capable of detecting mechanical separation of the muscle layers, which was found to occur more frequently in w1118 than yw (0.41 ± 0.04 vs. 0.00 ± 0.02 μm separation, respectively, 1 week flies). Detection of mechanical separation between muscle layers via nanoindentation was modeled and verified in a microfabricated polydimethylsiloxane system. This first in situ mechanical analysis of a living myocardium revealed differences in cardiac mechanics due to age and suggest that aspects of the mechanical properties of the aging phenotype differ between Drosophila strains. We investigation on other laboratory Drosophila wildtype strains to assess the impact of diverse genetic backgrounds or mutations on age-related myocardial stiffening and cardiomyopathy.

1782-Pos Board B552

In Situ Mechanical Analysis of Genetic Modification and Aging on Soft, Bilayered Drosophila Myocardium

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Drosophila melanogaster is a genetically malleable organism with a short life span, making it a tractable system in which to study mechanical effects of genetic perturbation and aging on tissues, such as impaired heart function. However, Drosophila heart tube studies can be hampered by its bilayered structure: a ventral muscle layer covers the contractile cardiomyocytes. We have refined an atomic force microscopy-based analysis to measure individual mechanical components of soft composite materials. The technique was verified using bilayered polydimethylsiloxane. Its biological utility was further demonstrated by its ability to resolve stiffness changes due to cardiac-specific RNA interference to reduce cardiomyocyte myofibrillar assembly or due to aging in Drosophila myocardial layers. Female yellow-white (yw) flies experience decreased diastolic diameter with age (>20%) while cardiomyocytes stiffened more than two-fold with age (1.8 ± 0.1 vs. 3.8 ± 0.3 kPa in 1 and 5 week old flies, respectively) at cell-cell junctions. Cardiac-specific RNA-interference against myosin heavy chain severely impaired contraction and reduced stiffness after 1 week (1.0 ± 0.1 vs. 1.8 ± 0.1 kPa) without altering ventral muscle stiffness. This method provides a platform to assess the mechanics of soft biological composite systems and for the first time permits direct measurement of how genetic perturbations, aging, and disease can impact cardiac function in situ.

1783-Pos Board B553

Exploration and Suppression of Cardiac Amyloidosis Induced by Huntington's Disease-Causing Amyloid in the Drosophila Heart Model

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Accumulation of amyloids is associated with cardiomyopathy; however, the precise mechanism that leads to defective heart structure and function is unknown. Amyloid-like inclusions have been detected in patients with Huntington's disease (HD), which is caused by an expanded polyglutamine (Poly-Q) repeat in the Huntington (HTT) protein. HD patients also demonstrate a greater occurrence of cardiovascular events, presumably as a result of toxic amyloid accumulation due to global protein misfolding and/or oxidative stress. To explore cardiac defects associated with HD-causing amyloid protein, we used the UAS-Gal4 system and a cardiac-specific driver (Hand-Gal4) to express mutant HTT with short (UAS-*Httex1*-PolyQ25) and disease-causing expanded (UAS-*Httex1*-PolyQ72) Poly-Q in the *Drosophila* heart. Expression of disease causing Poly-Q in 1 and 3 week old fly hearts resulted in severe cardiac defects as evidenced by prolonged diastolic and systolic intervals, a significantly increased incidence of arrhythmias and extreme cardiac dilation that was accompanied by a significant decrease in cardiac contractility (reduced fractional shortening). Structural analysis showed myocardial cells with noticeably reduced myofibrillar content, myofibrillar disorganization and the presence of amyloid-aggregates. No such physiological and structural defects were seen upon expression of short Poly-Q under similar conditions. To take advantage of our genetic model and to further explore the mechanism underlying the PolyQ-induced cardiac defects, we co-expressed expanded Poly-Q with either the antioxidant enzyme superoxide dismutase (SOD) or a chaperone protein UNC-45. Our preliminary results suggest that cardiac dilation is reduced and cardiac performance is enhanced upon co-expression of SOD or UNC-45. Thus we have developed a novel *Drosophila* model that allows us to explore cardiac defects associated with the accumulation of HD-causing amyloid and to elucidate the mechanisms underlying cardiac failure in HD patients.

