A Higher-Throughput Approach to Investigate Cardiac Contractility *In Vitro* Under Physiological Mechanical Conditions

M. Goßmann[1], U. Thomas[2], A. Horváth[2], E. Dragicevic[2], S. Stölzle-Feix[2], N. Fertig[2], A. Jung[3], A.H. Raman[3], M. Staat[3], P. Linder[1]

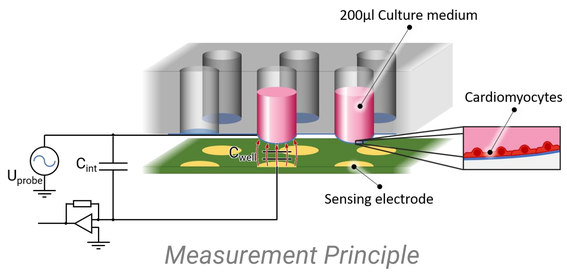
[1] innoVitro GmbH, Artilleriestraße 2, 52428 Jülich, Germany
[2] Nanion Technologies GmbH, Ganghoferstraße 70a, 80339 München, Germany
[3] Institute of Bioengineering, FH Aachen University of Applied Sciences, Heinrich-Mußmann-Str. 1, 52428 Jülich, Germany

Abstract

Despite increasing acceptance of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in safety pharmacology, controversy remains about the physiological relevance of existing in vitro models for their mechanical testing. We hypothesize that existing signs of immaturity of the cell models, e.g. negative inotropy upon adrenergic stimulation, result from an improper mechanical environment. We cultured hiPSC-CMs in a 96-well format on hyperelastic silicone membranes imitating their native mechanical environment, resulting in physiological responses to compound stimuli.

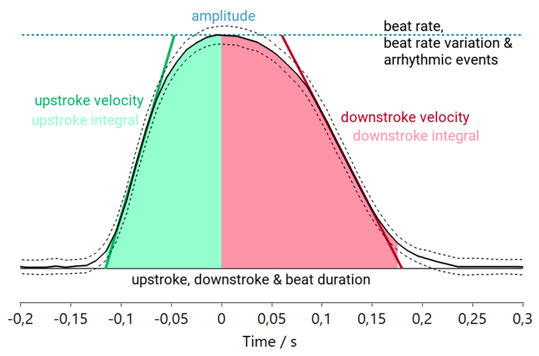
Technology

hiPSC-CMs from commercial sources were cultured on freely-swinging, ultra-thin and hyperelastic silicone membranes. Due to the weight of the cell culture medium, the membranes were deflected downwards. Rhythmic contraction of the hiPSC-CMs resulted in dynamic deflection changes which were quantified by means of capacitive distance sensing using the FLEXcyte 96 platform. From the recorded beats a broad set of temporal and shape related parameters is calculated. These allow a deep insight into the functional mechanical mechanisms of the cells.



Measurement Principle

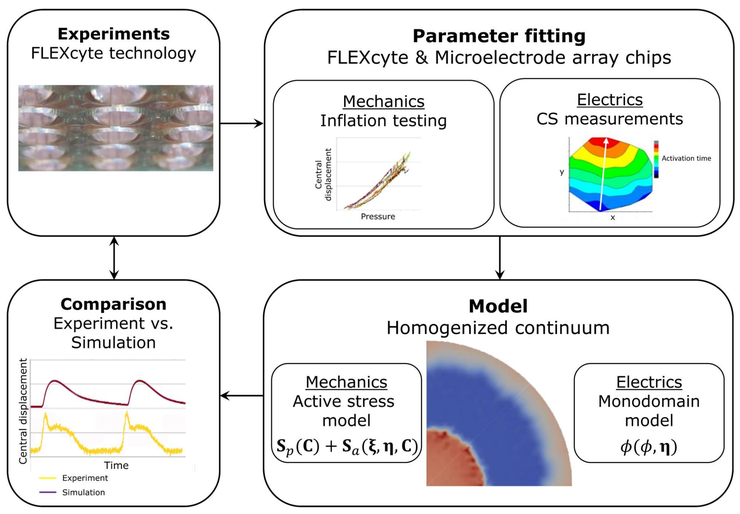
FLEXcyte 96 Platform



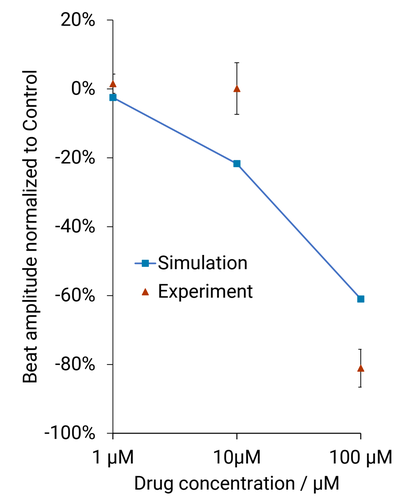
Mean Beat Analysis

Simulation

The measurements were complemented with electromechanical models based on electrophysiological recordings of the used cell types. Electrophysiological properties were recorded by automated patch-clamp (Patchliner) and the results were integrated into the electromechanical model (Jung & Staat, 2019). The Hill equation with given IC50 values and drug concentrations were used to account for the effect of drugs on the maximum conductance of affected ion channels. Effects of Sotalol on the maximum beat amplitude are shown as example.



