Monitoring drug-induced calcium flux in iPSC-derived cardiomyocytes

Introduction

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 tool for studying developmental biology and regenerative medicine [1,2]. The discovery of the ability to revert a terminally differentiated cell such as a dermal fibroblast back to a stem cell–like state has opened up the possibility of growing patient-specific tissue and organs that will originate from a patient's own cells and therefore not be rejected when transplanted [3,4]. Cells that have been reverted back to a pluripotent state in this manner are called induced PSCs (iPSCs). The ability of these cells to be sorted and differentiated into complex cell culture models, such as cardiomyocytes or dopaminergic neurons, using specialized differentiation media has made them an important research tool for cell biology discovery.

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

Improper function of the action potential in cardiomyocytes can lead to a variety of diseases in humans, such as high blood pressure. There are numerous drugs on the market, such as verapamil, which help control action potential in human diseases. Furthermore, several compounds are produced by the body to increase the rate of action potential in response to stimuli, such as the release of norepinephrine during the fight-or-flight response. Here we demonstrate a simple method to monitor drug-induced changes in the rate of action potentials in human iPSC–derived cardiomyocytes. This is achieved by quantifying the rate of calcium influx, measured by fluo-4 in a live-cell kinetic assay using the Invitrogen™ EVOS™ FL Auto 2 Imaging System and Celleste™ Image Analysis Software. This live-cell imaging and analysis enables quick and easy quantitative calcium imaging (Figure 1) that can be used in researching physiology and drug responses in muscle cells, neurons, and other cell systems, without the need for specialized equipment or techniques.

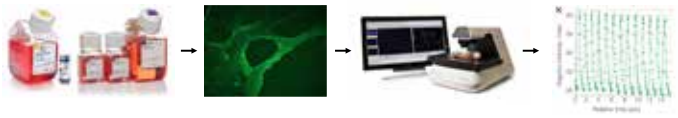

Figure 1. Simple workflow for monitoring drug-induced changes in cardiomyocyte contraction rate based on calcium flux.

Materials

- EVOS FL Auto 2 Imaging System (Cat. No. AMAFD2000)
- EVOS 10x Objective (Cat. No. AMEP4623)
- EVOS 4x Objective (Cat. No. AMEP4622)
- EVOS Light Cube, GFP (Cat. No. AMEP4651)
- Celleste Image Analysis Software (Cat. No. AMEP4816)
- Gibco™ PSC Cardiomyocyte Differentiation Kit (Cat. No. A2921201)
- Gibco™ Essential 8™ Medium (Cat. No. A1517001)
- Invitrogen™ Fluo-4 NW Calcium Assay Kit (Cat. No. F36206)
- Gibco™ Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A14700)
- Gibco™ Human Episomal iPSC Line (Cat. No. A18945)
- Acros Organics™ Verapamil HCl (Cat. No. 329330010)
- Sigma™ Norepinephrine, USP (Cat. No. N-5785)

Methods

iPSC culture and differentiation

Human iPSCs were cultured following the recommended protocol for Essential 8 Medium and were differentiated using the protocol for the PSC Cardiomyocyte Differentiation Kit to generate functional cardiomyocytes in 8 days.

Labeling and imaging

Mature cardiomyocytes were labeled with the Fluo-4 NW Calcium Assay Kit for 30 minutes under normal cell culture conditions. The labeling solution was then removed, and the cells were placed back in cardiomyocyte maintenance medium. Cardiomyocytes were then imaged on the EVOS FL Auto 2 Imaging System with an EVOS objective, either 4x or 10x, using the “Record video” function for 15 seconds to establish a baseline calcium flux rate. The cells were then treated with 50 nM verapamil, 1 µM verapamil, or 10 µM norepinephrine, and another video of the same field of view of the cardiomyocytes was recorded.

Quantification

Each video was analyzed with Celleste Image Analysis Software using the “measure:intensity” tracking function after creating a region of interest on a section of contracting cardiomyocytes. The region of interest was placed in the same area for the videos of the baseline sample and each of the drug-treated samples.

