Advanced 2D and 3D Cardiomyocyte-based Models for Use in Drug Discovery

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Abstract #622

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, imposing considerable health and economic burden. The lack of relevant in vitro models hinders the development of cardiovascular drugs or the prediction of cardiotoxicity from new drug candidates. Although human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) provide a promising source of CMs, with the current technology, these in vitro differentiated hiPSC-CMs often fail to recapitulate the phenotype and physiologically relevant functionality. The maturation severely impacts the use of iPSC-CMs in vitro modeling for pathological, pharmacological, or therapeutic purposes since the electrophysiology, mechanical function, and metabolism are suboptimal. Various studies have demonstrated the use of electrical or mechanical stimulation, as well as artificial tissue scaffolds, to promote the maturation of hiPSC-CMs in vitro. However, these techniques often require months and have low throughput.

To address these issues, we have focused our work on harnessing the power of the ATCC Maturation Reagent (AMR™) to effectively propel the maturation of hiPSC-CMs in 2D monolayer culture. This method paves the way for the mass production of high-quality and mature hiPSC-CMs that exhibit a mature phenotype with regard to morphology, structure, gene expression, metabolism, calcium handling, and contractile performance.

In addition, we are integrating this enhanced cardiomyocyte function methodology with the 3D technology Robot-Directed Organoid Deposition (RODEO) to further advance the creation of more in-vivo-like cardiac models. The combined power of these technologies holds the promise to revolutionize the creation of in vitro cardiac tissue that truly mimics the structural and biochemical properties of the cardiac environment. The resulting vascularized multichambered cardiac organoids are highly similar to adult cardiac muscle transcriptionally and respond to drugs that mimic physiological conditions. This disruptive high-throughput technology underscores the utility of microphysiological systems for use in the drug discovery process and improving clinical success rates.

Method

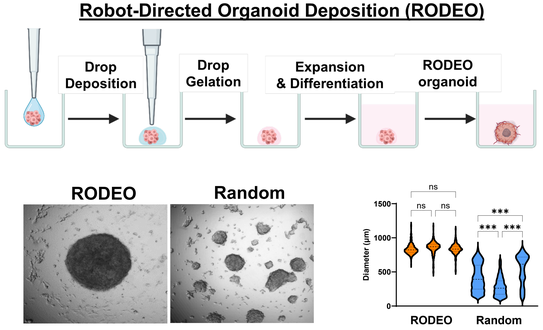

Figure 1: RODEO technology generated consistently sized organoids, facilitating the high-throughput manufacturing of organoids in a well-plate format for drug screening.

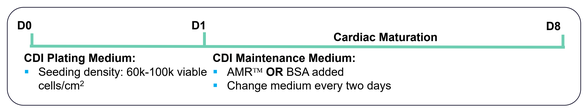

Figure 2: ATCC Maturation Reagent (AMR)™ tested on iCell® Cardiomyocytes²(Fujifilm® CDI).

Results

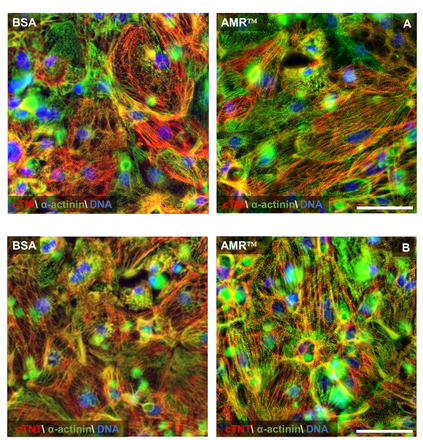

Figure 3 - AMR™ enhances cardiac tissue organization. iCell® Cardiomyocytes were and maintained for 7 days in CDI maintenance medium supplemented with BSA or AMR™. The cells were fixed and stained for DNA, cardiac troponin I (cTNI) or cardiac troponin T (cTNT), and alpha-actinin. (A) AMR-treated cells show organized and aligned cardiac fibers, while BSA-treated cells display a mix of organized and disorganized fibers. Scale bar = 50 µm. (B) Cells treated with AMR™ exhibit organized sarcomeres along the tissue, while BSA-treated cells show mixed sarcomere behavior. Scale bar = 20 µm. (C) AMR™ promotes sarcomere elongation and fiber organization with significantly organized ultrastructure and aligned mature cardiac fibers. Scale bar = 20 µm. (D) Statistical significance determined by Unpair student t-test, ***p<0.001; n=4; >9 fibers, >12 sarcomeres for each repeat. Error bars = S.E.M

