sustained VT or VF, from 90% to 12.5% of animals (p<0.001, Figure 1C).

**Conclusion:** Chronic VNS ameliorates post-MI electrophysiologic remodeling and reduces the risk of VT and VF.

**PO-645-04**

**GENERATION AND MULTIPARAMETRIC TESTING OF ENGINEERED HEART TISSUE**

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**Background:** Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have become an essential tool for disease modeling and drug screening, although their immature phenotype may limit their utility.

**Objective:** Our aim is to generate engineered heart tissues (EHTs) from hiPSC-CMs to promote maturation and to perform simultaneous multiparametric analyses that inform pathophysiology and drug responses.

**Methods:** hiPSC-CMs were expanded with high purity (Fig 1a). qPCR was employed to assess the expression of relevant cardiac markers. We used immunostaining to perform structural assessments, including sarcomere alignment (Fig 1b). We built a custom rig capable of measuring force and speed of contraction, measures of relaxation, voltage and calcium transients, and conduction velocity (Fig 1c, d). We incorporated a stimulation system to control frequency. We compared EHTs of a disease hiPSC line carrying a truncating variant in *Titin* (WT/TTNtv) and its isogenic control generated by CRISPR/Cas9-mediated genome editing (WT/WT), as well as an unrelated healthy hiPSC line.

**Results:** Based on the qPCR data, the EHTs were more mature than 2D hiPSC-CM cultures. Contractile force was dependent on frequency as demonstrated by pacing protocols. We demonstrated a decrease of 0.07 mN in force of contraction in the disease line compared to its isogenic control (Fig 1e). We are able to interrogate voltage and calcium transients in spontaneous and paced EHTs (Fig 1f).

**Conclusion:** We have successfully generated EHTs and performed multiparametric testing of normal and disease cell lines using a custom rig and stimulation system. We are currently performing drug screening assays using the aforementioned quantitative outputs.

**Figure 1.** Generation and characterization of patient-derived iPSC model of an inherited heart disease. a. Each iPSC line can be consistently differentiated to CMs as quantified by flow cytometry analysis for cardiac troponin T (cTnT)(n). b. Imaging of EHT sarcomere alignment using confocal immunofluorescence microscopy. EHT stained with Titin M-line and DAPI. 20X, AiryScan and 3x Zoom. c. Schematic of EHTs in an apparatus for live imaging. Organsoids are mounted on flexible posts in a 24-well plate, allowing for determination of contractile force by measuring deflection of the posts. d. Frames from video captured during a typical EHT experiment. The same organsoid is shown relaxed (top) and contracted (bottom). e. EHTs generated from the disease and control cell lines differ in their measured contractile force. Reduced force generation in the WT/TTNtv line is consistent with the clinical presentation of the patient from whom this line was derived. f. Representative voltage (blue) and calcium recordings (red) from spontaneously beating EHTs.

**PO-645-05**

**CARDIAC ALTERNANS AS A BIOMARKER OF DYNAMIC ELECTROPHYSIOLOGICAL CHANGES IN ARRHYTHMOGENIC ISCHEMIC SUBSTRATES**

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**Background:** T-wave alternans (TWA) has been associated with an increased predisposition to ventricular tachyarrhythmias (VT/VF). Dynamic electrophysiological changes due to acute ischemia and chronic myocardial infarction (MI) are a known substrate for VT/VF.

**Objective:** Evaluate the potential of cardiac alternans as a biomarker for dynamic electrophysiological changes in ischemic substrates enabling arrhythmogenesis in an ovine chronic MI model.

**Methods:** ECG signals were collected at baseline, during occlusion (acute MI) and 4-6 weeks (W) post MI (chronic MI), in sheep. TWA burden was compared between sham (n=5) and chronic MI (n=14) groups at all 3 time points. At 6W, ex-vivo optical mapping experiments were performed on the endocardium of the left ventricle (LV). Langendorff perfused wedge preparations (n=8) were paced at increasing rates (1Hz-5Hz) until loss of capture or VT/VF induction. 2D action potential duration (APD) and amplitude (APA) maps were analyzed to assess the spatiotemporal evolution of localized cardiac alternans in sham and MI hearts.

