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Advancing contractile tissue engineering: Insights from Ethica M, an automated and high-throughput solution for 3D contractile tissues

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Cells kindly provided by:
FUJIFILM Cellular Dynamics

Introduction

- **Cardiovascular diseases (CVDs)** account for around 32% of global deaths[1].
- **Challenges in drug development** for CVDs include limited access to human cardiac tissue, non-regenerative nature of cardiac cells, and species differences[2].
- **Advances in stem cell biology and Organ-on-Chip technologies** have led to the development of 3D culture systems, such as engineered heart tissues (EHT).
- **3D culture systems** better mimic the architecture of heart tissues and replicate complex extracellular matrix and cellular interactions[3].
- **Benefits of 3D cultures** include improved accuracy and efficiency in drug testing and disease modeling in cardiovascular research[4].
- **Current 3D culture systems** use a dual-pillar structure to support contractile tissues, but face challenges like high cell requirements, low throughput in functional characterization, and drug adsorption by materials like Polydimethylsiloxane(PDMS)[5].
- **Ethica M** is introduced to overcome these challenges, offering high-throughput scalability, automation, and non-reactive, biocompatible materials in a 96-well plate format.
- **Ethica M** uses human induced cardiac pluripotent stem cells to generate and measure contractile forces from EHT, providing maximum data points over a given assay duration.

Summary

- **Application of EHTs** in drug discovery and safety pharmacology has gained momentum and formation of functional mature EHTs is critical for any 3D cardiac contractility assays.
- **Sophion's Ethica M solution** generates EHTs with enhanced maturity and demonstrates the desired pharmacological response to inotropic compounds.
- **Ethica M solution** combines high-throughput capabilities and automated assay scheduling to generate functional EHTs.
- **Assay scheduler** is key in automating and establishing the robustness of the workflow of tissue engineering, with minimal user interference required.
- **Integrated pacing system** is unique to Ethica M and offers the flexibility and ease of pacing tissues with user defined pacing protocols at desired time window.
- **Automated tracking and analyzing** of tissue contractility improves research efficiency for cardiac safety, efficacy testing, disease modeling, and precision medicine.
- **Positive correlation** between cultivation duration and increased contractile force supports EHT maturation with Ethica M solution.
- **Positive force-frequency relationship** at physiological pacing and sensitivity to extracellular calcium confirm tissue maturity[6].
- **Positive and negative ionotropic and chronotropic responses** were observed with the generic inotropic modulators, reinforcing reliability of the contraction data from Ethica M[7].
- **The Ethica M solution** represents a significant advancement in cardiac tissue engineering for research, therapeutics, and drug discovery.

Methods

Isogenic Human induced pluripotent stem cell (hiPSC) iCell® Cardiomyocytes[2] and iCell Cardiac Fibroblasts were kindly provided by FUJIFILM Cellular Dynamics, Inc. Cell culture and handling for the assay was done according to internal Sophion Standard Operating Procedures.

Media and gel matrix: Please contact us for further details (info@sophion.com).

All the analysis and figures were prepared using the Ethica M Analyzer and Igor 6.3.7.2 (WaveMetrics, Inc. Oregon, USA).

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. Michele, D., et al. Cells in the 3D biomatrix on-chip: better mimicking the real micro-physiological system. *Next Materials* **5**, 100229 (2024).
4. Stein, J. M., Mummery, C. L. & Bellin, M. Engineered models of the human heart: Directions and challenges. *Stem Cell Rep*. **16**, 2049–2057 (2021).
5. Toepke, M. W. & Beebe, D. J. PDMS absorption of small molecules and consequences in microfluidic applications. *Lab. Chip* **6**, 1484–1486 (2006).
6. Shi, S., et al. Physiological calcium combined with electrical pacing accelerates maturation of human engineered heart tissue. *Stem Cell Reports* **17**, 2037-2049 (2022).
7. Mannhardt, I. et al. Comparison of 10 Control hPSC Lines for Drug Screening in an Engineered Heart Tissue Format. *Stem Cell Reports* **15**, 983–998 (2020).