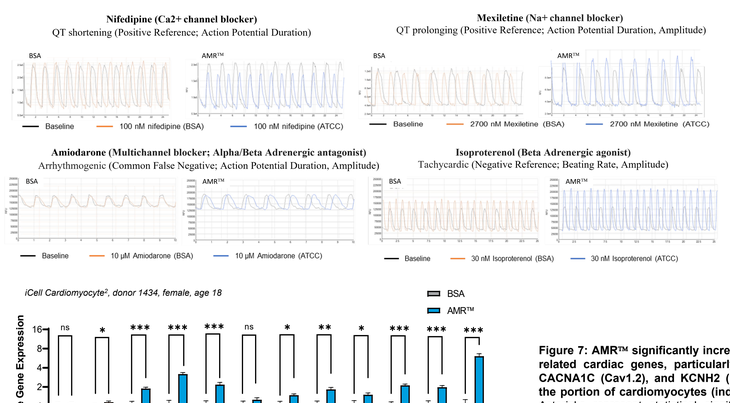

Figure 6: AMR™-treated CMs have increased drug response to cardio responsive compounds. iCell® Cardiomyocytes² were treated with a range of doses of cardio-responsive test compounds and evaluated for changes in their electrical activity. The activity was measured using a voltage-sensitive indicator FluoVolt® Membrane Potential dye or a calcium-sensitive indicator, EarlyTox™ Cardiotoxicity dye. Doses were selected according to the physiological concentration of each drug.

Figure 7: AMR™ significantly increases gene expression of key function-related cardiac genes, particularly the ion channels SCN5A (Nav1.5), CACNA1C (Cav1.2), and KCNH2 (hERG), without significantly affecting the portion of cardiomyocytes (indicated by early cardiac marker cTNT). Asterisks represent statistical significance over BSA in multiple unpaired student t-tests with a two-stage step-up method (Benjamini, Krieger, and Yekutieli) FDR correction for multiple comparisons.

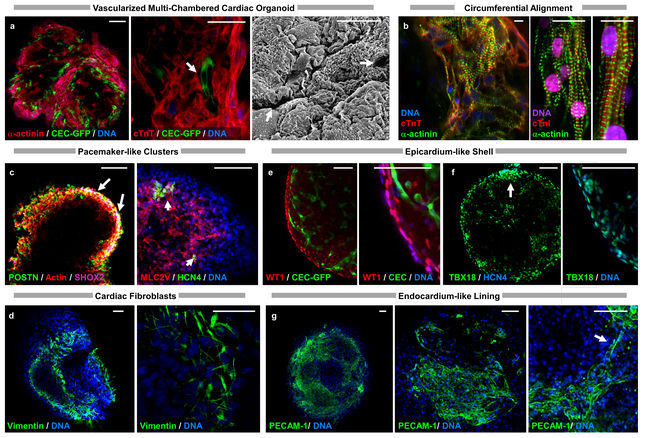

Figure 7: Generation of hiPSC-derived vascularized cardiac organoids. (A) "Vascularized cardiac organoids formed with cardiac endothelial cells (CEC-GFP) and stained for cardiac Troponin T (cTnT) and Alpha-actinin. The organoid shows aligned cardiac fibers surrounding the chambers embedded with a vascular network." The organoids ' wall displays aligned cardiac fibers (cTNT) pierced with a distinct microvasculature (white arrow). Scale bar, 50 µm. Scanning electron micrograph of vascularized cardiac organoid demonstrates lumen formation (white arrow). Scale bar = 10 µm. (B) High magnification image of a vascularized cardiac organoid stained for cardiac Troponin T (cTnT) and Alpha-actinin, showing mature cardiac tissue phenotype with aligned sarcomeres. Scale bar = 10 µm. (C) Confocal image of a vascularized cardiac organoid showing human Periostin (POSTN), Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4), and short stature homeobox protein 2 (SHOX2) staining. The organoid contains POSTN cardiac fibroblast-like cells and cell clusters expressing HCN4 and SHOX2 pacemaker-associated markers. Scale bar = 100 µm. (D) Vascularized cardiac organoid displaying human vimentin (VIM) staining. The organoid's myocardial layer is interwoven with VIM-positive cardiac fibroblast-like cells. Scale bar = 50 µm. (E) Confocal image of cardiac organoids stained for Wilms tumor protein (WT1) and the T-box transcription factor 18 (TBX18) with a single epicardium shell and nuclei showing positive staining for both markers. Scale bar = 100 µm. (G) Cardiac organoids stained for human platelet endothelial cell adhesion molecule (PECAM-1), showing membranal distribution within the chamber, indicating endocardial lining. The white arrow indicates a capillary-like structure. Scale bar = 50 µm.

