Functional Characterization of Bioreactor-Produced hiPSC-vCM Engineered Heart Tissues for Cardiac Safety Assessment

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Background:

Human-induced pluripotent stem cell-derived ventricular cardiomyocytes (hiPSC-vCMs) have emerged as promising human-relevant tools for cardiac safety assessment and preclinical drug screening. However, broader implementation requires scalable cell manufacturing together with reproducible and physiologically relevant functional outputs. In this study, hiPSC-vCMs (Ncyte® vCardiomyocytes) were incorporated into engineered heart tissues (EHTs) using decellularized myocardial scaffolds (MyoPod® platform), which promote cellular alignment and tissue maturation. Functional performance was evaluated using the MyoLab® platform, enabling continuous assessment of contractility and tissue dynamics.

Methods:

Ncyte® vCardiomyocytes - iPSCs are expanded and differentiated in fully controlled stirred-tank bioreactors (Fig. 1) under defined, serum-free conditions, with continuous monitoring of critical process parameters including dissolved oxygen, pH, agitation, and metabolite profiles from inoculation through harvest. Manufacturing is routinely performed at scales exceeding 1×10^9 to $>1\times10^{10}$ cells per batch, enabling the generation of large, well-characterized cell stocks intended to support screening campaigns spanning multiple years. Reproducibility is monitored through strict, predefined release criteria and data-driven process oversight supported by an internal manufacturing database comprising data from several hundred bioreactor runs across multiple cell types.

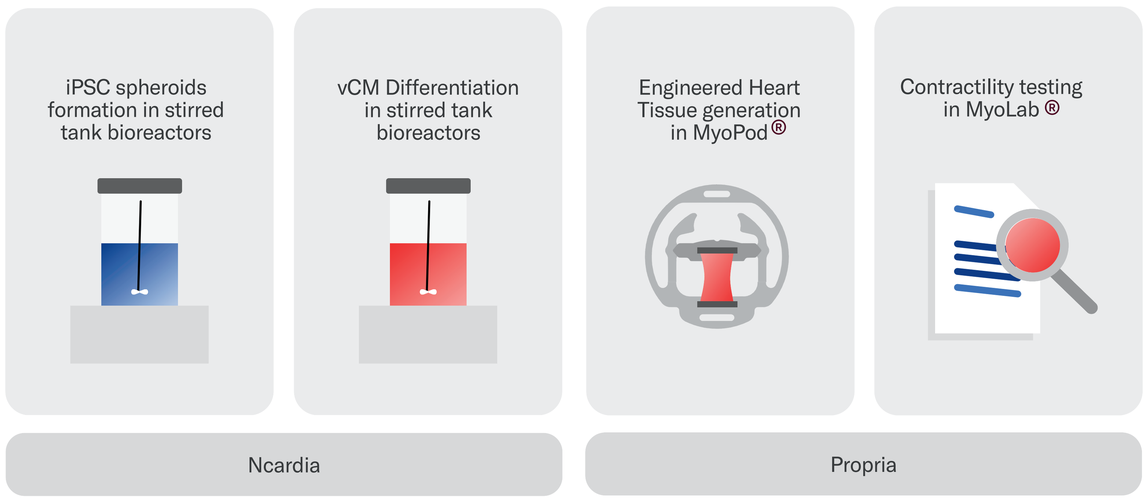

Figure 1. Workflow for generation and functional assessment of hiPSC-vCM EHTs.
Human iPSCs were expanded and differentiated into ventricular cardiomyocytes in stirred-tank bioreactors under dynamic culture conditions. The resulting hiPSC-vCMs were incorporated into engineered heart tissues using the MyoPod® platform and cultured under isometric load. Functional maturation and contractile performance were subsequently evaluated in real time using MyoLab® within a cardiac MPS.

