Electric Field Stimulation (EFS) of iPSC-derived cardiomyocytes using Hamamatsu FDSS/µCELL with fast data acquisition

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Abstract

Hamamatsu has developed a 96-channel electrode array system that is mounted on the FDSS/µCELL. It adds electric field stimulations (EFS) to all 96 wells in a microplate simultaneously while fluorescence/luminescence signals are monitored. Combining this EFS system with high sampling rates under temperature control, we measured oscillations of intracellular Ca^{2+} concentration, which occurs along with the beating of the cells, with a calcium sensitive fluorescent dye, Fluo-, in rat primary cardiomyocytes (Cosmo Bio), mouse ESC-derived thin-layered cardiomyocytes (Cor.At®, Axiogenesis), human iPSC-derived thin-layered cardimomyocytes (iCell® Cardiomyocytes, Cellular Dynamics International), and human iPSC-derived semi-clamped cardimomyocytes (ReproCardio2, ReproCELL). We observed that the Ca^{2+} oscillation was synchronized to the electric stimulation in all of four types of cardiomyocytes, which indicates that the EFS system is able to pace the beatings of cardiomyocytes. Such intracellular Ca^{2+} kinetics measurements coupled with electric stimulation would be useful in the assessment of cardiac toxicity of pharmacological compounds, in particular in the toxicity screening at the early stages of drug development.

Materials & Methods

Rat primary cardiomyocytes

- Rat primary cardiomyocytes (Cosmo Bio, Tokyo, Japan)

Mouse ESC-derived cardiomyocytes

- Cor.At® (Axiogenesis, Cologne, Germany)

Human iPSC-derived cardiomyocytes

- iCell® Cardiomyocytes (Cellular Dynamics Internationa, Madison, WI, USA)
- ReproCardio 2 (ReproCELL, Yokohama, Japan)

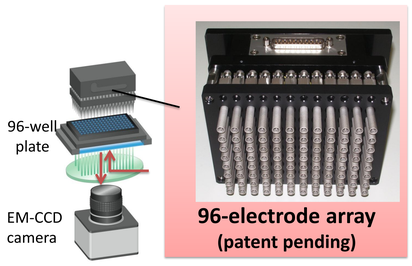
Intracellular Ca^{2+} measurements in cardiomyocytes using FDSS/µCELL

The cardiomyocytes were cultured in 96-well microplates (Coster). A calcium-sensitive fluorescent dye, Fluo- (AAT Bioquest), was loaded into cells with incubation of 2µM Fluo-/AM and 1.25mM probenecid (Sigma-Aldrich) for 1-2 h at 37˚C in 5% CO_2. The fluorescence images of all wells in a microplate were taken every 0.016 s to capture changes in intracellular Ca^{2+} concentration using FDSS/µCELL (Hamamatsu), a kinetic plate reader for cell-based fluorescent assays that can do the simultaneous kinetics measurements of fluorescent signals in all wells in a microplate.

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

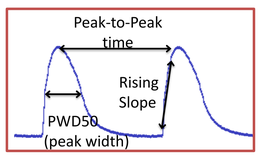
Our developed 96-channel electrode array can be used coupled with the FDSS/µCELL. The electric field stimulations were given to all 96 wells in a microplate simultaneously while fluorescent signals of calcium-sensitive dye were monitored.

FDSS/µCELL



Analysis of calcium waveform

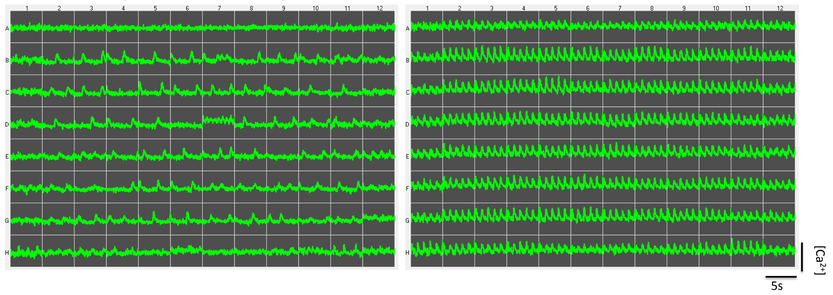
The intracellular Ca^{2+} concentration changes (calcium waveforms) were analyzed using the FDSS Waveform Analysis Software for Cardiomyocytes (Hamamatsu), which estimates peak rate, peak width, peak-to-peak time, rising slope, falling slope, and more.