Results

The EVOS FL Auto 2 Imaging System was used to record videos of pulsing cardiomyocytes that allowed visualization of rapid calcium flux based on changes in fluorescence intensity of the fluo-4 calcium indicator over time (Figure 2,A–J). By simply selecting the “measure:intensity” tracking function after defining the region of interest in each video of contracting cardiomyocytes, Celleste software was used to generate a graph of the calcium flux and contraction rate based on the fluorescence intensity changes observed in the video (Figure 2K). This function allowed quantification of the baseline calcium flux and contraction rate in cardiomyocytes, along with quantification of changes in the pulse rate with different treatments. Application of 10 µM norepinephrine doubled the rate of contraction compared to cardiomyocytes with no drug treatment (Figure 3). Treatment with 50 nM verapamil reduced the rate of contraction by half compared to the baseline cardiomyocyte contraction rate, while 1 µM verapamil prevented the calcium flux, resulting in no contractions (Figure 4).

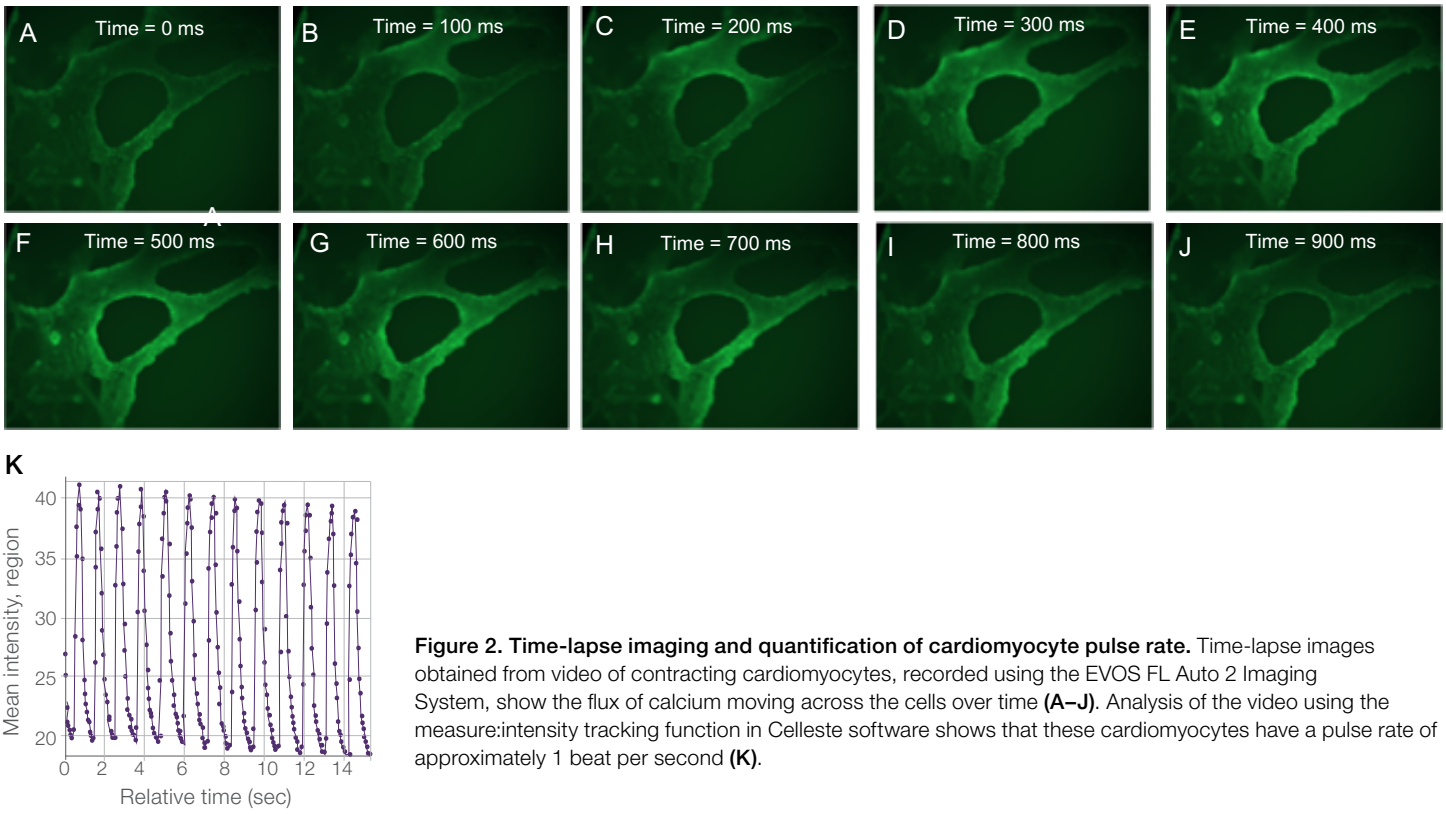

Figure 2. Time-lapse imaging and quantification of cardiomyocyte pulse rate. Time-lapse images obtained from video of contracting cardiomyocytes, recorded using the EVOS FL Auto 2 Imaging System, show the flux of calcium moving across the cells over time **(A–J)**. Analysis of the video using the measure:intensity tracking function in Celleste software shows that these cardiomyocytes have a pulse rate of approximately 1 beat per second **(K)**.

