Electric Field Stimulation (EFS) of cardiomyocytes using Hamamatsu FDSS/μCELL

Natsumi Kato[1)], Satoshi Yamamoto[1)], Taira Ito[1)], Masanori Matsubara[1)], Shouming Du[2)], Takuji Kataoka[1)] Fumio Iwase[1)]
1) Systems Division, Hamamatsu Photonics K.K., Hamamatsu 431-3196, Japan 2) Hamamatsu Corporation, Bridgewater, NJ 08807, USA
natsumi.saito@sys.hpk.co.jp SDu@hamamatsu.com

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. We measured oscillations of intracellular Ca^{2+} concentration, which occurs along with the beating of the cells, with a calcium sensitive fluorescent dye in mouse ESC-derived cardiomyocytes (Cor.At®, Axiogenesis). We observed that the Ca^{2+} oscillation was synchronized to the electric stimulation, which indicates that the EFS system is able to pace the beatings of cardiomyocytes. In the presence of some ion channel blockers, EFS was added at various frequencies to see frequency-dependent responses. 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 and Methods

Mouse ESC-derived cardiomyocytes

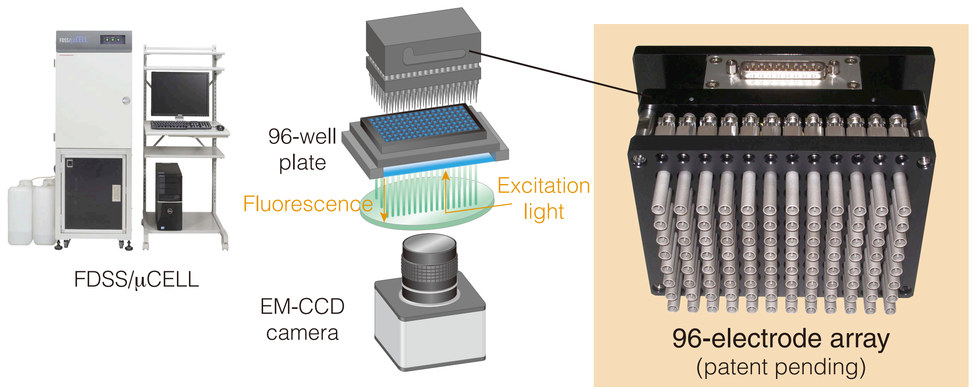
- Cor.At® (Axiogenesis, Cologne, Germany)

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

The cardiomyocytes were cultured in 96-well microplates (Coster). A calcium-sensitive fluorescent dye was loaded into cells with incubation of 2 μM Cal-520 AM (AAT Bioquest) and 1.25 mM 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 Photonics K.K.).

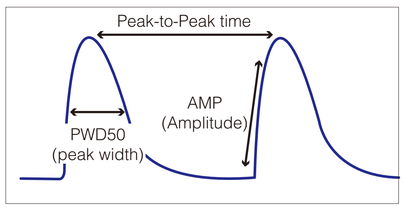
Electric stimulation of cardiomyocytes using the electrode array mounted on the FDSS/μCELL: the EFS system

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.