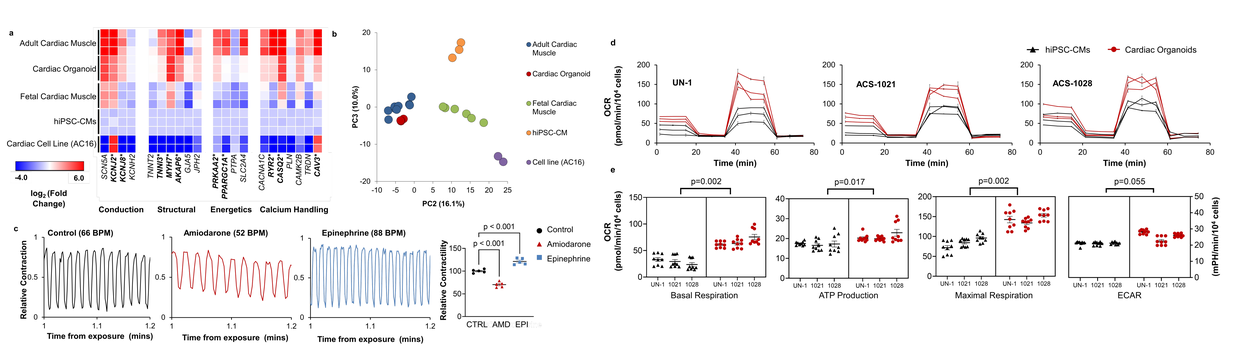

Figure 8: Functional characterization of human cardiac organoids generated by RODEO using ATCC iPSC lines. (A) Heatmap of key cardiac gene markers from RNA-seq analysis of hiPSC-derived cardiomyocytes (ATCC® ACS-1021™), fetal cardiomyocytes, vascularized cardiac organoids, and adult cardiomyocytes. Bold, asterisked genes represent differential expression between cardiac organoids and fetal cardiac muscle, 3 biological replicates, FDR<0.05 (*methods*). (B) Principal component analysis (PCA) of 513 genes differentially expressed between hiPSC-derived cardiomyocytes and vascularized cardiac organoids. Cardiac organoids cluster with adult, but not fetal cardiomyocytes. (C) Visual contraction analysis of UN-1 vascularized cardiac organoids treated with DMSO (Control), 10 µM amiodarone, or 100 µM epinephrine, normalized to the highest and lowest signal recorded through the entire measurement duration (30 seconds; *methods*). Analysis shows that untreated organoids acquire a homogenous synchronized spontaneous beating of 66±5 beats per minute. Stimulation with 100 µM epinephrine increases the contraction rate to 88±7 bpm and relative contraction by 18% (n=5, p<0.001), while stimulation with 10 µM amiodarone decreased the rate to 52±4 bpm and contraction by 28% (n=5, p<0.001) , resulting in a physiological-like response to the drugs. Mean of 5 biological replicates; Error bars = SEM. Significance was determined using a one-way ANOVA with Dunnett correction. (D) Seahorse MitoStress test comparing cardiac organoids and hiPSC-derived cardiomyocytes (hiPSC-CMs) from three independent hiPSC donors. Lines represents independent experiments; error bars mark standard error of mean among n=3 biological repeats. (E) Nested analysis shows that the basal respiration of cardiac was 132% higher than hiPSC-cardiomyocytes (n=9, p<0.01), oxidative phosphorylation increased by 23% (n=9, p<0.05), and maximal respiratory capacity increased by 70% (n=9, p<0.01). ECAR, a surrogate for glycolysis, did not change significantly. Middle represents mean of 3 biological repeats in 3 independent experiments for each line; Error bars = SEM. Significance was determined using a two-tailed nested t-test.

Summary

- AMR™ effectively enhances the maturation of iPSC-derived cardiomyocytes in 2D monolayer culture.
- RODEO, a 3D high-throughput technology, advances the creation of in-vivo–like in vitro cardiac models.
- These vascularized multichambered cardiac organoids underscore the utility of microphysiological systems for use in drug discovery.

Reference

Ghosheh M, et al. Electro-metabolic coupling in multi-chambered vascularized human cardiac organoids. Nat Biomed Eng 7(11):1493-1513, 2023.

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