Mechanical Stimulation in a 2D High-Throughput Contractility System Induces Functional Changes in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

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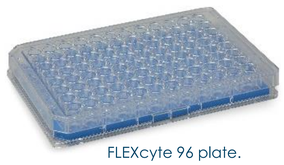
Maturation of hiPSC-CMs can be enhanced by mechanical stress

The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to assess efficacy and cardiac safety of new drug candidates continues to increase. In this context, a new 2D high-throughput system, FLEXcyte 96, allows for contractility measurements of cells cultured in a more physiological environment, reflecting the mechanical conditions of the native human heart[1].
This study aims to show the influence of mechanical stimulation on functional behavior of hiPSC-CMs. This aspect is of particular importance, as mechanical load acts as crucial growth regulator in the developing heart.

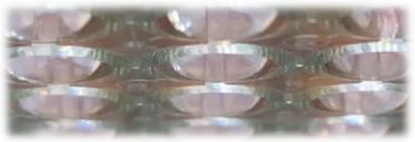
FLEXcyte 96 allows application of constant and cyclic stretching to cell monolayer

iCell Cardiomyocytes[2] (FUJIFILM Cellular Dynamics) were cultured on fibronectin-coated silicone membrane of the FLEXcyte plate (10^5 cells/well). The cell monolayer was further mechanically stimulated through application of pneumatic pressure, resulting in increased deflection and mechanical strain of the cell monolayer. Through this feature, the FLEXcyte 96 allows biaxial load conditions and simultaneous contractility evaluation of cell monolayers in a 96 well format. Different levels of strain (%) inducing membrane deflection were applied to the cell monolayer in order to test the reproducibility of Frank-Starling mechanism. In addition, 1Hz cyclic pressure stimulation, inducing 5% membrane deflection, was applied to investigate its effect on hiPSC-CMs monolayer during 3 days. This cyclic stretching of the silicone membranes aims to imitate the physiological stretching experienced by CMs in the beating human heart during systolic and diastolic phases.

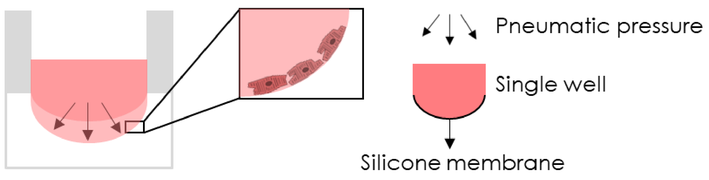


FLEXcyte 96 plate.

Picture of FLEXcyte 96 modified to have an active pressure regulation (a prototype). This system allows to apply a mechanical stress to the cell monolayer.



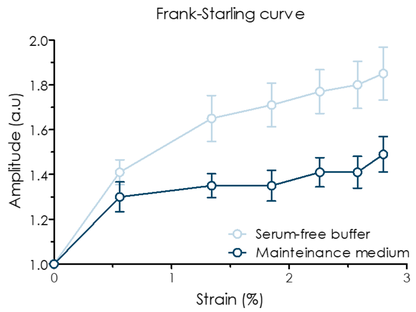
The bottom of a FLEXcyte 96 plate has silicone membranes characterized by a thickness ~6 µm and an elastic modulus 30KPa.



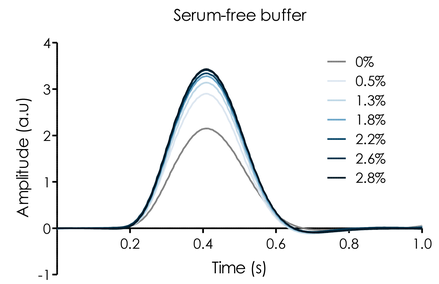
Pneumatic pressure mechanism in a single well of FLEXcyte 96.

FLEXcyte 96 can reproduce the Frank-Starling mechanism

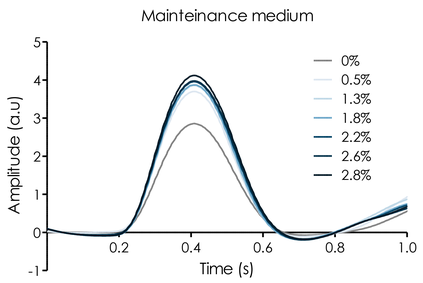
Increasing levels of applied strain resulted in higher contraction amplitudes of the cell monolayer. The amplitude increase under higher strain could be observed in both serum-free and maintenance medium culture conditions, but it was more prominent in serum-free conditions. This confirms the reproducibility of the Frank-Starling mechanism with the FLEXcyte 96.



Frank-Starling mechanism shows contraction amplitude of iCell Cardiomyocytes[2] cultured in maintenance medium and serum-free buffer at increasing level of membrane strain. The amplitude values were normalized to the amplitude at 0% strain.

