Stimulation Methodologies for Human iPSC-derived Cardiomyocytes on the FLEXcyte 96 System

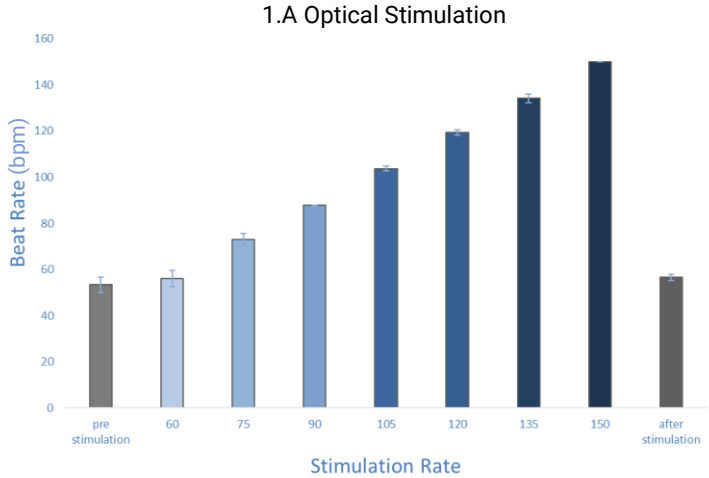
Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are being widely used for basic research, regenerative medicines and drug screening, but spontaneous contraction behaviour and immature drug-induced functional responses challenge this cell models' translational potential for these endeavors. Besides the need for a physiological environment, provided by the FLEXcyte 96 technology[1], stimulation of hiPSC-CM contractile behaviour is a key element in supporting the functional maturation process *in vitro*[2] and allowing synchronized cardiac effects[3]. Here, two FLEXcyte 96 system associated optical and mechanical pacing methodologies are presented to foster hiPSC-CM maturity and functional relevance *in vitro*, including a standardized cardiomyocyte beating behaviour for more reliable data calculation.

Optical Stimulation of hiPSC-CMs - Transfection with Channelrhodopsin2

- Light-induced stimulation with CardioExcyte 96 SOL - LEDs integrated into the lid of the FLEXcyte 96 incubation system
- Fuse-It- mRNA transfection kit (beniag GmbH) employed for transfection
- Channelrhodopsin2 transfection of hiPSC-CMs (iCell CM², FCDI) 4 days post seeding
- Optical stimulation analysis 24 hours post transfection
- Optical pacing with 470 nm for 30s for each recording (sweep)

CardioExcyte 96 SOL

1.A Optical Stimulation



1.B Optical Stimulation Raw Data

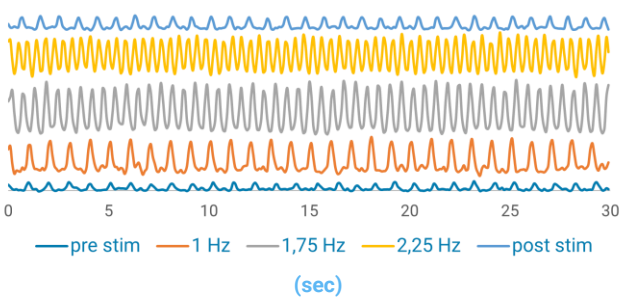
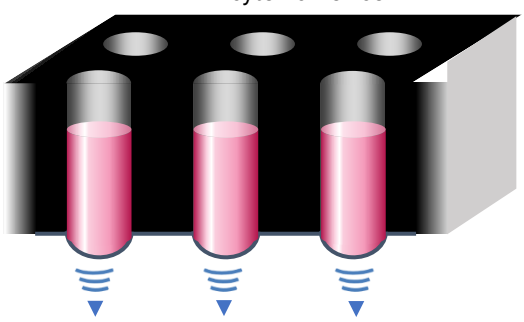


Figure 1. Analyzed and raw data of optical stimulation of hiPSC-CMs (iCell CM², FCDI) transfected with Channelrhodopsin2 on the FLEXcyte 96 system. (A) Bar graph shows beat rate of pre- and post-optical stimulation (grey) as well as rising stimulation effects (light blue - dark blue). (B) Raw traces of optically paced hiPSC-CM beating behaviour recorded for 30 seconds. Graph shows beating of cells before and after stimulation (blue) as well as stimulated cells at 1 Hz (orange), 1.75 Hz (grey) and 2.25 Hz (yellow).