MyoPod® engineered heart tissues and MyoLab® contractility testing – MyoPod®scaffolds are generated from decellularized porcine myocardium and seeded with hiPSC-cardiomyocytes and primary adult human cardiac fibroblasts to form spontaneously beating, 3D engineered heart tissues (EHTs). EHTs are cultured isometrically and can be maintained for weeks to months. Contractility testing is performed in the MyoLab®. EHTs are loaded into the instrument onto a force transducer. Isometric twitch contractions are recorded at physiological temperature and calcium under continuous perfusion and external electrical stimulation. Baseline contractility, response of contractile metrics to frequency and to stretch, and response to acute perturbation with small molecules can be recorded and observed in real time.

Differentiation and Characterization of Ncyte® vCM

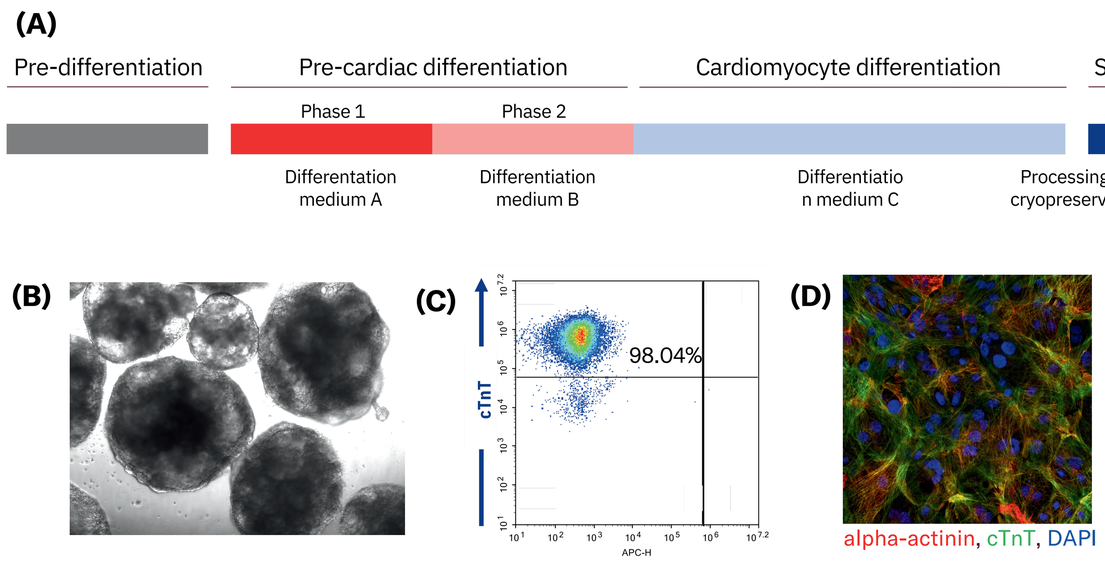

Figure 2. Characterization of Ncyte® human iPSC-derived vCardiomyocytes. (A) Schematic representation of the differentiation process used for Ncyte® vCardiomyocytes manufacturing. **(B)** Bright-field imaging of aggregates generated using stirred-tank controlled bioreactors at the end of differentiation. **(C)** Flow cytometry analysis showing high cardiomyocyte purity based on cTnT expression. **(D)** Immunofluorescence staining of ventricular-like cardiomyocytes demonstrating organized sarcomeric structure (α-actinin, cTnT, and DAPI).