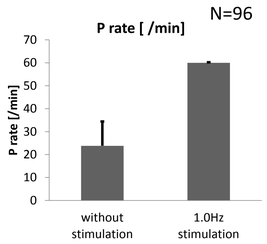


$$\text{Rising slope} = \frac{\text{90\% of peak height} - \text{10\% of peak height}}{\text{Time (ms)}}$$

Results ; Intracellular Ca^{2+} concentration changes in cardiomyocytes

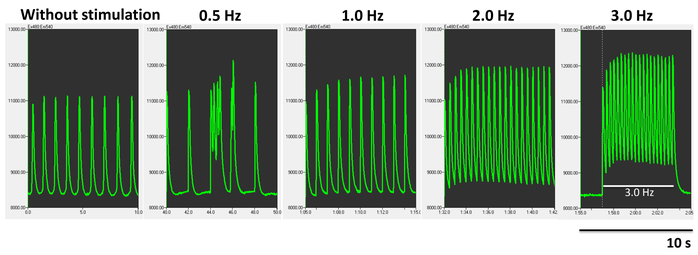
(1) Rat primary cardiomyocytes



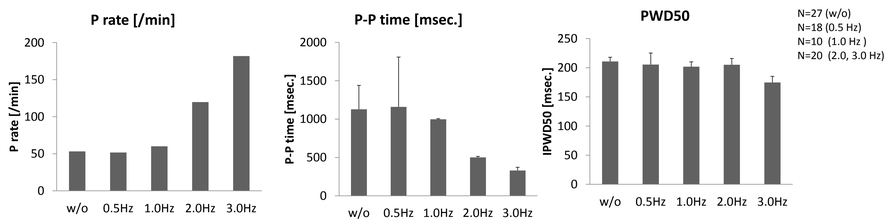


Rat primary cardiomyocytes (Cosmo Bio) were cultured in 96-well plate. The above figures show the intracellular Ca^{2+} concentration changes for 5 s in 96 wells in a microplate. In primary cultured cardiomyocytes, there is a case that cells in each well beat at each rate and timing (left). In such a case, adding of electric stimulation (1.0 Hz, voltage 5 V, duration 5 ms). resulted in the uniform Ca^{2+} oscillations between all wells, that is the synchronized beatings (right).

(2) Mouse ESC-derived cardiomyocytes

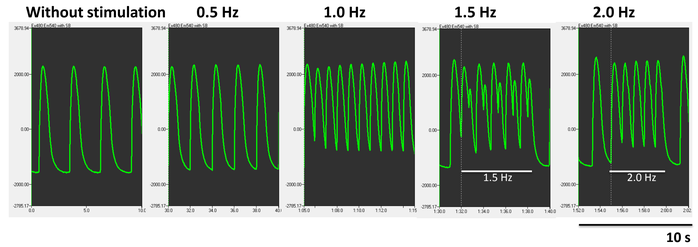


Mouse ES cell-derived thin-layered cardimomyocytes (Cor. At®) were cultured in a 96-well plate. Electric stimulations were added at frequencies of 0.5, 1.0, 1.5, and 2.0 Hz (voltage 5 V, duration 5 ms). The calcium waveforms in one well described above were analyzed to estimate P rate, Peak-to-Peak time, and PWD50.

