Automated Generation of 3D iPSC-Based Cardiac Microtissues for High-Throughput Calcium Transient Screening

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Background and Purpose

Transition towards in vitro compound screening for safety and efficacy in preclinical drug development needs physiologically relevant test systems reflecting complex biology while allowing for higher throughput applications. While iPSC technology integrates human biology, standard 2D high-throughput assays are often lacking complexity, whereas most 3D models are not allowing sufficient throughput. We have developed a physiologically relevant 3D iPSC-based cardiac microtissue (MT) model that is assembled and maintained by a robotic system and can be used for high-throughput measurement of calcium transients.

Methods

MTs were assembled in 384w plates using a Tecan Fluent robot by combining 70% ventricular cardiomyocytes, 15% endothelial cells, and 15% cardiac fibroblasts differentiated from either a control iPSC line, or an iPSC line harboring a RyR2 mutation recapitulating a phenotype of catecholaminergic polymorphic ventricular tachycardia (CPVT). See Fig. 1 for details.

For Calcium imaging, MTs where loaded with FLIPR Calcium6 dye and transferred to an FDSS µCell imager for subsequent compound addition and recording of calcium transients.
To assess re-oxygenation after ischemia and reperfusion, microtissues were loaded with MitoXpress Intra intracellular oxygen assay probe inside a Clariostar plate reader equipped with environment control (temperature and %O2).

Conclusion

The Heart in a Box cardiac microtissue model can be assembled and maintained at scale in an automated process, displays robust calcium handling properties and is suitable for high-throughput screening in safety / tox studies. Microtissues generated from a CPVT iPSC line recapitulated the phenotype, which could be rescued using clinically relevant compounds, demonstrating that the platform can be leveraged to advance cardiac drug screening

Ncardia's approach for HTS with Cardiac Microtissue

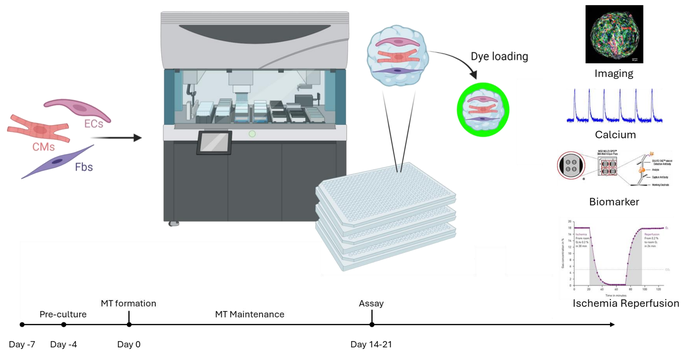

Figure 1. Workflow illustrating the preparation and use of 3D cardiac microtissues from Ncyte® iPSC-derived cardiomyocytes, endothelial cells, and cardiac fibroblasts for high throughput screening (Modified from https://doi.org/10.1016/j.tibtech.2025.11.016.)

Heart in a box microtissues show consistent morphology and functionality

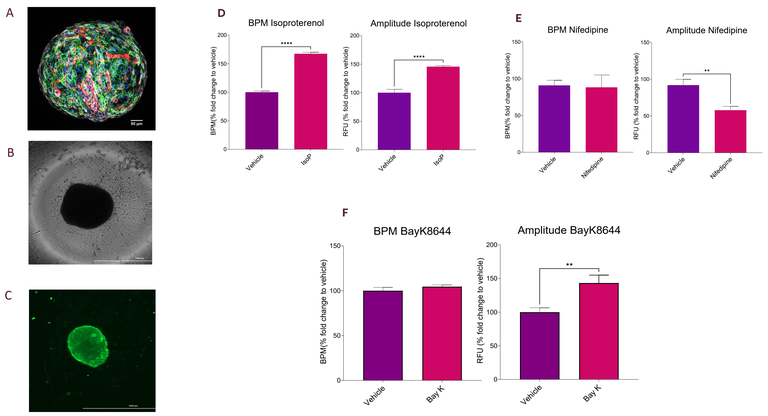

Figure 2. Identity, morphology, and pharmacology of Heart in Box™ Microtissue.