MyoPod® Engineered Heart Tissues and MyoLab® Tissue Analyzer

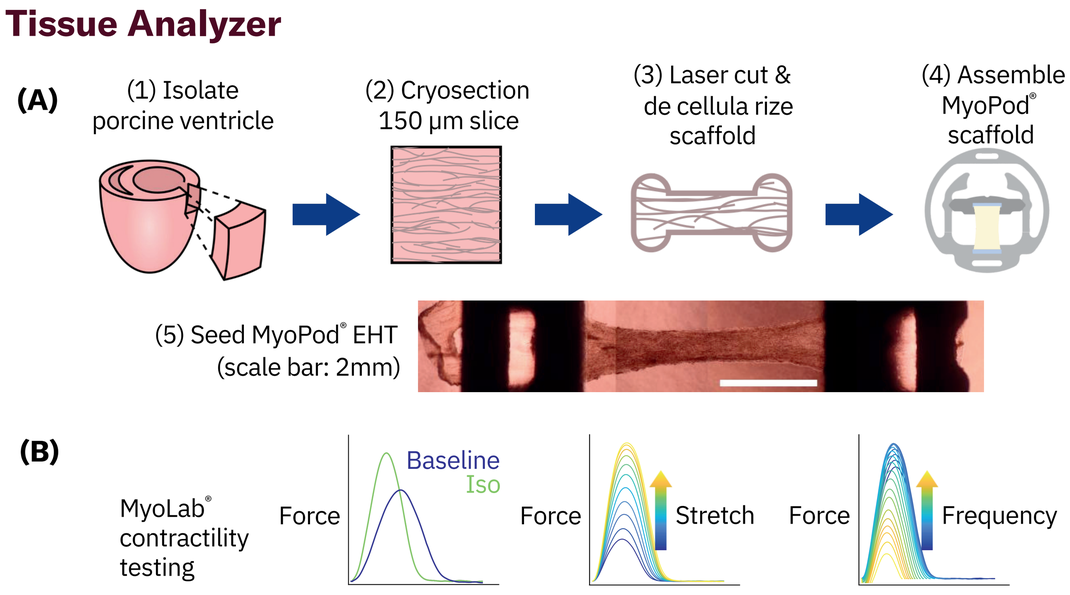

Figure 3. MyoPod® human engineered heart tissues. (A) Schematic of MyoPod® scaffold generation and engineered heart tissue (EHT) seeding. MyoPod® scaffolds are made from aligned, decellularized porcine left ventricular myocardium (steps 1-4). MyoPods® are seeded with hiPSC-CMs and human cardiac fibroblasts (hCFs, PromoCell) at a 10:1 ratio to form EHTs (step 5). EHTs beat spontaneously in culture and are tested after 3 weeks. **(B)** Ncyte® EHTs underwent contractility testing of length-dependent activation (LDA) and force-frequency response (FFR) at baseline and under ß-adrenergic stimulation with 10 µM isoproterenol (Iso).