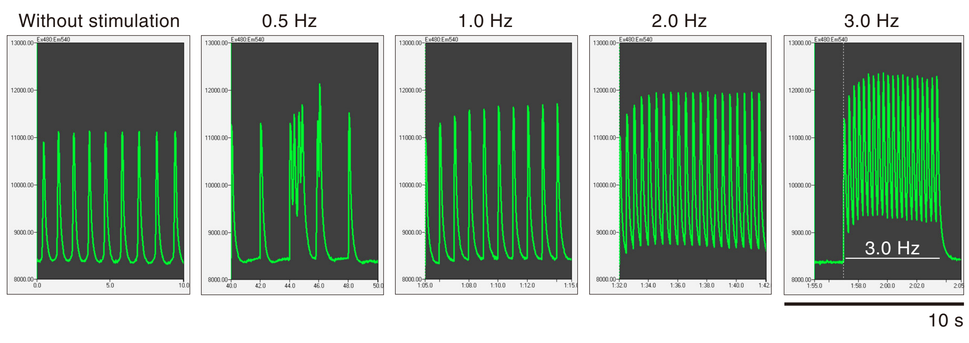
Analysis of calcium waveform

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

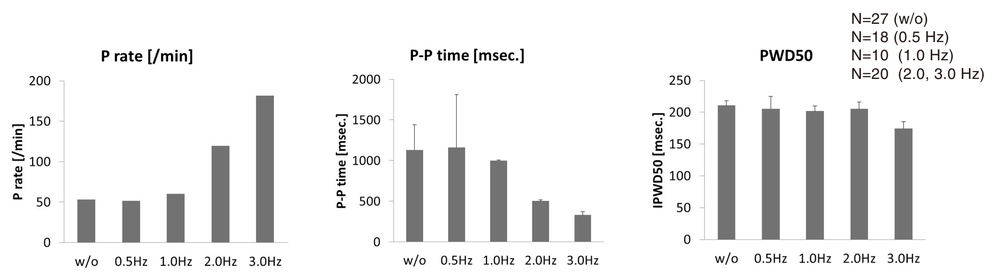


Results; Intracellular Ca^{2+} concentration changes in mouse ESC-deriverd cardiomyocytes

(1) Add EFS stimulation of various frequencies

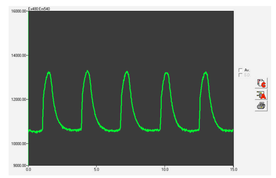
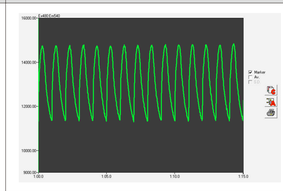
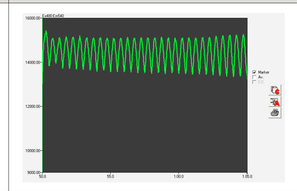
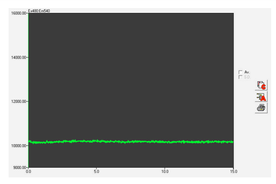
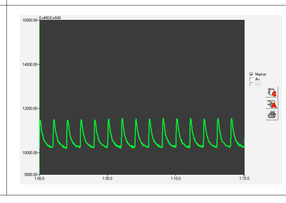
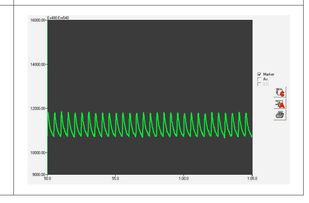


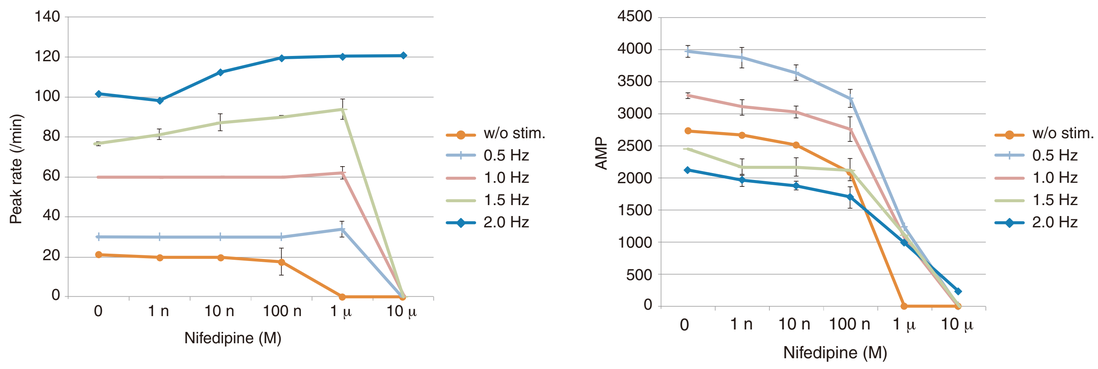
Mouse ESC-derived cardimomyocytes (Cor. At®) were cultured in a 96-well plate. Electric stimulations were added at frequencies of 0.5, 1.0, 2.0, and 3.0 Hz (voltage 5 V, duration 5 ms). The changes in fluorescence intensity (calcium concentration) in one well shown.



