 wells of a microplate simultaneously.

Using this instrumental setup, we measured Ca²⁺ transients in human iPSC-cardiomyocytes with a calcium-sensitive fluorescent dye with 16 ms time interval under temperature control (37 °C), under spontaneous beating of cardiomyocytes or while EFS was added at various frequencies. We also examined effects of some ion channel blockers on the Ca²⁺ transients in cardiomyocytes under spontaneous beating and with EFS.

Materials & Methods

The human iPSC-derived cardiomyocytes (Cor.4U®, Axiogenesis AG, Cologne, Germany or iCell® Cardiomyocytes, Cellular Dynamics International, Madison, WI, USA) were cultured in 96-well microplates (Costar). A calcium-sensitive fluorescent dye, Cal-520, was loaded into cells with incubation of 2 μM Cal-520 AM (AAT Bioquest) and 1.25 mM probenecid (Sigma-Aldrich) for 1 h at 37 °C in 5% CO₂. The fluorescence images of all wells in a microplate were taken every 0.016 s to capture changes in intracellular Ca²⁺ concentration using FDSS/iCELL (Hamamatsu).

Result 1  Fluorescence measurements of Ca²⁺ transients in cardiomyocytes in a 96-well plate: Ca²⁺ transients were synchronized to the electric field stimulation (EFS) with high uniformity

Intracellular Ca²⁺ concentration changes (Ca²⁺ transients) in human iPSC-derived cardiomyocytes (Cor.4U® Cardiomyocytes, CDD) were fluorescently measured with a calcium-sensitive fluorescent dye in a 96-well plate under spontaneous beating and while 1.0 Hz of EFS was added. The above figure shows the intracellular Ca²⁺ concentration changes for 10 s in a 96-well microplate when 1.0 Hz of EFS was applied.

FDSS/iCELL

FDSS/iCELL is a kinetic plate reader with an integrated dispensing head and an imaging-based detector. Simultaneous dispensing into the entire 968944-well plates and simultaneous detection of the kinetics of the fluorescence or luminescence intensity allow quick measurements with no time lag for the 96/384-well format.

Electric stimulation of cardiomyocytes using the electrode array mounted on the FDSS/iCELL: the EFS system

The 96-channel electrode array (patent pending) is used in coupling with the FDSS/iCELL. The electric field stimulations (EFS) are given to all 96 wells in a microplate simultaneously while fluorescent signals of calcium-sensitive dyes are monitored.

Analysis of calcium waveform

The measured calcium waveforms (intracellular Ca²⁺ concentration changes) are analyzed using the FDSS Waveform Analysis Software for Cardiomyocytes (Hamamatsu). Using this software, you can estimate peak rate, peak width, time to peak, rise time, fall time, and more.

FDSS/iCELL EFS system should not be used for optically detecting change in transmembrane potential of the cells, and should not be used with the cells in which you/somebody expressed the target ion channels.

Result 2  Effects of ion channel blockers on Ca²⁺ transients: Beating-rate dependency

We examined effects of ion channel blockers, Verapamil and Flecainide, on Ca²⁺ transients in human iPSC-derived cardiomyocytes (Cor.4U®, Axiogenesis AG). Ca²⁺ transients were measured at various concentrations of ion channel blockers under the spontaneous beating (0.7 Hz) and with 1.0 or 2.0 Hz of EFS.

(1) Ca²⁺ channel blocker: Verapamil

Some differences in disturbance of Ca²⁺ transients between beating-rates were observed. The dose response curves on peak number/min (beating rate) and amplitude of the Ca²⁺ transients are shown.

(2) Na⁺ channel blocker: Flecainide

We thank Drs. Ralf Kettenhofen and Felix von Haniel (Axiogenesis AG) for their helpful support on experiments using cardiomyocytes (Cor.4U®).

Summary

- We measured Ca²⁺ transients in human iPSC-derived cardiomyocytes with a calcium-sensitive fluorescent dye while electric field stimulations (EFS) were added, in the high-throughput measurement format using the FDSS/iCELL-EFS system.
- Using this instrumental setup, we examined effects of some ion channel blockers on the Ca²⁺ transients in cardiomyocytes under spontaneous beating and with EFS at various frequencies. Some different dose-dependences of ion channel blockers on disturbance of Ca²⁺ transients were observed between different beating rates. The FDSS/iCELL-EFS system could be used to examine beating-rate dependent phenomena.

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