Ethica M: A comprehensive solution for engineered contractile tissues

Fig. 1: Ethica M solution: The package includes: 1) An optical measurement system with high-speed camera and integrated pacing units, with slots for holding nine MChip consumables. The system is placed in a cell-culture incubator (not included with the system). 2) A 96-well plate format consumable, the MChip, has customizable mini-wells for casting contractile tissues and integrated pacing electrodes. 3) A pipetting robot is used for dispensing the cell/ gel matrix into the MChip for tissue formation. The pipetting robot has a HEPA filter and UV lamp for sterile handling of the pipetting process. 4) A user-friendly software package including an Assay scheduler, useful for long-term studies, to automatically tracks tissues and perform data acquisition and the Analyzer software that extracts key contractility parameters and presents results graphically or in an exportable format.

Ethica M solution key features:

- **High throughput screening (HTS):** Up to 9 x 96-well plate assays.
- **Low cell number:** Requires < 50,000 cells per tissue construct.
- **Label free assay:** Optical based readout with high-speed and high-resolution cameras.
- **Biocompatible materials:** Uses non-toxic and biocompatible materials for reliable tissue generation.
- **Fully automated workflow:** Includes automated casting of cell-gel matrix for tissue formation, assay scheduling according to user's experimental needs, data acquisition and analysis.
- **High success rate:** Achieves above 80% success in functional tissue formation.
- **Low volume:** Reduces culture media use with a minimum volume requirement of 200 µL per well.
- **Automated data handling and analysis:** Automatically tracks the contractility of the tissues from the acquired data and extracts key contraction parameters.

Workflow

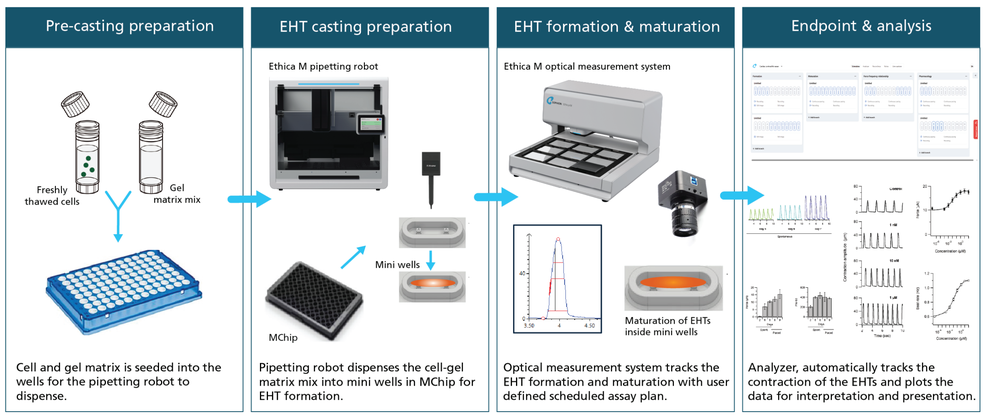

Fig. 2: Experimental workflow: **Step 1 (Pre-Casting preparation):** Mix freshly thawed isogenic human induced pluripotent stem cells (hiPSC) Cardiomyocytes (iCell® Cardiomyocytes[2], FUJIFILM Cellular Dynamics, Inc.) and Cardiac Fibroblast (iCell Cardiac Fibroblast, FUJIFILM Cellular Dynamics, Inc.) with gel matrix and pipette into a standard 96-well PCR plate. **Step 2 (EHT casting preparation):** Load the prepared PCR plate and MChip into the pipetting robot and initiate the EHT casting protocol in the software. The cell/gel mixture is automatically pipetted into each well in the MChip. **Step 3 (EHT formation and maturation):** The seeded MChip is manually placed onto a slot of the optical measurement system to automatically run user defined assays with planned scheduled events which includes timely recording of videos of tissues and pacing of the tissues. **Step 4 (Endpoint analysis):** Once the tissue has achieved desired maturity, various functional, pharmacological or molecular studies can be carried out on the EHTs. Analyze and plot the generated data using the Ethica M software package.

