Development of cardiac contractile function evaluation system by measuring impedance of human iPS cell-derived cardiomyocytes using a multipoint electrode array

多点電極アレイを用いたヒトiPS細胞由来心筋細胞のインピーダンス測定による心収縮機能評価系の開発

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Background

■ The importance of cardiac contraction evaluation in drug development

- Side effects on the cardiovascular system are one of the main reasons for terminating drug development. Therefore, it is crucial to accurately understand the effects of compounds on the cardiovascular system at an early stage.
- Potential side effects of drugs on cardiac systolic function may increase the risk of adverse cardiovascular events or heart failure.
- However, currently, there is no established in vitro evaluation methods for cardiac systolic function that can be utilized during the early stages of drug development.

■Trends in cardiac contraction evaluation using iPS-derived cardiomyocytes

- There are multiple evaluation methods available for assessing cardiac contractile function using human iPS cell-derived cardiomyocytes (iPSC-CMs) that involve both maturation techniques and detection methods. In this study, we opted for electrical stimulation due to its high throughput and practicality.
- We previously presented the findings of electrical stimulation and impedance measurements at JSOT2020. In this study, we aimed to validate the reproducibility of those results, expand the scope of drug evaluation data, and provide additional biological data such as calcium concentration measurements.

Maturation techniques			
Electrical stimulation	3D Co-culture	Biochemical factor	Biophysical factor

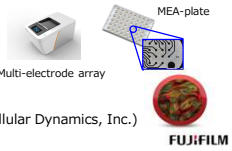
Detection methods			
Motion imaging	**Impedance measurement**		Intracellular calcium concentration measurement
	by cell resistance	by cell physical movement	

Purpose of research

We examined whether it is possible to evaluate the effect of drugs on cardiac contractile function by measuring the impedance of iPSC-CMs using a multi-electrode array (MEA) with electrical stimulation.

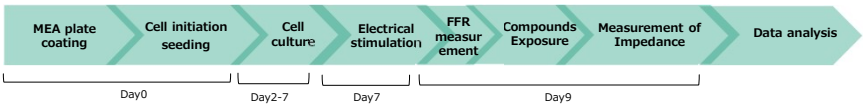
Materials and Methods

Measuring equipment
- ✓MEA : Maestro Pro (Axion BioSystems)
- ✓MEA plate: Cytoview (Axion BioSystems)

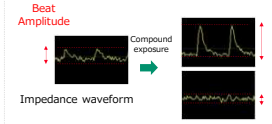


Reagents
- ✓Cell : iCell® Cardiomyocytes² (FUJIFILM Cellular Dynamics, Inc.)
- ✓Coating agent : Fibronectin
- ✓Media : iCell® CM Plating Medium , iCell® CM Maintenance Medium

Protocol



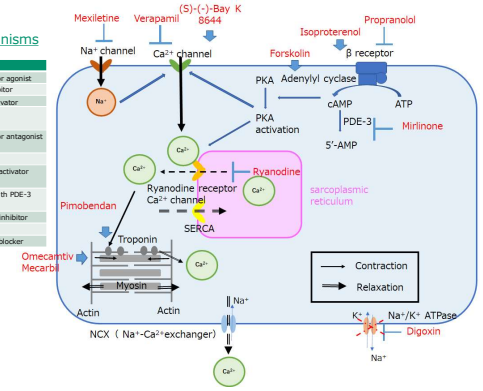
Analysis method



Calculate the change in Beat Amplitude after compounds exposure /before compounds exposure

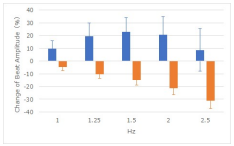
Test compounds and Mechanisms

Compounds	inotropy	Mechanisms
Isoproterenol	positive	β adrenergic receptor agonist
Digoxin	positive	Na⁺/K⁺ ATPase inhibitor
Forskolin	positive	Adenylyl cyclase activator
Omecamtiv mecabril	positive	Myosin activator
Propranolol	negative	β adrenergic receptor antagonist
Mexiletine	negative	Na⁺channel blocker
(S)-(-)-Bay K 8644	positive	L-type Ca²⁺channel activator
Pimobendan	positive	Calcium sensitizer with PDE-3 inhibition
Ryanodine	negative	Ryanodine receptor inhibitor
Milrinone	positive	PDE-3 inhibitor
Verapamil	negative	L-type Ca²⁺channel blocker



Result

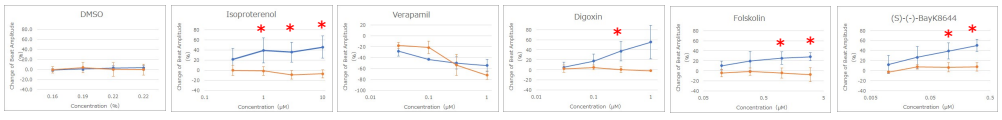
■ Study1 : FFR (Force Frequency Relationship)



Electrical stimulation improves FFR.

