SOPHION BIOSCIENCE | Discover more


# Application Report

## Ethica M: A Complete Solution for Engineered Contractile Tissues

Engineered Heart Tissues at Scale: Automated high-throughput 3D engineered heart tissue assay for disease modelling and drug discovery projects

### Introduction

Cardiovascular diseases (CVDs) account for around 32% of global deaths[1]. Even so, drug development has been hampered in laboratory models due to limited access to human cardiac tissue, the non-regenerative nature of cardiac cells, and species differences[2]. Recent advances in stem cell biology and Organ-On-Chip technologies have led to the creation of three-dimensional (3D) engineered human heart tissues (EHTs), offering a more physiologically relevant system for disease modelling and drug testing compared to traditional 2D models[3]. These 3D models, which often involve a dual-pillar structure and contractile tissue strips, provide valuable insights into contractility. However, challenges remain, such as low throughput and potential drug absorption by materials such as polydimethylsiloxane (PDMS), which collectively limit their use in drug screening[4].

To overcome these challenges, we introduce Ethica M, a cutting-edge automated high-throughput platform and solution that combines scalability, integration and ease of use, in a standard 96-well plate format to generate and measure contractile forces from engineered heart tissues, delivering maximum data points over a given assay duration.

Here we present an experimental workflow for generation of EHTs using the Ethica M solution. After EHT formation, we evaluated their contractility profile by assessing responses to electrical pacing, changes in pacing frequency, and ionotropic drugs. These benchmarks confirmed that Ethica M–derived EHTs can serve as a robust experimental model for assessing cardiac safety risks and supporting drug discovery efforts.

### Ethica M

Ethica M, offered by Sophion Bioscience, is a complete high-throughput, flexible solution for generating engineered contractile tissues (ECT) for functional and pharmacological assays at an industrial scale. As shown in figure 1, the system comprises a standalone pipetting robot, an optical measurement system with integrated pacing and temperature control unit that fits in a 240 L incubator (not provided by Sophion) and a PC with a software suite for system control, data acquisition, handling and analysis, as well as 96-well consumable plates, MChips.

**Fig. 1:** Schematic representation of the Ethica M solution including pipetting robot (left), PC (center) and the optical measurement unit in an incubator (right) (incubator not included).

### ECT formation and contractility measurement with Ethica M

The ECTs are generated in a MChip, a standard 96-well plate format consumable. As shown in figure 2, the MChip is an open-well design. Every well contains customized 3D scaffold with two mini-wells, each having two flexible pillars where cells are directed to form contractile tissues. The formed tissues either contract spontaneously or upon electrical or chemical stimulation. Tissue contractions result in a deflection of the pillars, which is automatically tracked by the optical measurement unit to generate contraction or force plots that further decipher the contractile properties of the tissues.