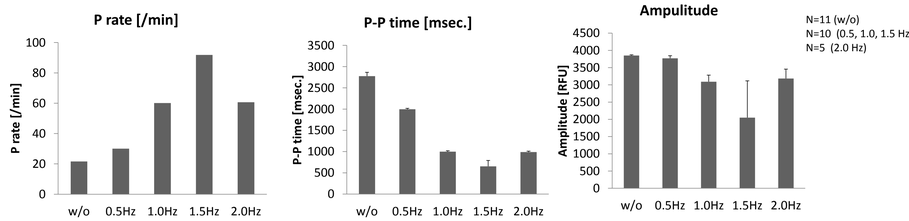


The calcium waveforms in one well described above were analyzed to estimate P rate, Peak-to-Peak time, PWD50, Amplitude, Rising slope, and Falling slope. The graphs show average values of all peaks in one well. The number of peaks in calcium oscillation (P rate) was synchronized to the electric stimulation at frequencies of 1.0, 2.0, and 3.0 Hz. At frequencies of 0.5 Hz, however, some multi-peaks of the calcium oscillations were seen.

(3) Human iPSC-derived thin-layered cardiomyocytes

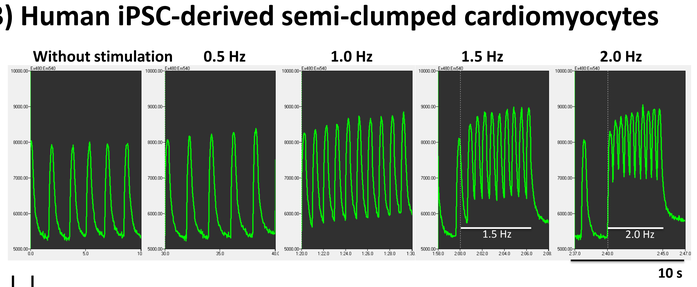


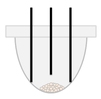
Human iPSC-derived thin-layered cardimomyocytes (iCell® Cardiomyocytes) were cultured in 96-well plate. Electric stimulations were added at frequencies of 1.0 Hz (voltage 10 V, duration 10 ms).



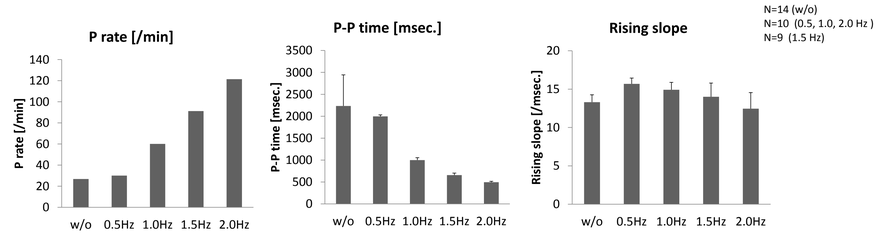
The graphs show average values of all peaks in one well. The number of peaks in calcium oscillation (P rate) was synchronized to the electric stimulation at frequencies of 0.5 and 1.0 Hz, but not at 2.0 Hz. At frequency of 1.5 Hz double-peaks of the calcium oscillations were seen.

(3) Human iPSC-derived semi-clumped cardiomyocytes





Human iPSC-derived semi-clamped cardimomyocytes (ReproCardio2) were cultured in a 96-well U-bottom plate. Electric stimulations were added at frequencies of 0.5, 1.0, 1.5, and 2.0 Hz (voltage 30 V, duration 50 ms).



The graphs show average values of all peaks in one well. The number of peaks in calcium oscillation (P rate) was synchronized to the electric stimulation at frequencies of 0.5, 1.0, 1.5, and 2.0 Hz. The rising slope slightly decreased as the frequency increased.

Conclusions

- The Ca^{2+} oscillations in rat primary, mouse ESC-derived, and human iPSC-derived cardiomyocytes were synchronized to the electric stimulation provided by the EFS system (a 96-channel electric array head) on FDSS/µCELL. **This result indicates that the EFS system is able to pace the beatings of cardiomyocytes.**
- The Ca^{2+} oscillations were regulated by the electric stimulation in the same manner in all 96 wells in a microplate using the EFS system on FDSS/µCELL.

Acknowledgmentes

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