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ratio of myosin heavy chain (MyHC) isoforms, α- and β-MyHC, expressed in the ventricles. Normal human ventricles express ~10% of the fast α-MyHC on a background of the slower β-MyHC, while in failing hearts α-MyHC is reduced to virtually undetectable levels with complete replacement by β-MyHC. Data from permeabilized myocardial preparations suggests that this isoform switch may be partly responsible for reduced myocardial twitch force and pressure development by failing ventricles since β-MyHC is a slower motor protein, yet most experiments have used non-human myosins and experimental conditions in which preparations were steadily activated, thus little is known about the response of human myosins to a time-varying Ca2+ transient. To address these limitations, we recently developed a human 3D engineered cardiac tissue (hECT) system in which we can express recombinant human muscle myosin motors. Using commercially available cloning and adenoviral expression systems, α- or β-MyHC isoform expressing adenoviral particles were used to transduce human cardiomyocytes produced from human iPS cells and construct hECTs. Preparations displayed well-defined cellular structure with elongated morphology aligned in the direction of preparation shortening during electrical pacing, while histological analysis of hECT revealed appropriate protein expression and localization within the sarcomere. In response to a Ca2+ transient, the time-course of twitch force development was accelerated in hECT expressing α-MyHC compared to β-MyHC, while peak twitch force was greater in hECT expressing α-MyHC. These results demonstrate the relative contribution of myosin isoforms to myocardial twitch kinetics in human engineered cardiac tissue constructs expressing a stable background of myofibrillar proteins.

1787-Pos Board B557
Cardiocyte Functional Data Analysis: A Novel Approach

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Current laboratory methods used to assess neonatal and adult cardiocyte function include measurement of gene and protein expression levels, calcium transients, and contractility. Our goal was to develop simple tools to analyze such data readily. We created two MATLAB®-based toolboxes; the Contraction Video Processing (CVP) and the Cardiocyte Functional Response Analysis (CFRA) Toolbox. Videos of contracting cultured cardiocytes are acquired using a digital camera attached to an inverted phase microscope. Video frames are analyzed using digital imaging processing techniques along with several contraction assessment methods available through the CVP toolbox. The CVP offers direct correlation, pixel intensity tracking and Polar Fourier transform methods for the analysis of neonatal cardiocyte contraction. Analysis of adult cardiocytes includes those implemented on neonatal cardiocytes in addition to area boundary tracking, Fourier descriptor analysis, and cell length tracking methods. The resulting contraction records are processed using the CFRA toolbox to provide quantitative analyses of cardiocyte contractility and calcium transient responses. Transient data are obtained by measuring the calcium fluxes using the fluorescent dye Fluo-3, and a Photon Technology fluorometer system running Felix software. Data analysis routines have been created and tailored exclusively to the characteristics and needs of cellular cardiovascular research investigators. The analytical methods created are used to find the onset of contraction, perform signal averaging, and acquire statistical information of functional data. CFRA toolbox contractility processing yields onset time, time-to-peak, duration, and fast and slow recovery times. CFRA toolbox calcium transient signal processing yields onset time, signal intensity, and fast and slow exponential recovery rates associated with SERCA and NCX channels respectively. The toolboxes allow examination of beat-to-beat contractility and calcium transient variations within the same cardiocyte as well as from cell population to population. Supported by NIH/NIGMS SDSU MARC Program 5T34GM008303-22

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Altered Cross-Bridge Relaxation Kinetics in Guinea Pig Cardiac Hypertrophy