Workflow of the Simulation Process

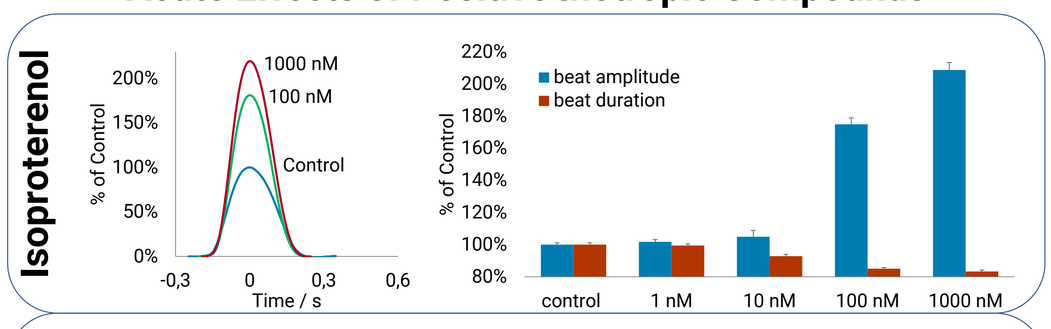


Simulation vs. Experiment

Results

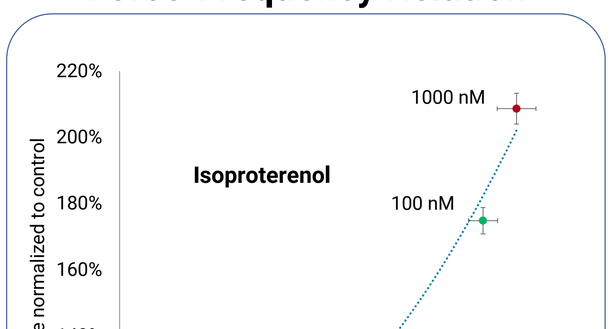
For the experiments hiPSC-CMs from different vendors were used. These were cultured in FLEXcyte 96 plates for 7 days prior to compound addition. Acute measurements were conducted in standard culture medium 10-30 minutes after compound addition. For chronic treatment, compound-containing medium was replaced daily for up to 5 days. All measurements were performed with a FLEXcyte 96 system from Nanion Technologies.

Acute Effects of Positive Inotropic Compounds



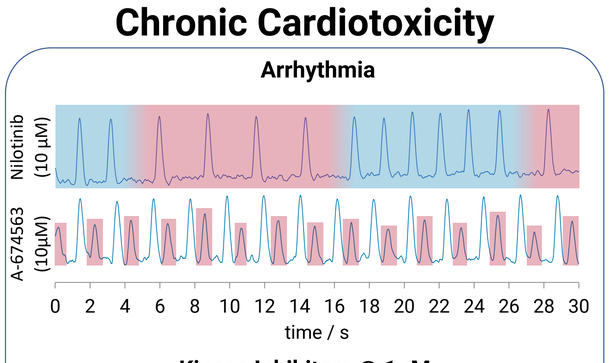
Positive inotropic compounds isoproterenol and S-Bay K8644 both induce an increase in beating amplitude but show distinct effects on beating shape.

Force-Frequency Relation



Stimulation with beta-adrenergic agonist isoproterenol for 7 minutes induced physiologic positive force-frequency relation.

Chronic Cardiotoxicity



Chronic treatment with kinase inhibitors induced arrythmia after 1 day (top) and decrease in beating amplitude with different profiles over 5 days (bottom).

Conclusion

In summary, the FLEXcyte 96 system is a reliable high throughput tool for in vitro cardiac contractility research, providing the user with data obtained under physiological conditions which resemble the native environment of the human heart. We show that the results obtained for both acute and chronic compounds are consistent with the respective physiological responses in humans.

References:

R. Frotscher, D. Muanghong, G. Dursun, M. Goßmann, A. Temiz-Artmann, M. Staat: Sample-specific adaption of an improved electro-mechanical model of in vitro cardiac tissue. *J Biomechanics*, 2016; 49(12):2428-2435.
M. Goßmann, R. Frotscher, P. Linder, S. Neumann, R. Bayer, M. Epple, M. Staat, A. (Temiz) Artmann, G.M. Artmann: Mechano-pharmacological characterization of cardiomyocytes derived from human induced pluripotent stem cells. *Cell Physiol Biochem*, 2016; 38(3):1182-1198.
R. Frotscher, J.-P. Koch, M. Staat: Computational investigation of drug action on human-induced stem cell derived cardiomyocytes. *J Biomech Eng.*, 2015; 137(7):071002-071002-7.
M. Paci, R.P. Pölönen, D. Cori, K. Penttinen, K. Aalto-Setälä, S. Severi, J. Hyttinen: Automatic optimization of an in silico model of human ipsc derived cardiomyocytes recapitulating calcium handling abnormalities. *Front Physiol*, 2018; 9:709.
A. Jung, M. Staat: Modeling and simulation of human induced pluripotent stem cell-derived cardiac tissue. *GAMM-Mitt.*, 2019; e201900002.

FLEXcyte 96 Whitepaper

Scan me

This research project was supported by:

Federal Ministry of Education and Research