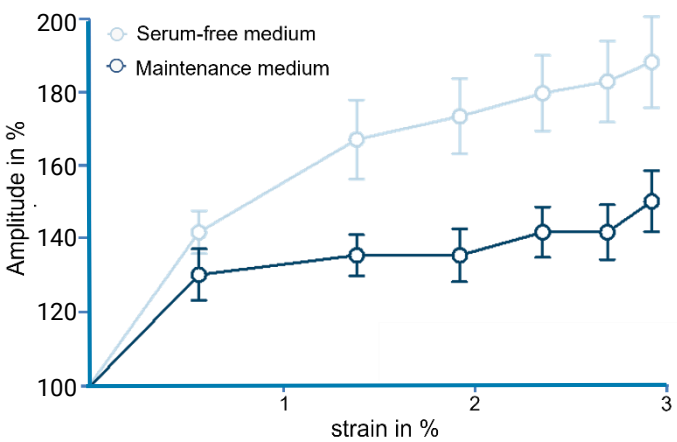
Mechanical Stimulation of hiPSC-CMs - Pneumatic Pressure

- Mechanical stimulation with pneumatic pressure application built into FLEXcyte 96 device
- Applied pressure pulls membranes down and stretches the cells
- Ramp pressure for replication of Frank Starling Curve - more pressure induced higher membrane strain, resulting in higher contraction amplitude
- Dynamic pressure for stimulation of hiPSC-CM maturity, leading to an increased contraction amplitude
- Static pressure for stress stimulation, e.g. hypertension

Pneumatic Pressure Built into FLEXcyte 96 Device



2A. Frank Starling Curve



2.B Mean Beat after Cyclic Stretching

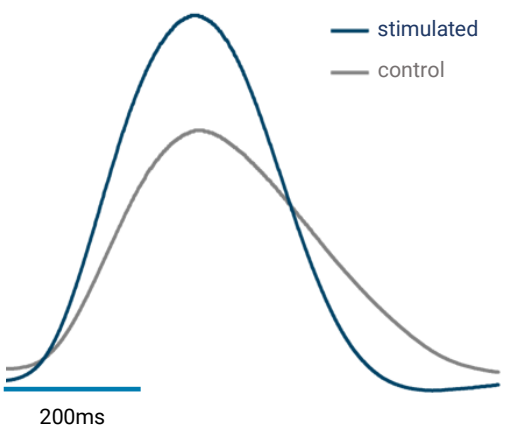


Figure 2. Analyzed data and mean beat of mechanically paced hiPSC-CM (iCell CM², FCDI) on the FLEXcyte 96 system. (A) Bar graph shows amplitude (in %) of hiPSC-CMs stimulated with increasing strain under serum-free and serum containing medium conditions, demonstrating the Frank Starling curve. (B) Mean beat of hiPSC-CMs after constant cyclic stretching over 3 days at 1 Hz (dark blue) in comparison to the non-paced control mean beat (grey).

Both pacing methodologies presented here feature a static, dynamic or ramp approach covering cardiac-related issues, such as hypertension, maturation or replication of the Frank Starling mechanism. Dependent on the approach, either a light-induced (Channelrhodopsin2) or label-free (pneumatic pressure) analysis of hiPSC-CMs is feasible. The FLEXcyte 96 system adds another dimension to basic research and drug screening methods, due to its flexible membrane technology and pacing features for increased maturity and simultaneous cardiomyocyte activation, allowing parallel recording of drug effects on a 96 well plate.

[1] Gossmann et al., *J. Pharm. and Tox. Methods*. 105, 106892, (2020)
[2] Mihic et al., *Biomaterials*. 35 (9), 2798-808, (2014)
[3]Lapp et al., *Scientific Reports*. 7, 9629, (2017)

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