Monitor drug-induced calcium flux in iPSC-derived cardiomyocytes

Fluo-4 meets the EVOS FL Auto 2 Imaging System and Celleste Image Analysis Software.

Pluripotent stem cells (PSCs) have the ability to differentiate into any one of the different cell types in the human body and are an important research tool for disciplines such as developmental biology, regenerative medicine, and oncology, to name a few [1,2]. The discovery that a terminally differentiated cell such as a dermal fibroblast can be reverted back to a stem cell–like state has opened up the possibility of growing patient-specific tissue and organs that originate from a patient's own cells and therefore have less chance of rejection when transplanted [3,4]. Differentiation of induced pluripotent stem cells (iPSCs)—using specialized media with the appropriate growth and signaling factors—into complex cell culture models such as cardiomyocytes or dopaminergic neurons has opened up new avenues for basic research and translational medicine.

Role of calcium in cardiac action potentials

During an action potential, cardiomyocytes contract by membrane depolarization, which is caused by the rapid influx of calcium into the cell through voltage-gated calcium channels. Ion indicators that increase in fluorescence upon binding calcium, such as Invitrogen™ fluo-4 dye, are commonly used to measure cardiomyocyte action potentials and contraction rate. As calcium enters the cell, it binds to intracellular fluo-4, causing the fluorescence of the calcium indicator to increase. Likewise, the fluorescence of the indicator is reduced as intracellular calcium concentration decreases when calcium is pumped back out of the cell during the repolarization phase of the action potential [5-8].

Improper functioning of action potentials in cardiomyocytes can lead to a variety of human diseases such as hypertension. Drugs for treating high blood pressure, including the calcium channel blocker verapamil, act by helping to control cardiac action potentials. Furthermore, several compounds are produced by the body to increase the action potential rate in response to stimuli, such as the release of norepinephrine during the fight-or-flight response. Here we demonstrate a simple method for monitoring drug-induced changes in the rate of action potentials in human iPSC-derived cardiomyocytes.

Quantify calcium influx rate

To quantify the rate of calcium influx in iPSCs, we used the calcium indicator fluo-4 in a live-cell kinetic assay. Cell fluorescence was detected and analyzed on the Invitrogen™ EVOS™ FL Auto 2 Imaging System using Invitrogen™ Celleste™ Image Analysis Software. This live-cell imaging and analysis system enables quick and easy quantitative calcium imaging (Figure 1)—which is required when studying physiological responses to drugs in muscle cells, neurons, and other cell systems—without the need for specialized equipment or techniques.

The EVOS FL Auto 2 system was used to record videos of pulsing cardiomyocytes, allowing visualization of rapid calcium flux based on changes in the fluorescence intensity of the fluo-4 calcium indicator over time (Figure 1A). By simply selecting the "measure:intensity" tracking function after defining the region of

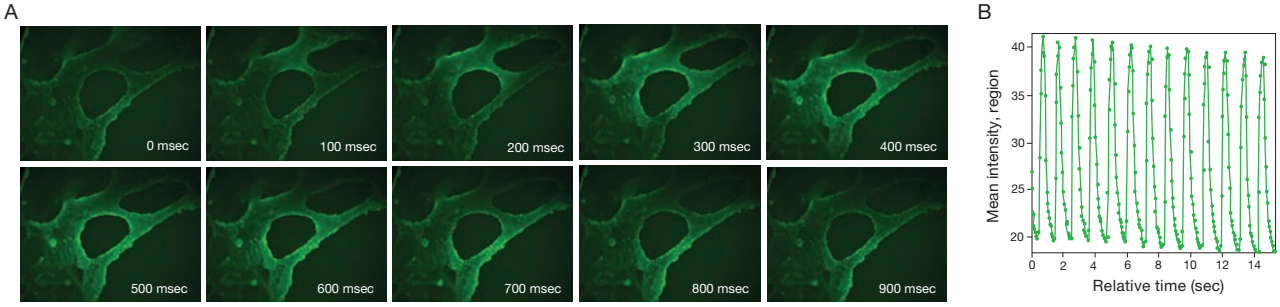

Figure 1. Time-lapse imaging and quantitation of cardiomyocyte pulse rate. (A) Time-lapse images (obtained from a video of contracting cardiomyocytes stained using the fluorescent fluo-4 calcium indicator provided in the Invitrogen™ Fluo-4 NW Calcium Assay Kit (Cat. No. F36206) and recorded using the Invitrogen™ EVOS™ FL Auto 2 Imaging System) show the flux of calcium moving across the cells over time. **(B)** Analysis of the video using the measure:intensity tracking function in Invitrogen™ Celleste™ Image Analysis Software shows that these cardiomyocytes have a pulse rate of approximately 1 beat per second.

interest in each video of contracting cardiomyocytes, Celleste software was used to generate a graph of the calcium flux and contraction rate based on fluorescence intensity changes observed (Figure 1B).

