*Drosophila melanogaster* possess a simple linear heart tube and constitute an excellent genetic model system with which to investigate the effects of cardiomyopathic mutation. The Mhc5 myosin heavy chain mutation is located in the 'transducer'domain and elicits hypercontractile function at the molecular level characterized by high ATPase activity and enhanced in vitro motility properties. Additionally, its expression impairs diastolic relaxation of the cardiac tube reminiscent of restrictive cardiomyopathy in humans. We have investigated the effect of the Mhc5 mutation on cardiac structure/function by quantitative proteomics using isobaric tags for relative quantification (iTRAQ). Excised fly hearts from yw (control) and Mhc5 strains were digested with trypsin, reduced, alkylated and labeled with ITRAQ reagent. Peptides from each pool were mixed together prior to fractionation by strong cation exchange chromatography and subsequent reversed-phase HPLC coupled to tandem mass spectrometry. This approach identified approximately 600 proteins, of which 94 were upregulated and 86 were downregulated in Mhc5 hearts relative to yw hearts ($p<0.05$). Ontological cluster analysis of the genes encoding the regulated proteins revealed that myofibrillar disarray in Mhc5 hearts likely stems from overexpression of actin with concomitant reduction of myofibrillar assembly proteins such as spectrin, and other actin-binding proteins. Structural remodeling was also characterized by increased expression of extracellular matrix proteins. Upregulation of proteins involved in mitochondrial oxidative phosphorylation and fatty acid catabolism suggests further bioenergetic remodeling. The proteomic, structural and ultrastructural data are consistent with a model whereby the elevated ATPase activity caused by Mhc5 mutation increases energetic demand, thereby stimulating a concerted compensatory metabolic response to maintain energetic homeostasis. Ongoing protein-network/interactome analysis will help to further refine the model.

**3733-Pos**

**Mesenchymal Stem Cells Protect Cardiomyocytes**

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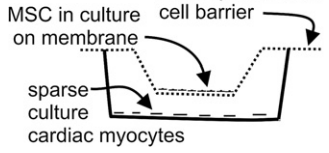
Possible therapeutic benefits of stem cell treatments have been widely investigated recently. We have presented initial reports that co-culturing mesenchymal stem cells (MSC, Lonza) with rat heart cells in primary culture can prevent the consequences of the treatment with a inflammatory bacterial endotoxin (LPS, Lipopolysaccharide-A). We now investigate how the MSC produce their beneficial actions. Using sparse primary cultures of neonatal rat ventricular or adult rat ventricular myocytes with either MSC or control cells (fibroblasts), we examine cardiac $Ca^{2+}$ signaling. LPS causes $Ca^{2+}$ signaling anomalies which include delayed afterdepolarizations (DADs) and $Ca^{2+}$-enhanced early afterdepolarizations (EADs). We find that co-cultures with cells co-mingled can prevent the untowards actions of LPS on the cardiac myocytes. The negative consequences of LPS are alterations in the normal $[Ca^{2+}]_i$ transient that is stimulated by field shocks as described above. Since the benefit of MSC co-culture are found even when a solute permeable / cell impermeant membrane separates the MSC from the LPS treated cardiac myocytes, we conclude that a paracrine action of the MSC can account for the treatment attributed to the MSCs. We continue to investigate possible beneficial signaling pathways that may explain the paracrine effect of MSCs.




**3734-Pos**

**Mechanical and Biochemical Characteristics of Human Stem Cell-Derived Cardiomyocytes**

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Cell-based cardiac repair following myocardial infarction has gained considerable interest recently, and the human pluripotent stem cell is an attractive cell source due its efficient differentiation into immature but functional cardiomyocytes. We examined the biophysical characteristics of cardiomyocytes generated from human embryonic stem cells (hESC-CMs) by measuring calcium transients, single cell contractions, and actomyosin interactions via flash photolysis. Furthermore, we compared these characteristics with those obtained from a second promising but still poorly characterized cell type, the human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM). We hypothesized that understanding fundamental biochemical and mechanical characteristics of these cells would provide insight into potential strategies to induce further cell maturation *in vitro*.