The calcium waveforms in one well shown 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 observed.

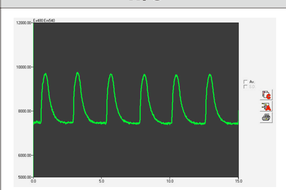
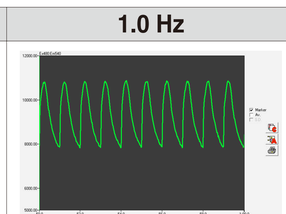
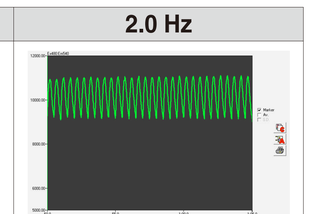
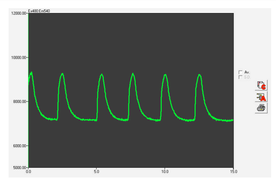
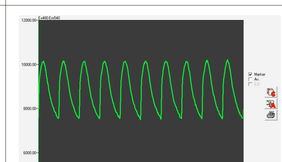
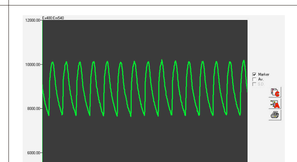
(2) L-type calcium channel blocker: Nifedipine

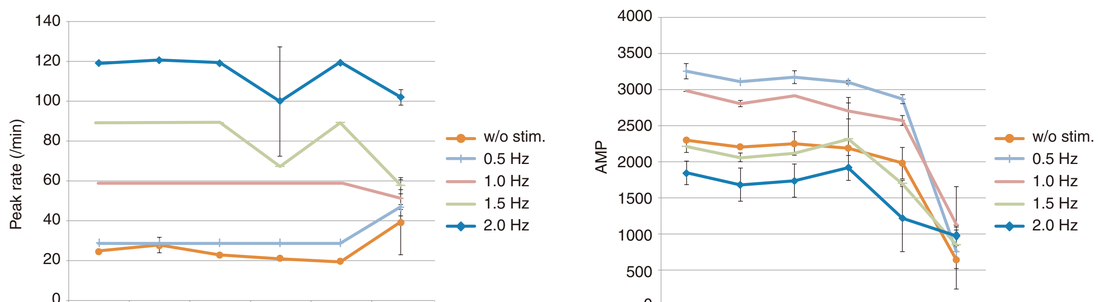
	w/o	1.0 Hz	2.0 Hz
-Nifedipine			
+1.0 μM Nifedipine			



The calcium waveforms shown above were analyzed to estimate peak rate and amplitude. The graphs show average values of all peaks in two wells. Nifedipine is a L-type calcium channel blocker and known as a drug which arrests beating. In the presence of 1.0 μM Nifedipine, any intracellular calcium oscillations were not observed without electric stimulations. In contrast, the calcium oscillations appeared when electric stimulations were given at frequencies of 0.5 -2.0 Hz.

(3) hERG blocker: Cisapride

	w/o	1.0 Hz	2.0 Hz
-Cisapride			
+3.0 μM Cisapride			



The calcium waveforms shown above were analyzed to estimate peak rate and amplitude. The graphs show average values of all peaks in two wells. We observed the effect of Cisapride on intracellular calcium oscillations more clearly with electric stimulations at 2.0 Hz than without stimulations.

Conclusions

- The Ca^{2+} oscillations in mouse ESC-derived 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.**
- We observed the effects of ion channel blockers on intracellular calcium oscillations with electric stimulations at various frequencies.

Acknowledgments

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