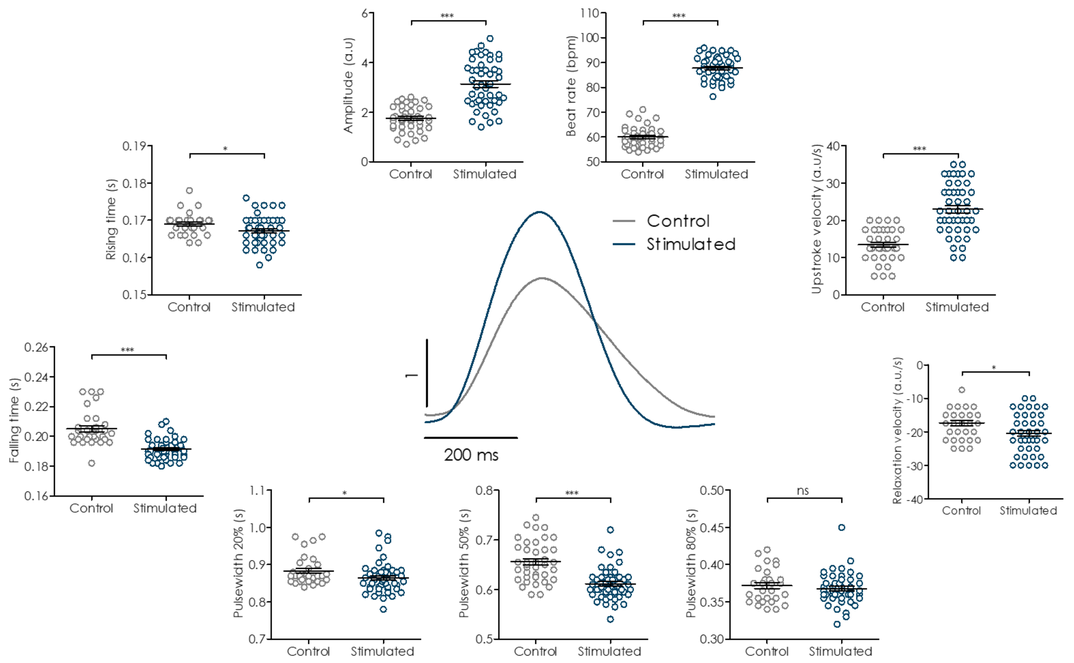


Contraction amplitude over time in serum-free buffer at increasing level of membrane stretching (%).



Contraction amplitude over time in maintenance medium at increasing level of membrane stretching (%).

Mechanical stimulation modulates the contractility of hiPSC-CMs



Comparison of iCell Cardiomyocytes[2] contractility parameters under control condition and after 3 days of 5% cyclic stretching: 3 days of cyclic stretching resulted in a significant increase of the contraction amplitude (3.1±0.14 a.u, n=47 vs. 1.7±0.07 a.u, n=41; p<0.0001), upstroke velocity (23±0.9 a.u/s, n=47 vs. 13.5±0.6 a.u/s, n=41; p<0.0001) and relaxation velocity (-20.4±0.8 a.u/s, n=47 vs. -17.3±0.8 a.u/s, n=41; p=0.0172) compared to non-stimulated cells. Accordingly, the rising and falling times decreased significantly. Unexpectedly, also the beat rate increased after 3 days of cyclic stretching (87.8±0.7 bpm, n=47 vs. 60±0.6 bpm, n=41; p<0.0001), which resulted in a shortening of the pulse width.

FLEXcyte 96 mechanical stimulation promotes maturation of hiPSC-CMs

The Frank-Starling mechanism could be successfully reproduced in the FLEXcyte 96. Applying higher steps of pressure induces higher membrane strain, which in turn resulted in higher contraction amplitude. The higher amplitude increase in serum-free buffer could be associated to the basal weaker contractile activity of the hiPSC-CMs when cultured without serum. In other words, hiPSC-CMs cultured in serum-free buffer offer a higher window of potential amplitude increase[2].

We conclude, that FLEXcyte 96 is a reliable high throughput tool for *in vitro* cardiac contractility research, which allows culturing hiPSC-CMs under more physiological conditions and introduces dynamic change to their mechanical environment. In this study, the applied mechanical stimulation resulted in higher contraction amplitude and faster upstroke/relaxation velocity. In hiPSC-CMs physiology, these changes indicate higher level of maturation towards adult hiPSC-CMs[3]. FLEXcyte 96 thereby offers the potential to measure real values of contraction force in an *in vitro* 2D high-throughput format and model cardiac pathological conditions, such as acute hypertension.

References

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2. H. Naito. Circulation. 2006;114:I-72–I-78
3. Yuxuan G., William T. P. Circulation Research. 2020;126:1086–1106

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