New Feature:

Contractile Force (mN/mm²) Analysis with the FLEXcyte 96

Contractile Force measurement is an important prerequisite for applications of human iPSC-CMs in disease modeling and *in vitro* drug screens. Hence, Contractile Force calculation was included as parameter for the FLEXcyte 96 by correlating the membrane movement with the stress of the cell-substrate-complex (mN/mm²) for each recording well.

Fig.1 Comparison of human iPSC-CM contractile force under various conditions in culture

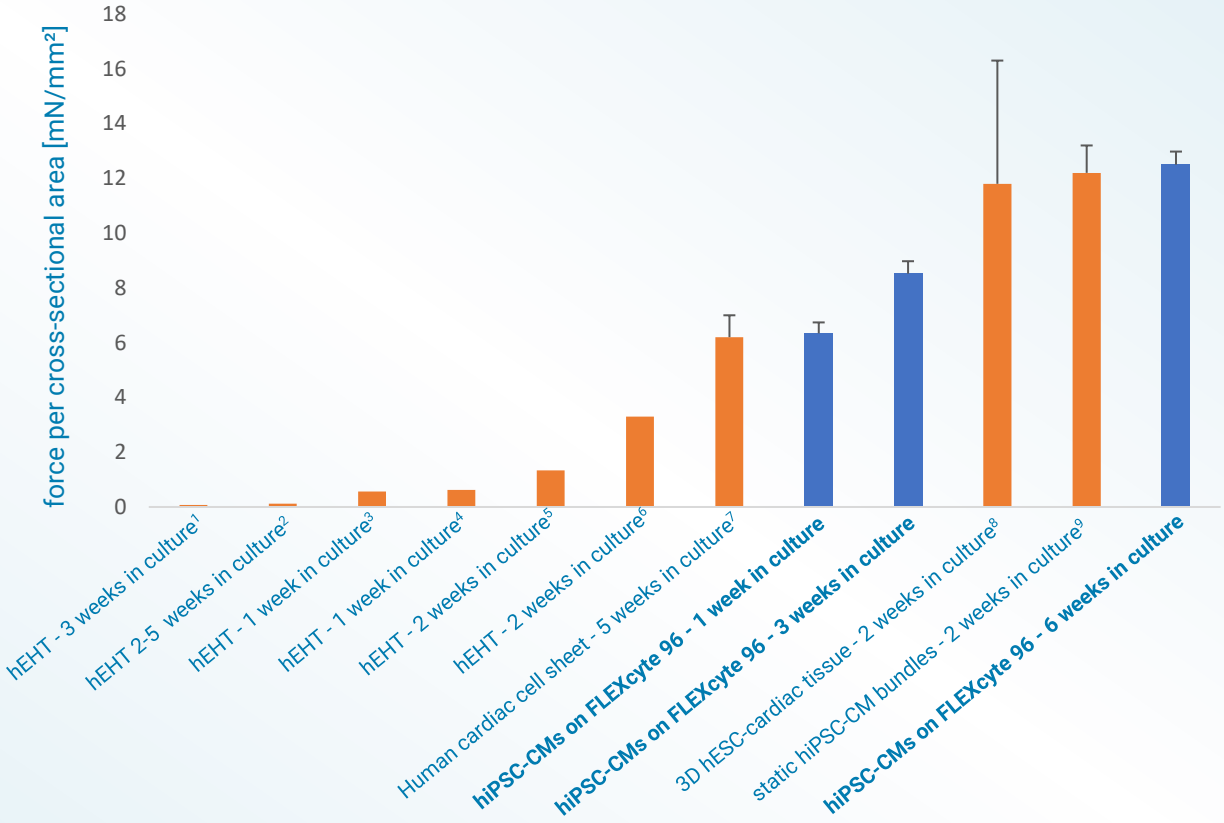


Figure 1. Bar graph shows comparative contractile force data of human iPSC- or ESC-derived cardiac tissues in 2D or 3D format with different cell culture times. HiPSC-CMs cultured on FLEXcyte 96 plates show increasing contractile force over prolonged culture times. [[1]Tulloch et al., 2011; [2]Schaaf et al., 2011; [3]Turnbull et al., 2014; [4]Masumoto et al., 2016; [5]Ruan et al., 2016; [6]Sasaki., 2018; [7]Tiburcy et al., 2017; [8]Zhang et al, 2013; [9]Jackman et al., 2018]

Literature comparison of FLEXcyte 96 contractile force

The contractile force measured for human iPSC-derved cardiomyocytes cultured on FLEXcyte plates for 7, 24 and 44 days demonstrates an increasing contraction force over time (Fig.1 and 2) as well as a decreasing beat rate (data not shown), both well known parameters for an adult cardiac phenotype [Yuxuan et al., 2020].