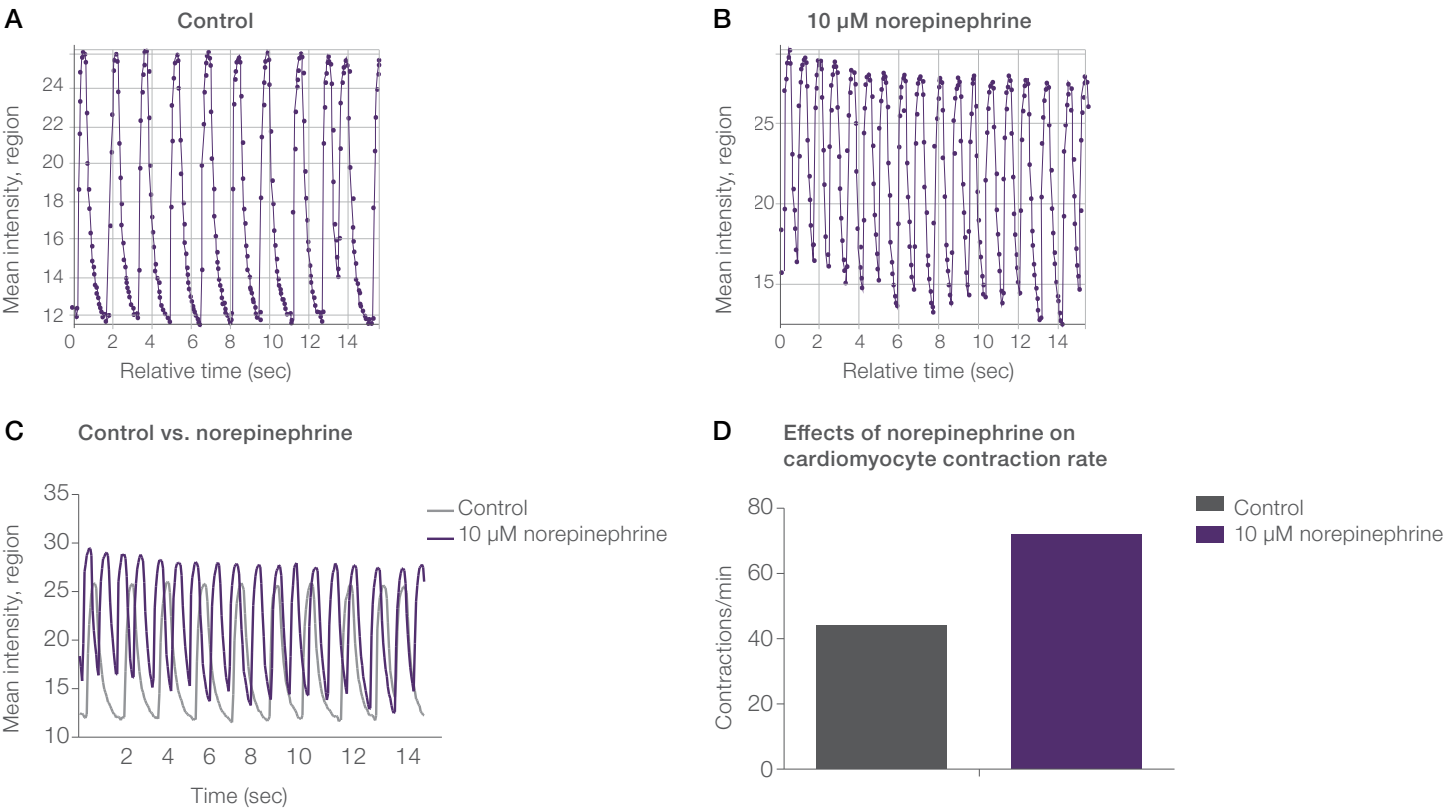

Figure 3. Effect of norepinephrine on cardiomyocyte pulse rate. Intensity measurements of the defined region of interest were obtained using Celleste software for **(A)** control and **(B)** 10 µM norepinephrine-treated cardiomyocytes. **(C)** Comparison of the intensity in the two conditions. **(D)** Quantification of the calcium flux measured with fluo-4 shows that the cardiomyocyte contraction rate is doubled with 10 µM norepinephrine, compared to the baseline contraction rate without any drug treatment (control).