Contractile and mature engineered heart tissues on Ethica M

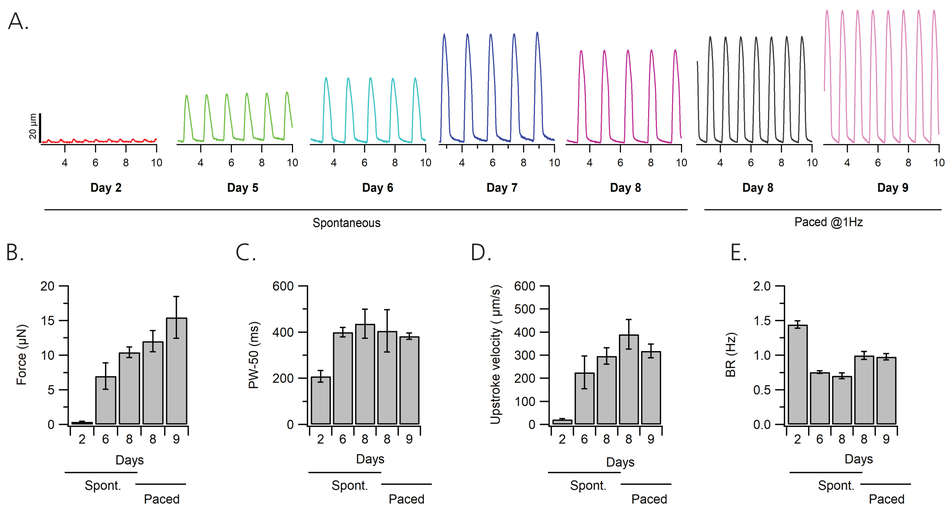

Fig. 3: Improvement of contractility profile of EHTs: A) Representative traces showing the improvement of contraction amplitude over days in culture both when spontaneously contracting and when paced at 1Hz. Qualitative comparison of parameters such as force (B), 50 % of pulse width (PW-50) (C), upstroke velocity (D) and beat rate (BR) (E) demonstrates an improvement in the contractility profile of EHTs over time that is further enhanced by the introduction of pacing.

Engineered heart tissues with positive force-frequency relationship

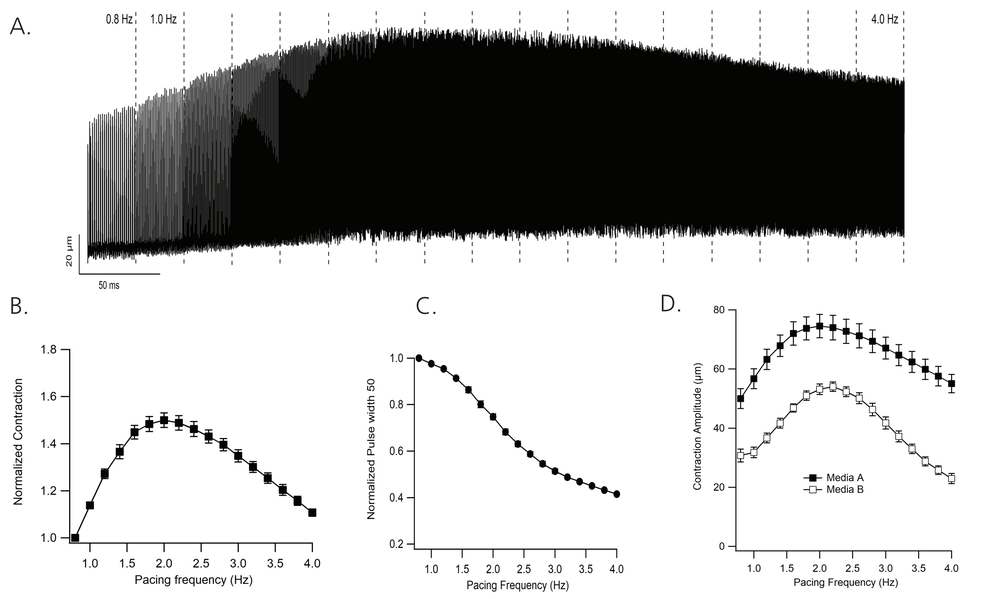

Fig. 4: Physiological adaptation of the EHTs with a positive force-frequency relationship. A) Representative contraction waveform of an EHT paced at increasing frequencies from 0.8 Hz to 4 Hz at increments of 0.2 Hz every 30 secs. B) and C) An average normalized contraction and pulse width of 20 tissues over varying pacing frequency demonstrating a positive force frequency relationship in the primary phase and negative in secondary phase mimicking the expected pattern observed in a non-failing human myocardium. D) Calcium is a key regulator of contractility and the response of EHTs to varying extracellular concentrations of calcium is critical for functional benchmarking. EHTs cultured in media with either physiological calcium concentration (Media A) or lower calcium concentration (Media B) demonstrates the anticipated difference in contraction amplitude in a calcium concentration-dependent manner.