This function allowed quantitation of the baseline calcium flux and contraction rate in cardiomyocytes, along with changes in the pulse rate with different treatments. Application of 10 µM norepinephrine nearly doubled the rate of contraction compared with that seen in cardiomyocytes receiving no drug treatment (Figures 2A and 2B). Treatment with 50 nM verapamil reduced the rate of contraction to about half of the baseline cardiomyocyte contraction rate, whereas 1 µM verapamil prevented calcium flux, resulting in no contractions (Figures 2C and 2D).

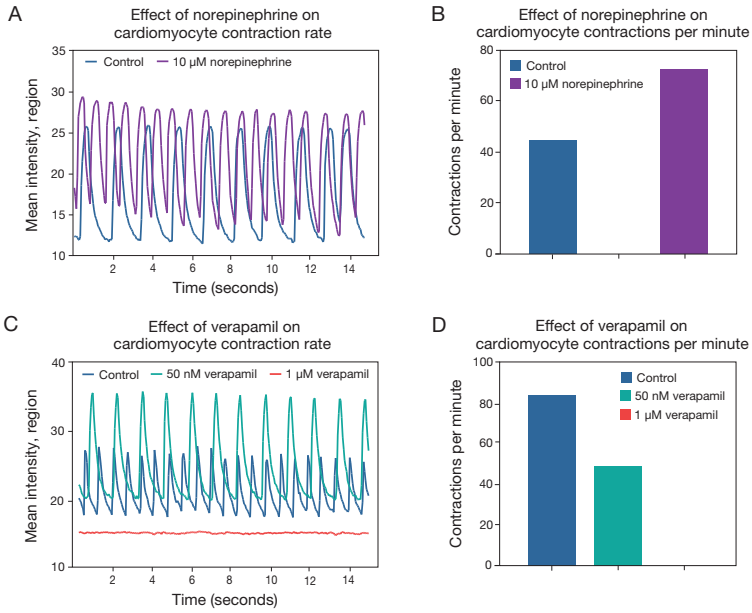

Figure 2. Effect of norepinephrine and verapamil on cardiomyocyte pulse rate. Overlays of intensity measurements of the defined region of interest were obtained using Celleste software for **(A)** control and 10 µM norepinephrine–treated cardiomyocytes and **(C)** control, 50 nM verapamil–treated, and 1 µM verapamil–treated cardiomyocytes. Quantitation of the calcium flux using fluo-4 shows that cardiomyocyte contraction rate is **(B)** nearly doubled with 10 µM norepinephrine and **(D)** reduced by about half with 50 nM verapamil or stopped completely with 1 µM verapamil, as compared with the baseline contraction rate with no drug treatment (control).

Fluorescence imaging in real time

Drug-induced changes in cardiomyocyte calcium flux, measured with the fluorescent calcium indicator fluo-4, can easily be imaged and quantified using the EVOS FL Auto 2 Imaging System and Celleste Image Analysis Software. Real-time fluorescence videos of calcium flux in pulsing cardiomyocytes are recorded by selecting the "record video" option on the EVOS FL Auto 2 system, and then changes in fluorescence intensity are quantified by defining a region of interest and using the "measure:intensity" tracking function with the Celleste software. This simple quantitation system can be applied to cell physiology and drug discovery assays using other fluorescent indicators. EVOS microscopes incorporate high-resolution cameras, bright and digitally controlled LED light cubes, and interfaces structured for usability that allow the acquisition of stunning, publication-quality images and data. Find out more about EVOS microscopes or request an in-lab demonstration at thermofisher.com/evos. ■

References

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Product	Quantity	Cat. No.
Invitrogen™ fluorescent ion indicator		
Fluo-4 NW Calcium Assay Kit	10 microplates 100 microplates	F36206 F36205
Gibco™ cells and media		
Essential 8™ Medium	500 mL	A1517001
Human Episomal iPSC Line	1 x 10^6 cells/vial	A18945
PSC Cardiomyocyte Differentiation Kit	1 kit	A2921201
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	1 mL 10 mL	A14700 A31804
Invitrogen™ fluorescence instrumentation		
EVOS™ FL Auto 2 Imaging System	1 system	AMAFD2000
EVOS™ 4x Objective, fluorite, LWD	1 each	AMEP4622
EVOS™ 10x Objective, fluorite, LWD	1 each	AMEP4623
EVOS™ Light Cube, GFP	1 each	AMEP4651
Celleste™ Image Analysis Software	1 each	AMEP4816