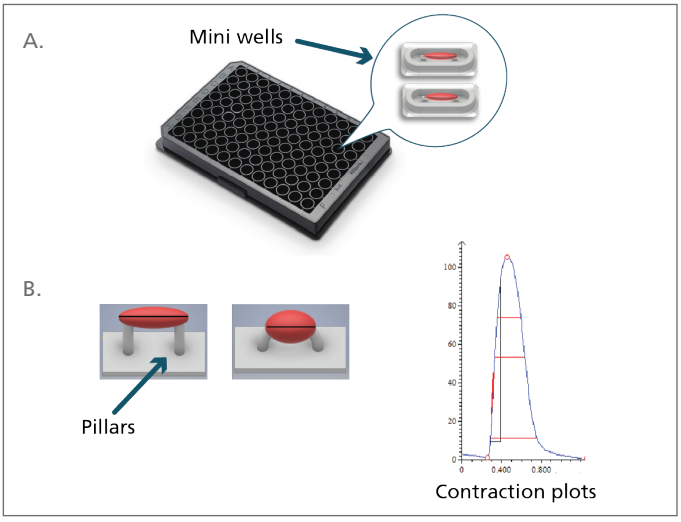
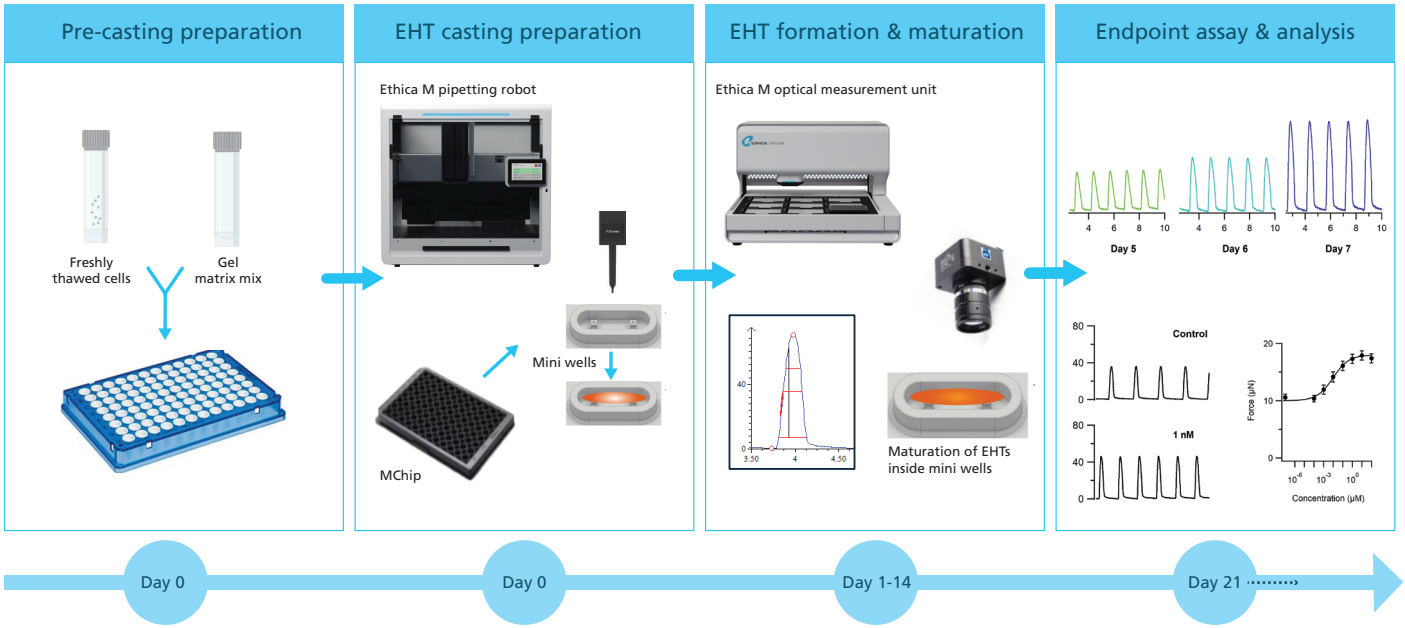



**Fig. 2: A)** Schematic illustration of an MChip containing 3D-engineered scaffolds designed to generate engineered tissues around two flexible pillars. **B)** The deflection of the two pillars is optically tracked and graphically represented as force or contraction plots.

## Key Features:

- **High-Throughput Screening (HTS)**: Supports simultaneous assays on nine standard format 96-well plates.
- **Low cell number:** Requires < 50,000 cells per tissue.
- **Biocompatible materials**: Uses non-PDMS, non-toxic and biocompatible materials for reliable tissue generation and drug studies.
- **Fully automated workflow**: Includes automated tissue casting, assay scheduling and data acquisition.
- **Integrated pacing unit**: The MChip has integrated pacing electrodes and pacing hardware is included in the optical measurement system. Individual pacing regimes can be applied to each column of the consumable giving the user maximum flexibility.
- **High success rate**: Functional tissue formation in more than 80% of the wells.
- **Low volume**: Reduces culture media and compound use with a minimum volume requirement of 200 µL per well.
- **Automated data handling and analysis:** Continuous and fully automated data analysis running in the background as data is acquired.

## Experimental setup with Ethica M




In this study, 3D EHTs were generated using donor-matched human induced pluripotent stem cell-derived cardiomyocytes (iCell® Cardiomyocytes², FUJIFILM Cellular Dynamics) and cardiac fibroblasts (iCell® Cardiac Fibroblasts, FUJIFILM Cellular Dynamics) following the workflow shown in figure above. After thawing the cells are mixed with a gel matrix and aliquoted into a PCR plate and loaded into the pipetting robot. The robot dispenses 3 µL of the cell-matrix suspension (< 50.000 cells) into the mini wells of the MChip to initiate tissue formation.

The seeded MChip is placed in the Ethica M optical measurement unit within an incubator, where user-defined assays are executed automatically. The assay scheduler records videos, applies electrical pacing, and monitors tissue maturation over time, including responses to ionotropic drugs.