Our results suggest that hESC-CMs and hiPSC-CMs exhibit spontaneous contractions and calcium transients with similar kinetics, including time to peak $[Ca^{2+}]_i$ ($116 \pm 34$ms vs. $155 \pm 40$ms) and time to 50% $[Ca^{2+}]_i$ decay ($352 \pm 87$ms vs. $296 \pm 49$ms). Furthermore, quantitative videomicroscopy of resulting single cell contractions suggests that cardiomyocytes from both sources demonstrate similar resting cell size ($17.1 \pm 1.4$um vs. $16.5 \pm 4.6$um), contraction amplitude ($4.2 \pm 1.6\%$ vs. $4.4 \pm 2.1\%$), time to peak contraction ($0.346 \pm 0.135$sec vs. $0.339 \pm 0.214$sec), maximum contraction velocity ($6.34 \pm 3.50$um/sec vs. $7.46 \pm 4.81$um/sec), and maximum relaxation velocity ($3.21 \pm 2.49$um/sec vs. $3.40 \pm 2.49$um/sec).

We have also successfully isolated and purified 20 ug of myosin per million hESC-CMs. Using flash photolysis to liberate ATP in a solution of actomyosin, we have shown that the myosin binds actin and is dissociated from the complex by ATP with the expected 2nd order rate constant (~1 $uM^{-1}sec^{-1}$).

In summary, the contractile properties of hESC-CMs and hiPSC-CMs are similar to each other but differ from values published for adult human cardiomyocytes, suggesting that they are functionally immature and may benefit from *in vitro* maturation efforts.

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**3735-Pos**

**Cell-Seeded Fibrin Scaffolds for Cardiac Tissue Engineering**

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Cellular cardiomyoplasty to replace non-functional tissue following cardiac infarction appears clinically viable. Current strategies utilizing direct injection of cell suspensions are limited by low cell retention, poor cell localization, and high cell death. Synthetic biomaterials developed to enhance cell delivery can lead to problems with immune rejection, degradation, and mechanical mismatch, preventing functional integration of constructs with host myocardium. The goal of this project is to develop a functional cardiac tissue construct with enhanced host integration capabilities as a novel strategy to replace damaged myocardium. We have developed a novel templated fibrin scaffold seeded with cells to promote functional integration. Fibrin is an ideal scaffold material because it can be autologous, improves cell attachment and growth, and degrades into natural byproducts that can induce angiogenesis. The novel scaffold architecture includes 1) microchannels spanning the length of the scaffold, allowing alignment and organization of cells to mimic native cardiac tissue structure, and 2) micropores to enhance construct survival by improving nutrient delivery and waste removal. The dense fibrin scaffolds (stiffness = 16.0 ± 3.0 kPa) had mechanical properties closer to native myocardium than fibrin gels (0.5 to < 7 kPa). Centrifuge seeding with a tri-cell mixture of cardiomyocytes, endothelial cells, and fibroblasts increased scaffold stiffness (38.3 ± 8.9 kPa) to values near neonatal myocardial tissue (~40 kPa). Stiffness decreased over time in culture (25.2 ± 3.1 kPa, Day 6), which may indicate ECM formation and scaffold degradation. Patches of beating cells were observed inside channels within two days in culture. After three days in culture, histology showed cardiomyocyte and fibroblast alignment and immature lumen formation. These results indicate micro-templated fibrin scaffolds are a unique and viable platform for cardiac tissue engineering. This work supported by NIH HL064387 (MR, MS, BR) and NSF GRFP (KT).

**3736-Pos**

**Cardiac Specific Overexpression of N-RAP in Transgenic Mice**

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The muscle specific protein NRAP plays a role in myofibril assembly and is upregulated in mouse models of dilated cardiomyopathy. We sought to determine if increased N-RAP expression would directly lead to a cardiomyopathy phenotype. Novel transgenic lines were developed using the tet-off system with transgenic N-RAP expression requiring the tetracycline transactivator (tTA). tTA was introduced by mating the N-RAP transgenic animals with well-characterized animals carrying the tTA transgene controlled by the cardiac specific alpha-myosin heavy chain promoter. Multiple founder lines were examined and lines showing the most significant increase in NRAP expression were used for further investigation. N-RAP expression in theses animals was up to 2.5 times greater than control littermates as determined by western blot analysis. Histological examination of hearts from ~12 week old transgenic mice

showed no structural defects compared to control littermates. Additionally, examination of these hearts by immunofluorescence microscopy revealed normal myofibrillar structure and localization of the transgenic protein to intercalated disks, as normally seen with the endogenous protein. Protein markers for cardiomyopathy were examined by qPCR and revealed no difference between non-transgenic and transgenic animals. Echocardiography and magnetic resonance imaging of the N-RAP transgenic animals revealed no significant structural or functional differences when compared to control littermates at 12 weeks of age. Based on these data, it does not appear that overexpression of N-RAP directly leads to an observable cardiac phenotype. The alternative hypothesis that upregulation of N-RAP in dilated cardiomyopathy is compensatory remains to explored.