■ Study3 : Change of Contractility by compounds Comparison with and without electrical stimulation

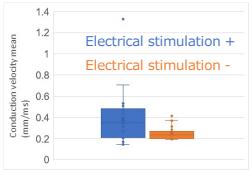
* Test for significant differences in Beat Amplitude change with and without pacing at each dose (t test, p<0.05)



Negative Control | β adrenergic receptor agonist | L-type Ca²⁺ channel blocker | N⁺/K⁺ ATPase inhibitor | Adenylyl cyclase activator | L-type Ca²⁺ channel activator

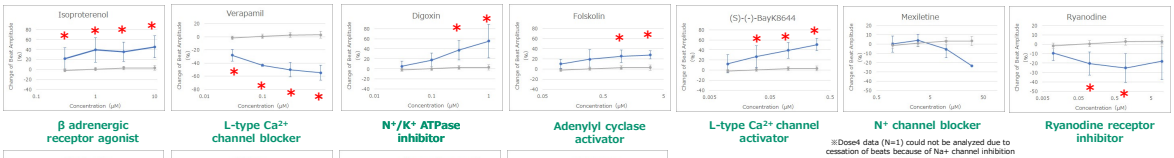
It was confirmed that the response increased when electrical stimulation was applied for five of these compounds.

■ Study2 : Conduction velocity



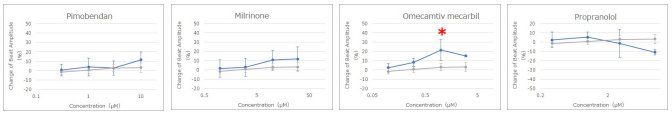
Electrical stimulation improves Conduction velocity.

■ Study4 : Change of Contractility by compounds with electrical stimulation comparison to negative control DMSO



β adrenergic receptor agonist | L-type Ca²⁺ channel blocker | N⁺/K⁺ ATPase inhibitor | Adenylyl cyclase activator | L-type Ca²⁺ channel activator | N⁺ channel blocker | Ryanodine receptor inhibitor

※Dose4 data (N=1) could not be analyzed due to cessation of beats because of Na+ channel inhibition



Calcium sensitizer with PDE-3 inhibition | PDE-3 inhibitor | Myosin activator | β adrenergic receptor antagonist

* Test for significant differences in Beat Amplitude change at each dose between compound group and vehicle control (DMSO) group (Dunnet's test, p<0.05)

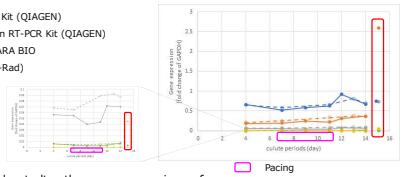
The responses of 11 compounds with different mechanisms of action on the contractility of iPSC-CMs were evaluated.

One-way ANOVA Dunnett's test was employed, because all compounds were compared to the same DMSO background data.

■ Study5 : Effect of electrical stimulation on biology of iPSC-CMs

1. Gene expression

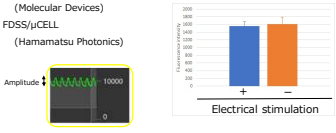
RNA extraction : RNeasy Mini Kit (QIAGEN)
qPCR : QuantiTect SYBR Green RT-PCR Kit (QIAGEN)
Primers : Obtained from TAKARA BIO
Measurements : CFX384 (Bio-Rad)



- Electrical stimulation did not alter the gene expressions of iPSC-CMs.
- The expression levels of ATP2A2 and PDE3B were found to be comparable to those of the human heart.
- The expression levels of TNNI3, RYR2, and PDE3A were lower than those of the human heart.

2. Ca2+ concentration

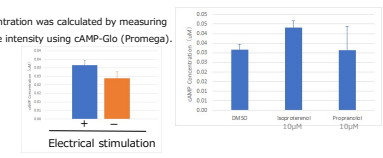
Calcium staining : EarlyTox Cardiotoxicity Kit (Molecular Devices)
Measurements : FDSS/μCELL (Hamamatsu Photonics)



- The effect of electrical stimulation on the amplitude of intracellular calcium concentration was evaluated.
- Electrical stimulation did not increase the intensity of the intracellular calcium peak.

3. cAMP concentration

The cAMP concentration was calculated by measuring the luminescence intensity using cAMP-Glo (Promega).



- Electrical stimulation led to an increase in intracellular cAMP concentration.
- Exposure to isoproterenol was found to significantly elevate the cAMP concentration compared to DMSO.
- The intracellular concentration of cAMP remained unaffected by propranolol.

Discussion

We confirmed that electrical stimulation significantly enhances the maturation of iPSC-CMs, improving factors such as FFR, conduction velocity, cAMP concentration, and drug responses. This method effectively detected the effects of compounds with diverse pharmacological actions on cardiomyocyte contractility. However, we observed that certain compounds had less impact on iPSC-CMs than anticipated. We hypothesize that low gene expression levels, inadequate intracellular calcium concentrations, and insufficient cAMP concentrations could contribute to the diminished compound responses.
Further research is necessary to promote the maturation of iPSC-CMs concerning morphology (myofibril alignment, sarcomere structure, T-tubule organization, etc.), electrophysiology and calcium handling. These improvements will enable a more comprehensive assessment of drugs with a wider range of actions using this evaluation system.

Conclusion

The MEA assay of iPSC-CMs with electrical stimulation is a promising method for promoting the maturation of iPSC-CMs and detecting the effects of various compounds on cardiomyocyte contractility.

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