Acquired data are processed by the Ethica M analyzer tool, which automatically tracks tissue contractility. Key parameters including contraction amplitude, force, duration and velocity are extracted and displayed in graphs. Raw data are also exportable in .csv format for customized analysis and graph generation using external software.

## Results and Discussion

### Engineered heart tissue formation and maturation:

Five days post -casting, cells had compacted around the measurement posts to form functional EHTs with spontaneous and uniform contractions. Spontaneous tissue contractility was assessed at regular intervals to observe the improvement of contractile force in the EHTs over time. As presented in figure 3A, contraction plots generated by the analyzer show an increase in amplitude over days in culture. To further facilitate maturation of the EHTs, continuous pacing at 1Hz was introduced on day 8 post-casting (figure 3A). The EHTs present an enhanced maturity profile, with an increase in the contractile force generation and narrower pulse width, with days in culture (Figure 3B-E). In a comparative study of EHTs that were either spontaneously beating or subjected to pacing for two weeks, the paced group showed an increase in contraction displacement and a reduction in pulse width (Figure 3F, and G) confirming the EHTs improvement of maturity with pacing.

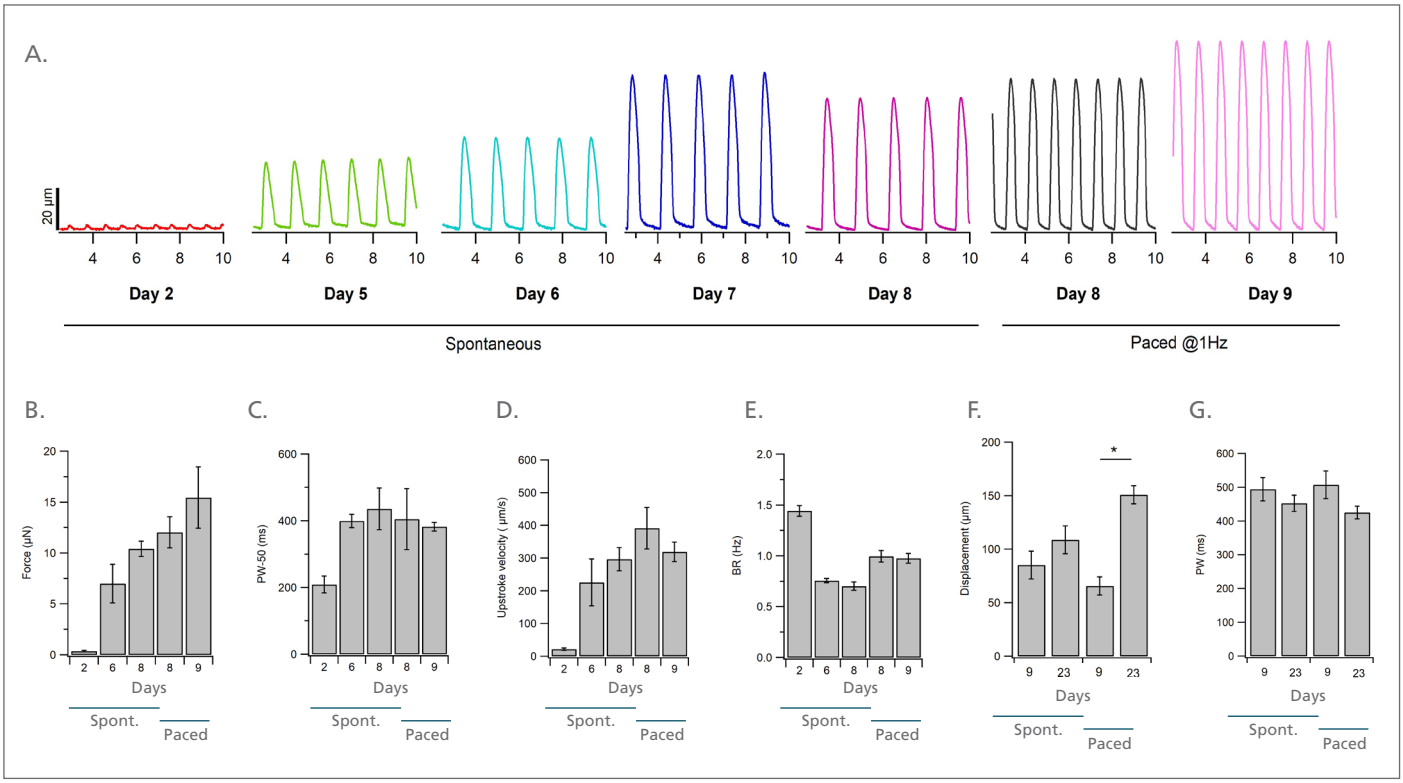



**Fig. 3: A)** Representative contraction waveform acquired from EHTs on different days post casting with either spontaneous (Day 2-8) or paced (Day 8 and 9) contractions. Qualitative comparison of parameters such as the force of contraction **(B)**, pulse width at 50% from peak (PW-50) **(C)**, upstroke velocity **(D)** and beat rate (BR) **(E)** as analyzed from the contraction plots. (F) Effect of pacing on the contraction displacement between day 9 and 23. (G) Effect of pacing on the pulse width between day 9 and 23.

### Physiological force adaptation of EHTs:

In continuous recordings, engineered heart tissues (EHTs) demonstrated a positive force–frequency relationship (FFR) (Figure 4A). When paced at the physiological human heart rate range of 0.8–2 Hz, contraction amplitude increased by approximately 50% compared to the baseline at 0.8 Hz. However, pacing frequencies exceeding 2 Hz resulted in a decline in contraction amplitude and a shortening of pulse duration (Figures 4B, 4C). This biphasic pattern - characterized by a positive FFR in the initial phase and a negative FFR at higher frequencies - closely parallels the response observed in native human myocardium[5]. Intricate balance of calcium handling and tissue composition is key in achieving the adaptation of the force to changes in the pacing frequency in EHTs[6]. We established a study matrix to assess the EHTs responses to the FFR pacing protocol under varying fibroblast sources and extracellular calcium concentrations. As shown in Figure 4D, modulation of extracellular calcium concentration markedly influenced the force–frequency relationship (FFR) response. EHTs maintained in media A, containing physiological calcium levels, exhibited significantly greater contraction amplitudes compared with those cultured in media B under reduced calcium conditions. In addition, the fibroblast source was identified as an additional determinant of the EHTs' response to the FFR pacing protocol.