Ncyte® vCMs form EHTs with a robust ß-adrenergic response

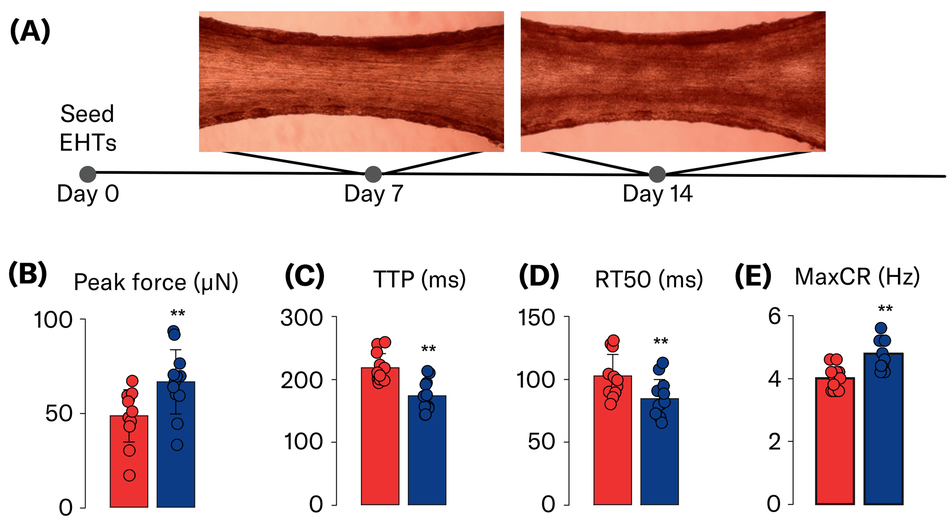

Figure 4. Ncyte® MyoPod® formation, culture, and baseline contractility testing. (A) Timeline of EHT formation, growth, and endpoint testing. **(B-E)** Peak force **(B)**, time to peak force (TTP, **(C)**) and time from peak force to 50% relaxation (RT50, (D)), of EHTs at culture length and minimum capture rate (1-1.2 Hz). **(E)** Maximum capture rate (MaxCR) of EHTs at culture length. Bars are average measure in baseline testing solution (red) and in the presence of 10 µM isoproterenol (blue). Error is standard deviation, circular markers represent average measure for each tissue; n = 12 EHTs. **p < 0.01 vs baseline by student's paired *t*-test.

Ncyte® EHTs exhibit mature length & frequency sensitivity

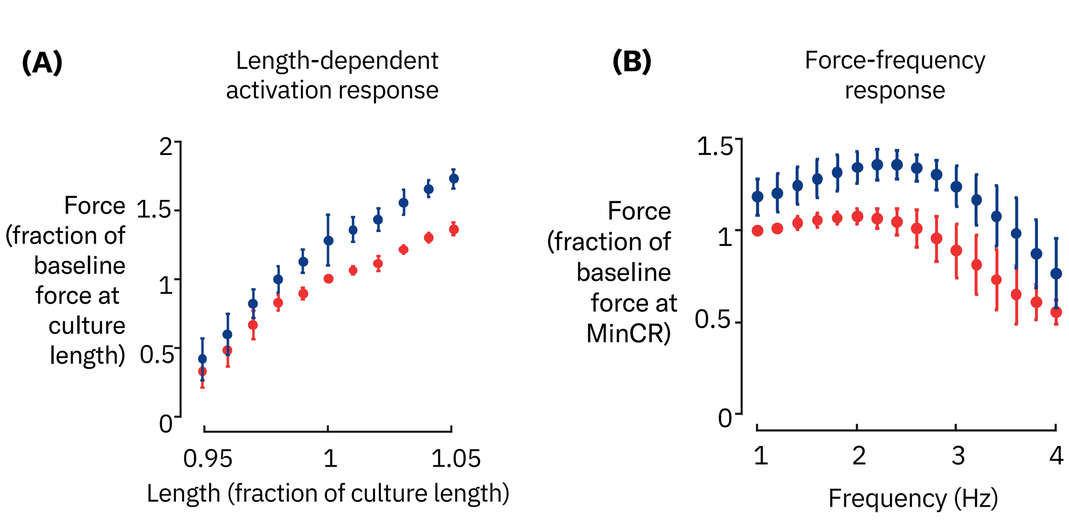

Figure 5. Length and frequency dependence of Ncyte® MyoPod® EHT contractile force. (A) Each marker represents the average peak force internally normalized by the average peak force of each EHT at culture length (length = 1) in baseline testing solution. Error is standard deviation. **(B)** Each marker represents the average peak force internally normalized by the average peak force of each EHT at the minimum capture rate (MinCR) in baseline testing solution. Error is standard deviation.

Conclusion:

vCM-derived EHTs demonstrated a robust inotropic response to β-adrenergic stimulation, with peak force significantly increased by 41.0% ± 20.8% relative to baseline. Kinetic parameters were significantly accelerated in the presence of isoproterenol, including time to peak force, time to 50% relaxation, and time to 90% relaxation, consistent with preserved adrenergic signaling. In addition, tissues exhibited positive force-frequency behavior and physiological length-dependent activation, consistent with hallmark features of mature ventricular myocardium.Collectively, these findings demonstrate that bioreactor-manufactured hiPSC-vCMs generate functionally robust and pharmacologically responsive cardiac tissues with reproducible contractile behavior. The combined MyoPod® and MyoLab® workflow supports physiologically relevant assessment of cardiac function and drug responsiveness, highlighting its utility as a scalable platform for translational cardiac safety pharmacology applications

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