**3737-Pos**

**Extracellular Matrix Remodelling in an Ovine Model of Ageing and Heart Failure**

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We sought to establish a model of heart failure (HF) encompassing young and aged animals to determine if alterations in the amount of cardiac extracellular matrix (ECM) occur in ageing and whether these changes are similar to those in HF.

HF was induced in sheep aged 18 months (young) and those over 8 years (old) by 4 weeks rapid right ventricular pacing (3.5Hz). Paraffin-embedded left ventricular (LV) samples were stained with picro-sirius red. Interstitial collagen was visualised by polarised light microscopy. LV protein extracts were assessed for MMP activity using gelatin zymography. Statistical significance was calculated using the mean ± SEM and a t-test or by 2-way ANOVA.

LV diameter increased with age (OC) compared to young controls (YC) ($3.04 \pm 0.2$cm *vs*.$2.44 \pm 0.14$cm, $n$=4-13, $P<0.05$), in young heart failure (YF) compared pre-pacing ($3.82 \pm 0.1$cm *vs.* $2.44 \pm 0.1$cm, $n$=13, $P<0.001$) and in old heart failure (OF) compared to pre-pacing ($3.87 \pm 0.1$cm *vs*. $3.04 \pm 0.2$cm, $n$=4, $P<0.05$). Collagen content increased in OC compared to YC ($2.27 \pm 0.4\%$ *vs*. $0.96 \pm 0.1\%$, $n$=5, $P<0.05$) and in YF compared to YC ($2.62 \pm 0.3\%$ *vs*. $0.96 \pm 0.1\%$, $n$=5-8, $P<0.001$), and decreased in OF compared to YF ($1.26 \pm 0.4\%$ *vs*. $2.62 \pm 0.3\%$, $n$=4-8, $P<0.01$). Normalised MMP-2 activity increased in OC ($1.52 \pm 0.08$), YF ($1.66 \pm 0.14$) and OF ($1.74 \pm 0.17$) compared to YC ($0.98 \pm 0.22$, $P<0.05$, $n$=6-8).

The commonality of these changes seen in ageing and HF may indicate that cardiac ECM remodelling is important in the predisposition of ageing to the development of HF.

All procedures accord to The UK Animals (Scientific Procedures) Act, 1986.

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**3738-Pos**

**Involvement of Calcineurin/STAT3 Pathways in Heart Hypertrophy during Pregnancy**

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Previously we have characterized the physiological heart hypertrophy which occurs during pregnancy. However, the underlying molecular mechanisms of pregnancy-induced hypertrophy are still not elucidated. Here we performed Western Blot analysis together with high resolution confocal microscopy to identify the key signaling molecules involved in the pregnancy-induced heart hypertrophy in non-pregnant in diestrus stage (NP), late pregnant (LP), 24 hours post partum (PP1) and 7 days post partum (PP7) mice. Western Blot analysis of heart lysates showed that phospho-AKT protein levels were decreased ~ 7 fold at the end of pregnancy (N=4 NP and N=4 LP mice). Interestingly, AKT activity was restored one day post-partum to levels comparable to NP. There were no significant changes in total or phosphorylated levels of ERK1/2 with pregnancy. The calcium/calmodulin-dependent serine-threonine phosphatase Calcineurin, which has been shown to be upregulated in pathological cardiac hypertrophy, was significantly downregulated at the end of pregnancy, and this downregulation was reversed 1 day after partum. The phosphorylation of the signal transducer and activator of transcription 3 (STAT3), but not its total protein levels, was also significantly lower at the end of pregnancy and was restored completely one day post-partum. Although there was a tendency in reduction of phospho-GSK protein levels in the LP group, this reduction was not statistically significant. High resolution confocal microscopy demonstrated that pregnancy is associated with relocalization of AKT and pAKT to the nucleus, which is partially reversed 24 hours post-partum. While the subcellular distribution of ERK1 and pERK was not regulated by pregnancy, the nuclear labeling of P38, JNK1 and pJNK was significantly upregulated at the end of pregnancy. Although pJNK localization disappeared completely from the nucleus in the PP1 group, P38 and JNK1 nuclear labeling remained high 24 hours PP.