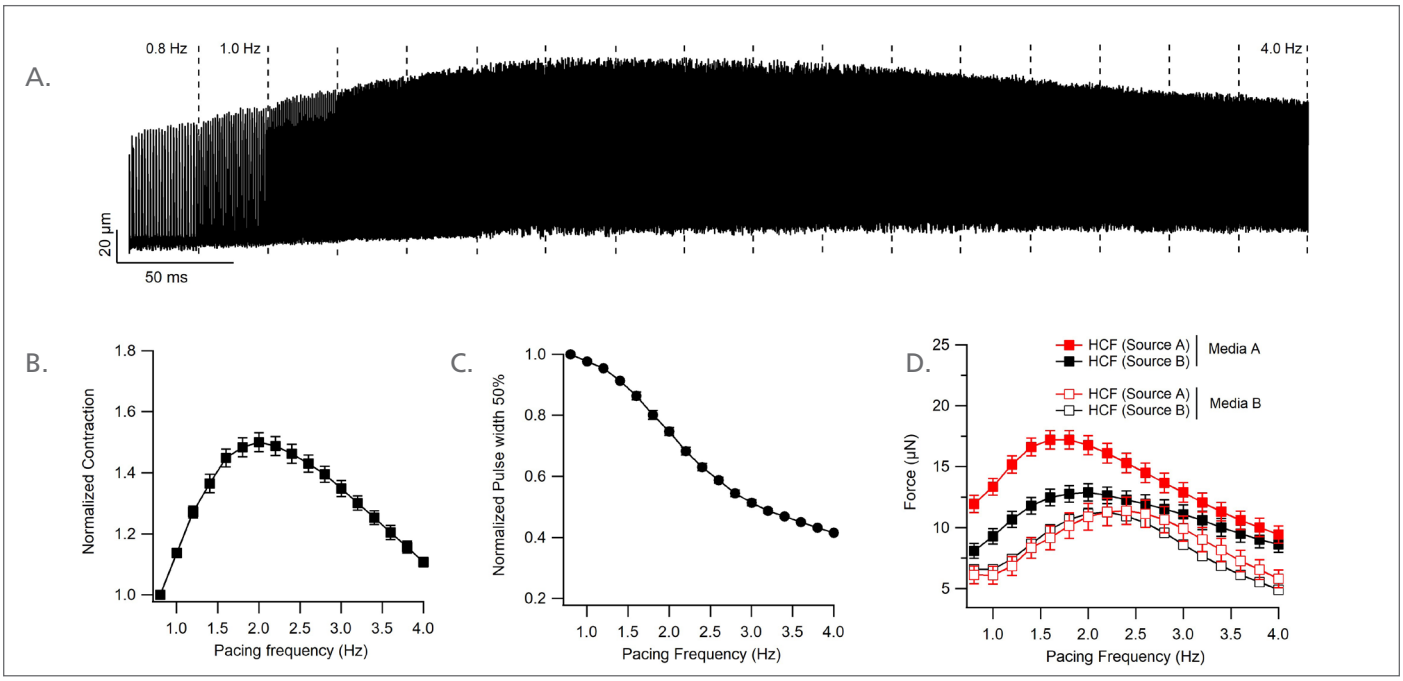



**Fig. 4: A)** Representative contraction waveform from an EHT continuously paced with pacing frequencies increasing from 0.8 Hz to 4 Hz in increments of 0.2 Hz every 30 secs. **B)** Normalized contraction at varying pacing frequencies. **C)** Normalized pulse width at 50 % from peak at varying pacing frequencies. **D)** Force of contraction in response to varying pacing frequency recorded in EHTs containing two different sources fibroblast sources (source A and B), both exposed to either physiological (media A) or reduced (media B) calcium concentration media.

## Pharmacological response of EHTs to ionotropic compounds

To evaluate the responsiveness of EHTs to standard inotropic agents, cumulative dose–response experiments were performed. Spontaneously beating EHTs were exposed to increasing concentrations of isoprenaline, a β-adrenergic receptor agonist and established positive inotrope. Isoprenaline elicited a concentration-dependent