1784-Pos Board B554

Multi-Scale Modeling of Patient-Specific Ventricular Geometry, Fiber Structure, and Biomechanics

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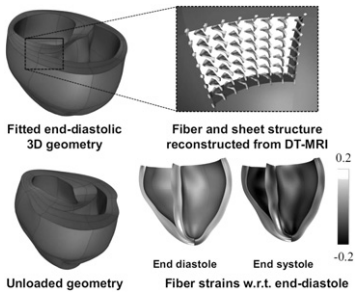
Patient-specific image data of the heart can now be obtained through advanced medical imaging. This data combined with clinical measurements can potentially be integrated into patient-specific computational models of regional cardiac function. We have developed a pipeline for patient-specific ventricular biomechanics simulations in the failing heart. Three-dimensional ventricular geometry was segmented from CT or MRI data at end-diastole from patients with congestive heart failure. Human myofiber and sheet architecture was estimated using eigenvectors computed from Diffusion Tensor MRI obtained in an isolated, fixed human organ donor heart and mapped to the patient-specific geometric model using large-deformation diffeomorphic mapping. Passive myocardial properties were optimized using semi-automated methods while simultaneously computing the unloaded reference geometry. Active cardiac-muscle contraction properties were optimized to match ventricular pressures measured by cardiac catheterization. Finally, echocardiographic data and an adaptation algorithm (CircAdapt) were used to estimate parameters of a lumped-parameter closed-loop model of the circulation. These methods were validated in three heart failure patients who gave informed consent at the San Diego VA Medical Center by comparing simulation results with echocardiographic measurements of regional wall motion and with predictions of empirical formulas derived from previous clinical studies.



1785-Pos Board B555

Cross-Bridge Cycling Kinetics in Intact Multicellular Cardiac Muscle at Physiological Temperature: Impact of Muscle Length

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The contractility of the heart is physiologically determined by load, frequency and β-adrenergic stimulation. It has been shown that these regulatory mechanisms involve post-translational modifications of myofilament proteins that can potentially influence the rate of cross-bridge cycling, an important determinant of cardiac output. We set out to develop a method for measuring cross-bridge cycling rate in intact cardiac muscle preparations where the cascades of post-translational signaling are functionally intact. With the use of a K^+ contracture protocol, we were able to induce a steady-state tension in intact trabeculae and measure the rate of tension redevelopment (k_{tr}), an index for cross-bridge cycling rate. We utilized this technique in order to investigate the effect of load on cross-bridge cycling rate. In cardiac trabeculae isolated from Brown Norway rats (n=11), the rate of tension redevelopment was measured twice at L_{opt} (optimal length) and at L_{90} (corresponding to 90% of optimal length) in each muscle. The k_{tr} for the L_{90} was 45.1 ± 7.6 s^{-1} and it was significantly decreased to 27.7 ± 3.3 s^{-1} as the muscles were stretched to their L_{opt} ($P < 0.05$). The k_{tr} for each length was measured a second time in order to show the reproducibility of the system. There was no significant difference between the duplicate measurements of each length ($P = 0.84$). In addition, we were able to apply these experiments in mammals that more closely reflect the human situation (such as the rabbit and dog) and muscle preparations isolated from explanted human hearts. This technique permits the studying of cross-bridge cycling kinetics in intact muscles in a reproducible and reliable manner, where the impact of signaling cascades leading to post-translational modifications can be studied.

1786-Pos Board B556

Differential Twitch Kinetics in Engineered Cardiac Tissue Expressing Human Cardiac Myosins

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Congestive heart failure is a debilitating disease in which the principal pathology is impaired ventricular contractility leading to diminished cardiac output, and previous work indicates that reduced contractility is based in part on the

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

1788-Pos Board B558

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.

1789-Pos Board B559

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.

1790-Pos Board B560

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