Pharmacological response to Inotropic drugs

Positive inotropic effect

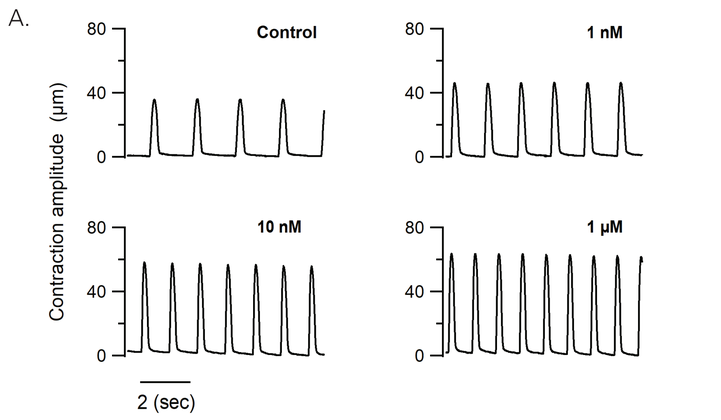

Fig. 5: Positive inotropic and chronotropic effect of isoprenaline: A) Representative traces showing an increase in contraction amplitude (ionotropic effect) and beat rate (chronotropic effect) of spontaneously beating EHTs in response to increasing concentrations. B) shows an increase in the force of contraction in EHTs in a concentration-dependent manner with an EC_{50} of 9 ± 2 nM C) shows an increase in the beat rate of EHTs in a concentration-dependent manner.

Negative inotropic effect

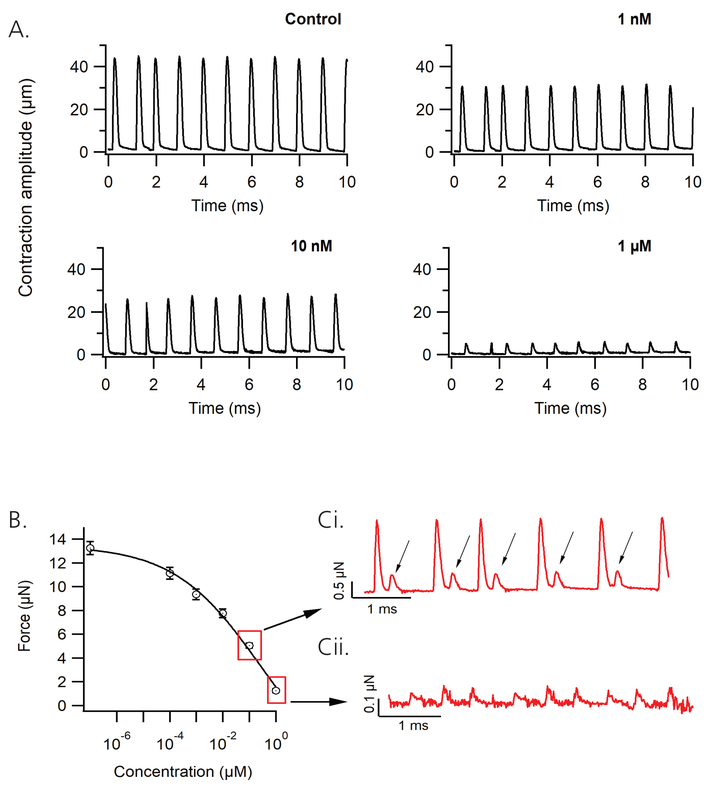

Fig. 6: Negative inotropic effect of nifidipine. A) Representative traces showing a decrease in the contraction amplitude of EHTs paced at 1 Hz in response to an increasing concentration of nifidipine. B) demonstrates a negative inotropic effect on the EHTs in a concentration-dependent manner with and IC_{50} of 41 ± 3 nM. Ci) 1 µM of nifedipine triggered an arrhythmia-like event in some of the EHTs as presented by extra peaks after the paced contraction peaks. Ci) The highest concentration tested, 10 µM, compromised the contractility of the EHTs as observed with lack of any contraction peaks even when electrically paced.