increase in contraction amplitude (Figure 5A, B), with an $EC_{50}$ of 9 ± 2 nM, accompanied by a corresponding increase in beat rate (Figure 5C). Conversely, nifedipine, a calcium channel blocker and negative inotropic agent, induced a concentration-dependent reduction in contraction amplitude in EHTs paced at 1 Hz, with an $IC_{50}$ of 41 ± 3 nM (Figure 6A, B). The characteristic responses to these reference inotropes confirm the physiological relevance of the EHT model and underscore its utility in assessing inotropic drug liabilities using the Ethica M solution[7].

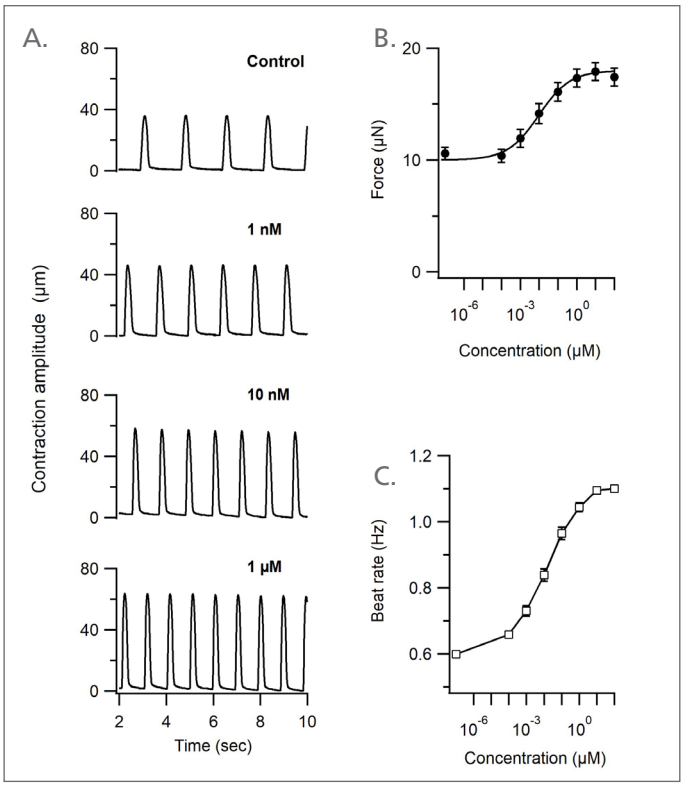



**Fig. 5: A)** Representative traces showing an increase in contraction waveform of spontaneously beating EHTs in response to the positive inotropic drug isoprenaline in a concentration-dependent manner. **B)** Cumulative concentration-response curve for isoprenaline. **C)** Change in beat rate of EHTs in response to increasing concentrations of isoprenaline.