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Cardiac hypertrophy is associated with Diastolic Heart Failure (DHF), a syndrome in which systolic function is preserved, but cardiac filling dynamics are depressed. The molecular mechanisms underlying DHF and the potential role of altered cross-bridge cycling in this syndrome are poorly understood. Accordingly, we induced chronic pressure overload by surgically banding the thoracic ascending aorta in female Dunkin Hartley Guinea pigs weighing 400g, for 12-16 weeks. Guinea pigs were chosen to avoid the confounding effects of myosin isoform switch that occurs in other small rodent models. Left ventricular (LV) samples were frozen in liquid N2. Aortic banding resulted in (+31%) LV hypertrophy (LV/BW ratio) and reduced diastolic cardiac function, but normal systolic function. Single myofibrils were prepared by mechanical dissociation and subsequently attached between two glass microneedles that were positioned on the stage of an inverted phase-contrast microscope. While the maximum calcium saturated force development was depressed (−18%), the time required for force relaxation was increased (+8%) in parallel to a significant decrease in the rate of relaxation (−25%) in DHF myofibrils; Myosin Heavy Chain (MHC) isoform distribution was unaltered. We conclude that slower cross-bridge relaxation kinetics contribute to diastolic dysfunction in cardiac hypertrophy.

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Transmural Heterogeneity and Depressed Function in the Mechanical Properties of Ventricular Tissue from Patients with End-Stage Heart Failure

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Heart failure is a progressive condition in which the ventricles can no longer pump enough blood to meet the body's basal demands. Our laboratory is investigating whether the transmural variation in cellular contractile properties that occurs in normal hearts (and which is thought to be important for ventricular function) is altered in heart failure. We procured through wall samples of failing left ventricle from patients receiving transplants at the University of Kentucky and non-failing samples from brain dead organ donors. The tissue was divided into epicardial, midmyocardial and endocardial layers and frozen in liquid nitrogen within 30 minutes. Multicellular chemically permeabilized preparations were subsequently obtained from these samples by mechanical homogenization and triton treatment. The samples were attached between a force transducer and a motor and subjected to two mechanical protocols: 1) a stretch-restretch protocol in solutions with different activating Ca2+ concentrations and 2) a force-velocity protocol in which maximally-activated preparations were allowed to shorten against pre-set loads. Parameters including steady-state force, short-range stiffness, short-range force and maximum power output were measured using these two protocols. The results suggested a 30% decrease in maximum power output (p-value = 0.01) and steady-state force (p-value=0.005) in heart failure patients (n=8, total of 72 preparations) as compared to non-failing (n=4, total of 36 preparations). Short-range stiffness (p-value=0.003) and short-range force (p-value=0.002) also significantly decreased in heart failure vs. non-failing. Transmurally there was a significant difference in maximum power output between the regions (p-value=0.02). The data suggests that mechanically the mid myocardium maybe affected the most in heart failure. Further studies need to be done to understand the protein modifications that may be responsible for these variations.

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Ca^{2+}-Independent Decrease in Resting Sarcomere Length in Rat Failing Right Ventricular Myocytes

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Pulmonary artery hypertension (PAH) can cause right ventricular (RV) contractile dysfunction and failure by mechanisms that are not fully understood.
PAH and RV failure were induced in male Wistar rats (200g) 3-4 weeks after a single injection of 60 mg/kg monocrotaline (MCT). Single RV myocytes were isolated from extracted hearts and fast Fourier transform of their video image was used to compare resting sarcomere lengths (SL) of myocytes from MCT and saline injected control animals (CON) during superfusion with a physiological saline solution.
There was no difference in resting intracellular Ca^{2+} levels in cells loaded with the Ca^{2+}-indicator Fura-4 (MCT 0.31 ± 0.02 ratio units n= 10 cells; CON, 0.30 ± 0.01 ratio units n= 11 cells, $P > 0.05$, unpaired t-test). However, resting SL was significantly shorter in MCT myocytes (1.78 ± 0.01 μm) than CON (1.90 ± 0.01 μm) $P < 0.001$. When Ca^{2+}-dependant cross-bridges were inhibited by exposure to the Ca^{2+} buffer BAPTA-AM (100 μM for 10 min) SL increased in both groups by similar amounts (MCT 0.01 ± 0.003 μm vs CON 0.03 ± 0.01 μm, $P > 0.05$). Inhibition of Ca^{2+}-independent cross-bridges by exposure to BAPTA plus the actin-myosin inhibitor BDM (40 mM for 5 min) further increased SL, this effect was significantly greater in MCT