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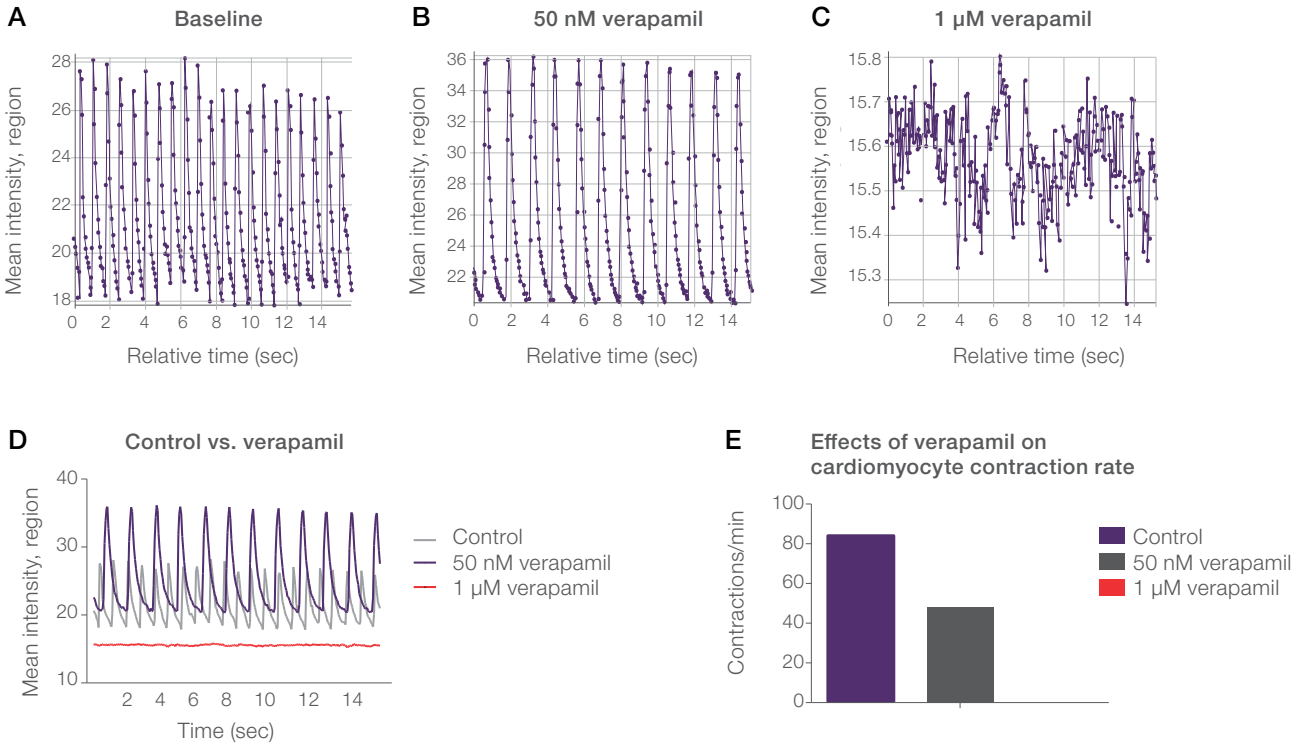

Figure 4. Effect of verapamil on cardiomyocyte pulse rate. Intensity measurements of the defined region of interest were obtained using Celleste software for **(A)** control, **(B)** 50 nM verapamil-treated, and **(C)** 1 µM verapamil-treated cardiomyocytes. **(D)** Comparison of the intensity in the three conditions. **(E)** Quantification of the calcium flux measured with fluo-4 shows that the cardiomyocyte contraction rate is reduced by half with 50 nM verapamil or stopped completely with 1 µM verapamil, compared to the baseline contraction rate with no drug treatment (control).

Conclusion

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 video of calcium flux in pulsing cardiomyocytes can be recorded by selecting the “record video” option on the EVOS FL Auto 2 system. The changes in fluorescence intensity in the videos can then be quantified using Celleste Image Analysis Software by simply defining a region of interest and using the “measure:intensity” tracking function. This simple system allows quick and easy quantification that can be applied to analysis of calcium flux in a variety of cell types and systems, and opens up many possibilities for examination of cellular physiology and drug discovery assays using other fluorescent indicators, without the need for complex instrumentation or techniques.

References

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