A comparison of literature values derived from force measurements of human iPSC- or ESC-CMs cultured under various conditions such as human engineered heart tissue (hEHT), 3D cardiac tissue, cardiac cell sheets or cardiac bundles to FLEXcyte data was performed. The comparison demonstrates that cardiac force analysis performed with the FLEXcyte 96 are in line with cardiac tissue of 3-dimensional standard, especially when cells were cultured for longer time periods on FLEXcyte 96 plates.

Hence, the pro-maturation effect of the FLEXcyte 96 plates on hiPSC-CMs in combination with proper contractile force analysis demonstrates this technologies' potential for reliable drug-induced cardiac risk analysis for drug discovery up to preclinical safety and toxicity.

Fig. 2A Mean beat comparison of CMs

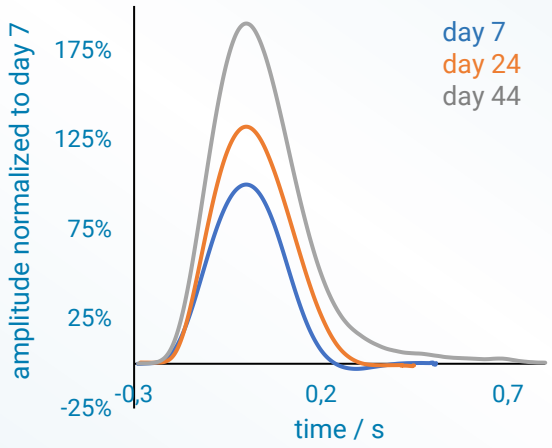


Fig.2B Contractile force long-term analysis of human iPSC-CMs

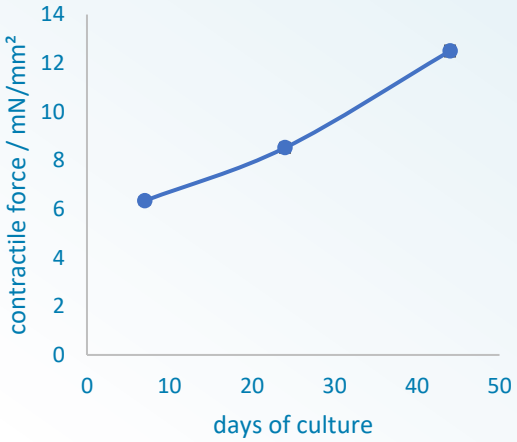
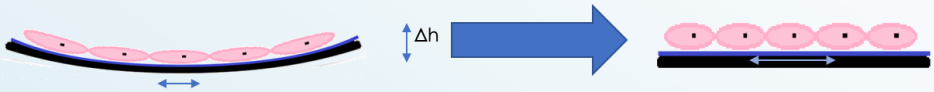


Figure 2A. Graph shows mean beat comparison of iCell® CM² cultured for 7, 24 and 44 days on FLEXcyte 96 plates. Data is normalized to amplitude on day 7. (2B) Graph shows contractile force of iCell® CM² over a time span from 7 – 44 days in culture.

Contractile force calculation:

The cells are cultured on flexible silicone membranes less than 10 µM in thickness and sealing the bottom of the FLEXcyte 96-well plate. The membrane deflects downwards due to the weight of the cell culture medium. The inner side of the membrane is covered with a monolayer of hiPSC-CMs. Mechanical rhythmic cell contraction and relaxation of the auto-contractile cardiomyocytes lifts the membrane up and down, respectively. The changes in deflection are measured by capacitive sensing (see Fig.3).

Fig. 3 Contractile force calculation with the FLEXcyte 96 technology



The tension σ in N/mm² is determined from the membrane deflection together with known systemic parameters using Laplace's equation::

$$\sigma = \frac{p}{h} \cdot \frac{r^2}{4s} \cdot \left(1 + \frac{h^2}{r^2}\right)$$

h = Deflection in FLEXcyte 96 well

r = Radius of the well

s = Membrane thickness

p = Hydrostatic Pressure

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