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into host tissue. To address functional integration, the physiological interactions between muscle cells and their microenvironment need to be further elucidated. We are using microfabricated post array detectors (mPADs) as novel functional assays to assess contractile forces transferred through focal adhesions to the extracellular environment from cardiomyocytes. mPADs utilize an array of microscale posts that deflect as cantilever springs in response to forces applied by cells cultured upon them. Contractile forces of neonatal rat cardiomyocytes (NRCs) are calculated by analyzing the deflection of the posts and multiplying by their spring constant. The measured forces are compared to vascular smooth muscle cells (VSMCs), cells with similarly disorganized contractile apparatus, and adult rat cardiomyocytes (ARCs). Using immunofluorescence of vinculin, both NRCs and VSMCs demonstrate the ability to create focal adhesions to the fibronectin coatings on the tips of the posts. Preliminary results indicate that VSMC produced a total force of 570 ± 175 nN/cell which is almost 6-fold greater than NRCs. However, NRCs produce 50% more force per unit area than VSMC. Furthermore, spontaneous beating of NRCs yielded an additional maximal contraction of 55.6 nN per post per beat above the baseline which is about two orders of magnitude lower than reported forces produced by ARCs. The use of mPADs provides a tool to further the understanding of the stress and strains created by cardiomyocytes onto their local environments through their focal adhesions. With this new technique to assess muscle adhesion and contractility, we seek to characterize the physiological interactions that implanted muscle cells must recapitulate to advance new therapies for cardiovascular regenerative medicine. HL61683 & UWRRF

2567-Pos Board B537

Integrin Response To Altered Actin-Myosin Mechanochemistry In Cardiac Myocytes

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Altered contractility in cardiac myocytes associated with motor protein mutations has been implicated in several pathologies such as familial hypertrophic (FHC) and dilated (DCM) cardiomyopathies. The mechanism(s) by which these altered contractile forces result in cell phenotype changes are poorly understood. However, they are generally believed to involve mechanotransduction of force by cell adhesion molecules such as the integrin class of proteins. Here we investigate the use of blebbistatin and other small molecule effectors of actin-myosin mechanochemistry as a chemical model for the reduced contractile force associated with the pathogenesis of DCM. In order to establish intercalated-disk cell associations in vitro that are representative of in vivo conditions, we employ micropatterned cardiac myocyte cell culture, where the extracellular matrix proteins collagen and laminin are printed onto the culture vessel surface in 10µm wide lines. Fluorescent polymer microspheres (0.25µm dia.) are embedded in the collagen layer. As the beads move from myocyte contraction, the movement amplitude is measured by nanometer-resolution position analysis, both in the presence of blebbistatin and in the rescue state where blebbistatin is removed. Relative force generation is calculated from the position analysis data and matrix modulus. Cells are then fixed and prepared with fluorescent antibodies for observation of connexin-43 and β1 integrin expression and localization by confocal microscopy. By employing micropatterned cardiac myocyte cell culture, optical contractile force measurement, and subsequent observation of the expression and localization of connexin-43 and β1 integrin, we assess the changes in integrin activity due to blebbistatin-induced reduction in contractile force.

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A Direct Method to Measure the Restoring Force and Slack Sarcomere Length of Intact Cardiomyocytes

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Molecular mechanisms underlying diastolic suction are poorly understood. Several proteins have been implicated to play a role, including extra-cellular proteins, titin, and cytoskeletal proteins. An in vitro measurement of diastolic suction at the cell level is restoring force (or stiffness), a force which is difficult to measure as it requires a cell to be passive below the slack sarcomere length (SL). Previous restoring force studies were made utilizing calcium independent shortening of cardiomyocytes to below their slack length and then upon relaxation measuring the force that developed as the cell is stretched back to its slack length. However, these studies used chemically permeablized cells and, thus, eliminated the membrane and soluble intracellular proteins as possible contributors to restoring force. In the current study we developed a novel method to determine restoring force and the slack sarcomere length of mouse cardiomyocytes that were intact. Intact cardiac myocytes that were below their slack length due to a low level of active force development were attached to flexible carbon fibers. We then added butanedione monoxime (BDM) to inhibit actomyosin interactions and abolish active tension. This led to an increase in the sarcomere length and a negative force. From this new baseline force (-1.34 ± 0.34 mN/mm^2 (mean ± SE)), we stretched the cells across the physiological range from ~1.8-2.2 µm. We determined the sarcomere length at which force is zero (slack SL) to be 1.93 ± 0.019 µm. Plotting the stress-SL relationship we then determined the restoring stiffness from the slope of this plot as 16.1 $mN/mm^2/\mu m$. Thus we successfully measured the restoring force - SL relation of intact cardiac myocytes.

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Titin Isoform Transitions and Passive Stiffness During Skeletal Muscle Development

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During postnatal striated muscle development, multiple changes in active and passive properties occur, reflecting an altered mechanical demand. For instance, during postnatal cardiac development titin isoform expression switches from large isoforms to small stiffer isoforms, likely affecting diastolic filling behavior. In the present study, we investigated whether titin isoform transitions also take place during *skeletal* muscle development. We used gel-electrophoresis to determine changes in titin isoform size in mice and rabbits of various ages. A titin exon microarray was used to evaluate transcript expression of all of titin's exons. To investigate the mechanical effect of titin isoform transitions, passive properties of neonatal and adult skeletal muscles were determined.

Neonatal mice were found to express large titin isoforms, which are gradually replaced by smaller isoforms during skeletal muscle development. The half transition time of the isoform for tibialis cranialis (TC), soleus, extensor digitorum longus, gastrocnemius and diaphragm were 6, 17, 17, 12, and 10 days, respectively. Essentially similar findings were obtained from NZW rabbits, with the exception that the half-life of the isoform transitions was slightly longer compared to murine skeletal muscles. Titin exon analysis in neonatal murine gastrocnemius muscle revealed increased expression of a large group of exons when compared to adult muscle transcripts, with all upregulated exons coding for exons of the elastic PEVK region of titin. In line with these observations, we found ~50% lower titin-based passive stiffness of murine neonatal soleus and TC when compared to adult muscle.These data demonstrate that during skeletal muscle development titin isoform transitions occur from large compliant isoforms to smaller and stiffer isoforms in adult muscle, likely due to changed expression of PEVK exons.

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Vinculin Contributes to the Passive Stiffness of Myocardium

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In cardiomyocytes, the costamere links the Z disc to the surrounding extracellular matrix. Proteins at these junctions include integrins, talin, and vinculin (Vin). Vin is also found in the intercalated discs. Cardiac-specific Vin knockout (VinKO) mice have a sudden death phenotype early in life (<12 weeks of age) with a progressive dilated cardiomyopathy leading to 100% mortality by 32 wks. At 7 weeks of age, systolic ventricular function is normal.

We hypothesize that deletion of the costameric protein Vin leads to changes in passive stiffness prior to the onset of systolic dysfunction. Vin deletion may disrupt the normal force transmission pathways from ECM to cytoskeleton through the integrin-based costamere or cell-to-cell force transmission along the myocyte axis, which may manifest in altered passive material properties of the myocardium.

To test the mechanical properties of myocardial tissue, a system was developed in which murine right ventricular papillary muscles could be passively strained in the axial direction while simultaneously measuring force. Papillary muscles from 7 week old VinKO mice and WT controls were isolated and stretched. Stress-strain analysis was used to measure passive stiffness in the direction of myocardial fibers.

Stress-strain curves were significantly different between WT and VinKO papillary muscles ($p<0.05$). The slope of the VinKO curve was less than in the WT curve, indicating that VinKO muscles are more compliant in the fiber