**3739-Pos**

**Gper Activation Inhibits Mitochondria Permeability Transition Pore Opening Via Erk Phosphorylation and Provides Cardioprotection after Ischemia-Reperfusion**

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Recently, several studies have demonstrated G protein coupled receptor 30 (GPER) can directly bind to estrogen and mediate its action. We investigated the role and the mechanism of estrogen-induced cardioprotection after ischemia-reperfusion using a specific GPER agonist G1. Isolated hearts from male mice were perfused using Langendorff technique with oxygenated (95% O2 and 5% CO2) Krebs Henseleit buffer (control), with addition of G1 (1μM), and G1 (1μM)+PD98059 (10μM) to investigate the involvement of Erk pathway. After 20 min of perfusion, hearts were subjected to 20 min global normothermic (37°C) ischemia followed by 40min reperfusion. During the course of experiment cardiac function was measured and myocardial necrosis was evaluated by triphenyltetrazolium chloride (TTC) staining at the end of the reperfusion. Mitochondria were isolated after 10 minutes of reperfusion to assess the calcium load required to induce mPTP opening. G1 treated hearts developed better functional recovery with higher rate pressure product (RPP, $6140 \pm 264$ *vs*. $2640 \pm 334$ mmHgxbeats/min, $p<0.05$). The infarct size decreased significantly in G1 treated hearts ($21 \pm 2\%$ *vs.* $46 \pm 3\%$ $p<0.001$) and the $Ca^{2+}$ load required to induce mPTP opening increased ($2.4 \pm 0.06$ *vs.* $1.6 \pm 0.11$ μM/mg mitochondrial protein, $p<0.05$) as compared to the controls. The addition of PD 98059 significantly prevents G1 effect on heart function RPP ($4120 \pm 46$ mmHgxbeats/min, $p<0.05$), infarct size ($53 \pm 2\%$) and calcium retention capacity ($1.4 \pm 0.11$μM/mg mitochondrial protein $p<0.05$) These results suggest that GPER activation inhibits the mPTP opening and provide a cardioprotective effect after ischemia-reperfusion and this effect is mediated by Erk pathway. Supported by NIH and AHA.

**3740-Pos**

**Opposite Production of Reactive Oxygen Species by Complexes I and III during Heart Ischemia/Reperfusion**

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In mitochondria, the main reactive oxygen species (ROS) generators are complexes I and III. Mitochondrial ROS generation has been implicated in cellular damage occurring in a variety of pathologies including ischemia/reperfusion (I/R). Most of the studies have observed an increase of ROS production after I/R. However, there is evidence of an increase of ROS production after cardioprotection by preconditioning interventions.

We investigated the differential production of ROS by complex I and III in I/R and sham isolated mitochondria heart mice, and the action of pro-apoptotic drugs (rotenone and antimycin A) on ROS production by these complexes in sham animals.

Mitochondrial ROS generation by both complexes was measured using amplex red in the presence of horseradish-peroxidase. Specific substrates for complex I (glutmate/malate) and complex II (succinate), and the inhibitors (rotenone and antimycin-A) were used. Mitochondria from I/R mice produced more ROS than mitochondria from sham when the substrate for complex I was used. In contrast, with the substrate of complex II, mitochondria from I/R mice produced less ROS than sham. Application of rotenone and antimycin-A in mitochondria from sham heart significantly increased ROS production when the substrate for the complex I was used. Surprisingly, these inhibitors decreased the ROS production when the substrate for the complex II was used. This data indicate the ambivalent production of ROS by the respiratory chain complexes I and III, and suggest opposite role of ROS depending on the complex being cardio-deleterious for complex I and cardio-protective for complex III. Supported by NIH and AHA.

**3741-Pos**

**Sub-Proteomic Fractionation of Rat Cardiac Tissue: Comparing Ischemic Vs Normal Remote Region with In-Solution Based Proteomics**

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