Fontan procedure, includes connection of the vena cava (VC) to the pulmonary artery (PA) using a bio-inert conduit to reduce work required by the right ventricle (RV). While this operation greatly extends the lives of HLHS patients, the Fontan circuit eventually fails, and the only solution is a scarcely available donor heart. This failed circuit is explained by the "Fontan paradox" where central venous pressures build up over time, causing increased systemic resistance and congestion. The absence of the sub-pulmonary ventricle leads to abnormal hemodynamics associated with life-threatening complications. We believe that decreasing central venous pressures through the use of a tissue engineered contractile, patient specific conduit will decrease the amount and severity of complications caused by the "Fontan paradox." We will use amniotic fluid derived induced pluripotent stem cells (AF-iPSCs) differentiated into cardiomyocytes (CMs) to generate flow within a biodegradable conduit. Additionally, AF-iPSC will be differentiated into structural support cells (SSCs), including cardiac fibroblasts and epicardium. Several studies suggest advanced contraction and structure of CMs in specific ratios with SSCs, particularly mouse and human fetal fibroblasts. In combination, these cells have shown advanced tissue organization and function through mechanically and electrically aligned junctions. This allows them to have a magnitude higher contractile force than CMs alone, making them ideal for increasing pressure within a tissue engineered construct. This poster focuses on the differentiation and selection of SSCs. METHODS/STUDY POPULATION: AF-iPSCs differentiation began at roughly 80% confluency. Mesoderm formation occurred via WNT pathway modulation by supplementing RPMI+insulin media with 0.5 ng/mL BMP4 at day 0, followed by 3 ng/mL BMP4, 2 ng/mL Activin A, and 5 ng/mL BFGF for four days. Then, RPMI+insulin media was supplemented with 10 ng/mL of BMP4 until day fifteen for epicardial formation. Cells were lifted to induce epithelial-to-mesenchymal transition (EMT) and RPMI-insulin media was supplemented with 10 ng/mL BFGF for cardiac fibroblasts. They were then harvested and characterized using immunofluorescence. Planned experiments include RT-qPCR for further characterization of cardiac fibroblasts. Additionally, a fibroblast isolation plating technique will be utilized to obtain cardiac fibroblast from AF-iPSC CMs and AF-iPSC epicardium. Commercially obtained human cardiac fibroblasts will be utilized as a control for all studies. RESULTS/ANTICIPATED RESULTS: Immunofluorescence (IF) revealed positive expression of vimentin and α-SMA indicating a fibroblast and vascular smooth muscle phenotype after supplementation with 10 ng/mL of BMP4 after EMT induction. It is expected that IF of epicardial formation at day 15 will show positive expression of WT1, a well-known epicardial marker. We also suspect RT-qPCR will reveal high expression of cardiac fibroblast specific markers COL1A1, PDGFA, TCF21, and THSB1. We expect to yield a higher number of cardiac fibroblast from the small molecule AF-iPSC differentiation compared to a timed plating technique of AF-iPSC CMs and AF-iPSC epicardium (separately plated). Results will be quantified and compared using the aforementioned techniques. DISCUSSION/SIGNIFICANCE OF IMPACT: Discussion/significance of impact: Although fibroblasts make up a large portion of cells in the heart and greatly enhance CM function, they are poorly characterized in the literature and not easily obtained. This study will provide an efficiency comparison on the best method for acquiring cardiac fibroblast for cardiac tissue engineering applications as we move forward with translational cardiac pediatric research.

3444

Development of human engineered cardiac tissue (hECT)-based screening assay to explore cardiac contractile properties in response to pharmacological challenge with proarrhythmic drugs

Francesca Stillitano, Irene C. Turnbull, Jaydev Dave, Jean-Sébastien Hulot and Roger J. Hajjar
Mount Sinai School of Medicine; Icahn School of Medicine at Mount Sinai; Institute of Cardiometabolism and Nutrition (ICAN); Icahn School of Medicine at Mount Sinai

OBJECTIVES/SPECIFIC AIMS: The goals of this study were (1) to evaluate the effect of proarrhythmic drugs on calcium transient and (2) to use three-dimensional human engineered cardiac tissue (hECT) technology to evaluate cardiac contractile properties in response to pharmacological challenge with proarrhythmic drugs. METHODS/STUDY POPULATION: Calcium transient was measured in subject-specific iPSC-CMs by using the IonOptix system in Sotalol treated vs. untreated conditions. We fabricated human engineered cardiac tissues (hECT) in a custom designed bioreactor using low- and high-sentitive subject-specific iPSC-CMs. Contractile function of the hECT was evaluated at baseline and after Sotalol [300 μM] administration. The change in beat rate was recorded under spontaneous beating conditions; changes in other twitch parameters, including time to relaxation, were recorded under electrical stimulation. Time to relaxation served as an indicator of action potential duration (APD), which has a temporal correlation with the QT interval. RESULTS/ANTICIPATED RESULTS: The low-sensitive iPSC-CM showed a considerable drop in overall peak height of the calcium transient, in the presence of 100 μM Sotalol. The high-sensitive line, however, showed a more pronounced drop in peak height. Sotalol treatment also induced a more pronounced increase in the exponential decay time constant (tau) in the high-sensitive line compared to the low-sensitive line. The hECT fabricated with high sensitive hiPSC-CM showed a larger decrease in spontaneous beat rate in response to Sotalol (0.41 vs 0.23 fold decrease), with a higher increase in time to relaxation (1.8 vs 1.3 fold increase), compared to hECT from low sensitive hiPSC-CM. Moreover, while the low-sensitive hECT showed a positive correlation between time to relaxation and developed force, as expected after Sotalol stimulation; the high-sensitive hECT failed to show a positive inotropic response. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest subject-specific iPSC-CMs and hECT, can be used to model functional abnormalities observed in diLQTS in response to Sotalol, and offer novel insights into human-based screening assays for toxic drug reactions. Success of this study may help identify key components underlying diLQT susceptibility to ultimately develop novel therapeutic agents.

3521

Distinct single cell gene expression in peripheral blood monocytes correlates with treatment response groups to TNF-alpha inhibition in rheumatoid arthritis

Theresa Wampler Muskardin[1], Zhongbo Jin[2], Jessica M. Dorschner[3], Yogita Ghodke-Puranik[1] and Timothy Niewold[1]
[1]New York University - H+H Clinical and Translational Science Institute; [2]University of Florida and [3]Mayo Clinic

OBJECTIVES/SPECIFIC AIMS: The cellular mechanisms that underlie the IFNβ/α ratio that predicts response are not known. Effects of IFN on single immune cells may be masked in whole blood

https://doi.org/10.1017/cts.2019.23 Published online by Cambridge University Press