A. Immunostaining of Ncyte® Heart in a Box. Nucleus (blue), Cardiac troponin T (green), CD31 (red), α SMA (white). 20X Image.
B. Brightfield image showing the morphology and structure of a 3D cardiac microtissue
C. 3D cardiac microtissue loaded with FLIPR Calcium6 dye for calcium transient recordings.
D. - F. Microtissues response to tool compounds. Isoproterenol, a β-adrenergic agonist, significantly increases the beating rate BPM) and amplitude, indicating a positive chronotropic effect. Nifedipine, a calcium channel blocker, shows no effect on BPM but significantly decreases the amplitude of contraction in CMs, demonstrating a negative inotropic effect

Additional Application of Ncyte® Heart in a Box™

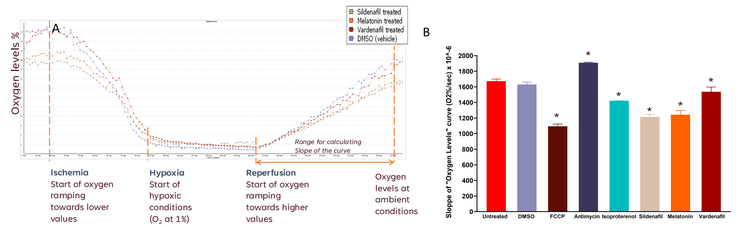

Figure 3.The Ncyte® Heart in a boxTM is model effectively models cellular oxygen consumption during ischemia-reperfusion.

A. Real-time changes in oxygenation (% O2) over time for microtissues treated with DMSO (control), FCCP (uncoupler, increasing oxygen consumption), and Antimycin (complex III inhibitor, decreasing oxygen consumption). Phases of ischemia (oxygen depletion), hypoxia (sustained low oxygen), and reperfusion (oxygen reintroduction) are indicated, highlighting the period used for oxygen consumption rate calculations. B. Bar graph showing the slope of oxygen consumption (O_2%/sec × 10^{-6}) in cells treated with the indicated compounds. Data represent mean ± SEM; *p < 0.05 versus untreated.

Partnership with LUMC – Disease modeling and Drug screening

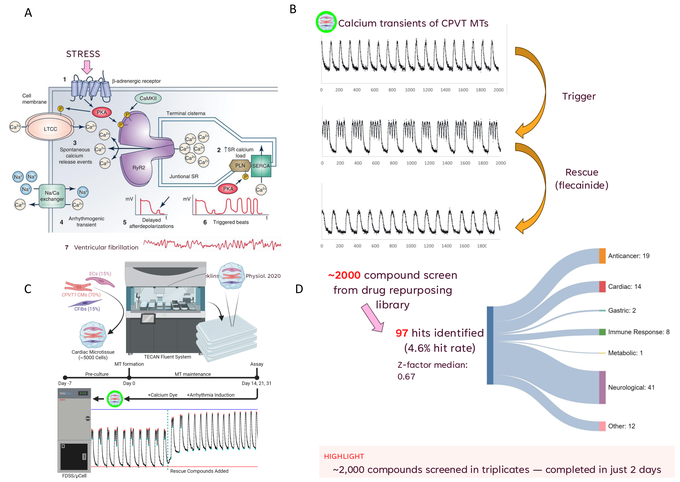

Figure 4. Disease modeling and High-throughput screen in CPVT1.

A. Schematic showing how β-adrenergic stimulation in CPVT1 leads to PKA-mediated phosphorylation of RYR2 on the sarcoplasmic reticulum, resulting in leaky channels, abnormal calcium release, and ventricular arrhythmias.
B. Representative calcium traces from CPVT MTs highlight arrhythmic activity and pharmacological correction.
C. Overview of the fully automated system for forming, maintaining, treating, recording, and analyzing calcium transients in CPVT microtissues.a
D. Alluvial diagram summarizing results from ~2,000-compound screen. 97 hits identified (4.6% hit rate); median Z'-factor of 0.67 confirms assay robustness.

Advancing high-throughput cardiac screening with physiologically relevant 3D iPSC-based models

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