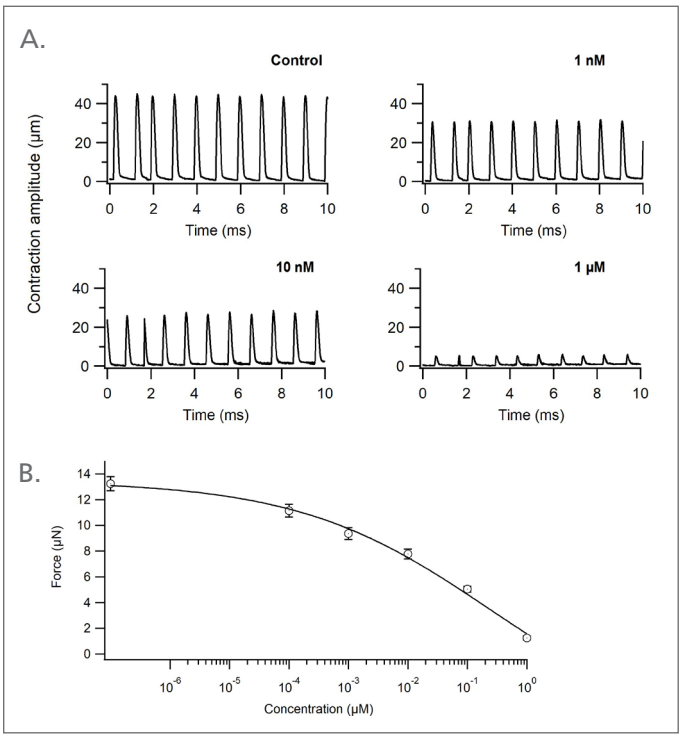



**Fig. 6: A)** Representative contraction waveform showing the decrease in contraction amplitude of EHTs paced at 1Hz in response to the negative inotropic drug nifedipine in a concentration-dependent manner. **B)** Cumulative concentration-response curve for nifedipine at 1Hz pacing.

## Conclusion

We introduce Sophion's Ethica M solution for generating EHTs with advanced maturation and pharmacological characteristics. The correlation between culture duration and pacing-induced increases in contractile force demonstrates progressive EHT maturation on this solution. Furthermore, the positive force–frequency relationship at physiological pacing rates, together with sensitivity to extracellular calcium, highlights the enhanced maturation state of the EHTs and their suitability for downstream applications such as pharmacological testing and disease modeling. The dose-dependent response of EHTs to inotropic agents further confirms functional calcium handling and pharmacological responsiveness. This application note presents key data showing how the Ethica M solution integrates high-throughput capacity and automation to produce robust EHTs that closely replicate human contractile tissues. The integrated assay scheduler and analyzer software enable automated monitoring of tissue contractility, thereby increasing efficiency and accelerating research in cardiac safety assessment, efficacy testing, disease modeling, and precision medicine.

## Methods

All the data presented here is Average ± SEM. Analysis and figures were prepared using the Ethica M Analyzer and Igor 6.3.7.2 (WaveMetrics, Inc. Oregon, USA). Cell culture and handling for the assay was done according to internal Sophion Standard Operating Procedures.

For detailed methods please contact at info@sophion.com

## References


1. Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Fermini, B., Coyne, K. P. & Coyne, S. T. Challenges in designing and executing clinical trials in a dish studies. *J. Pharmacol. Toxicol. Methods* **94**, 73–82 (2018).
3. Stein, J. M., Mummery, C. L. & Bellin, M. Engineered models of the human heart: Directions and challenges. *Stem Cell Rep.* **16**, 2049–2057 (2021).
4. Toepke, M. W. & Beebe, D. J. PDMS absorption of small molecules and consequences in microfluidic applications. *Lab. Chip* **6**, 1484–1486 (2006).
5. Rate-Response Programming Tailored to the Force-Frequency Relationship Improves Exercise in Chronic Heart Failure https://www.jacc.org/doi/abs/10.1016/j.jchf.2017.09.018.
6. Saleem, U. *et al*. Force and Calcium Transients Analysis in Human Engineered Heart Tissues Reveals Positive Force-Frequency Relation at Physiological Frequency. *Stem Cell Reports* **14**, 312 (2020).
7. Mannhardt, I. *et al.* Comparison of 10 Control hPSC Lines for Drug Screening in an Engineered Heart Tissue Format. *Stem Cell Rep*. **15**, 983–998 (2020).



Thank you to our collaborative partner:

FUJIFILM Cellular Dynamics



Sophion Bioscience A/S
info@sophion.com
sophion.com