**Results:** Chronic MI led to increased heart rate (Fig.1A) and significant ST segment elevation during occlusion, which normalized 6W post MI (Fig.1B). Rise in TWA burden was observed during the occlusion which returned to baseline at 6W (Fig.1C). In chronic MI hearts, at mean frequencies of 2.2±0.5Hz and 3.3±0.1Hz, spatially concordant and discordant APA and APD alternans were observed in multiple discrete regions. Localized APA and APD alternans preceded onset of VT/VF (Fig.1D). APA alternans spatially evolved with an increase in pacing frequency, while APD alternans were sporadic and independent of pacing rate. Mean area of the LV
endocardium covered by APA alternans increased significantly from 14.15±6.5% at 1Hz to 32.97±7.3% at 5Hz (Fig.1E).

**Conclusion:** TWA can serve as a biomarker of dynamic changes in ischemic substrates and localized APA and APD alternans underlie arrhythmogenesis in chronic MI ovine hearts.

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**PO-645-06**

**EPICARDIAL ELECTRICAL HETEROGENEITY AFTER AMIODARONE TREATMENT INCREASES VULNERABILITY TO VENTRICULAR ARRHYTHMIAS IN A SWINE MODEL UNDER THERAPEUTIC HYPOTHERMIA**

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**Background:** Amiodarone is commonly used during therapeutic hypothermia (TH) following cardiac arrest due to ventricular arrhythmias.

**Objective:** Electrophysiological changes and proarrhythmic risk after amiodarone treatment have not yet been explored in TH.

**Methods:** Epicardial high-density bi-ventricular mapping was performed in pigs under baseline temperature (BT), TH (32-34°C), and amiodarone treatment during TH. The total activation time (TAT), conduction velocity (CV), local electrogram (LE) duration, and wavefront propagation from pre-specified segments were analyzed during sinus rhythm (SR) or right ventricular (RV) pacing (RVP), along with tissue expression of connexin 43. The vulnerability to ventricular arrhythmias was assessed.

**Results:** Compared to BT, TH increased the global TAT, decreased the CV, and generated heterogeneous electrical substrate during SR and RVP. During TH, the CV reduction and LE duration prolongation were greater in the anterior mid RV than in the other areas, which changed the wavefront propagation in all animals. Compared to TH alone, amiodarone treatment during TH further increased the QTc/ Tpeak-Tend, TAT and LE duration and decreased the CV in both the global heart and regional areas during SR and RVP. Heterogeneous conduction was partially attenuated after amiodarone treatment. After TH and amiodarone treatment, the connexin 43 expression in the anterior mid RV was lower than that in the other areas, compatible with the heterogeneous CV reduction. The animals under TH and amiodarone treatment had a higher incidence of inducible ventricular arrhythmias than those under BT or TH.

**Conclusion:** Electrical heterogeneity during amiodarone treatment and TH was associated with vulnerability to ventricular arrhythmias.

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**PO-645-07**

**SLEEP-RELATED CHANGES IN CARDIOVASCULAR AUTONOMIC REGULATION IN APOE KNOCKOUT RATS: NEURAL MECHANISM FACILITATING ARRHYTHMIA IN HYPERCHOLESTEREMIA**

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**Background:** The plasma levels of total cholesterol and low-density lipoprotein (LDL) cholesterol are important risk factors for coronary heart disease. Several reports suggest that elevated plasma cholesterol also leads to fetal arrhythmias.

**Objective:** We aimed to evaluate the LDL cholesterol and its relationship to cardiac autonomic activity, especially its relationship to sleep cycle.

**Methods:** Wireless transmission of polysomnographic recording was performed in wild type (n=5) and ApoE knockout (hypercholesteremia with elevated LDL-C, n=4) rats during 24 hours. Spectral analyses of the electroencephalogram (EEG) and electromyogram (EMG) were evaluated to define active waking (AW), quiet and paradoxical sleeps (QS, PS). Cardiac autonomic activities were measured by analyzing the power spectrum of heart rate variability (HRV).

**Results:** In the HRV analysis, we found that the LF/HF ratio and LF significantly increased in ApoE knockout rats during QS stage compared to that in normal rats (Fig A and C). The decreased QS times were noted in ApoE knockout rats compared to the normal rats (Fig E). Cardiac autonomic activities were measured by analyzing the power spectrum of heart rate variability (HRV).

**Conclusion:** We found that sympathetic hyperactivity developed in ApoE knockout rats, especially at QS stages. Those findings suggest that autonomic dysfunction is a possible mechanism for impairment of sleep quality and increased sudden cardiac death risk during QS stage in hypercholesteremia.