Heart failure was induced in adult male ferrets by ascending aortic coarctation. All procedures accorded with The United Kingdom Animals (Scientific Procedures) Act, 1986. Protein extracts from sham and failing hearts were separated by 2% SDS PAGE. The mean ratio of the major titin isoforms N2BA:N2B increased from 0.3 in control to 0.5 in failing hearts (n=6, $p<0.01$).
Titin molecules were isolated from left ventricle, aligned and stretched by a receding liquid meniscus (which applied a tensile force of ~60pN)[3] and visualised by atomic force microscopy. Combed titin molecules exhibited a straightened and beaded appearance. The mean molecular diameter of titin decreased in failing hearts compared to control (0.26 ± 0.001nm *vs* 0.33 ± 0.001nm, $p<0.001$, n=104-130 molecules, 3 animals per group). This difference was more pronounced in the shorter molecules (<3.5 μm). The mean distance between beads was increased in failing hearts (49.3 ± 1.5nm *vs* 126.8 ± 4.5nm, $p<0.001$, n=370-429, 3 animals per group). The decreased titin molecular diameter combined with an increased inter-bead distance suggests that titin from failing hearts is less resistant to tensile forces when compared to control, and may help to explain the decreased titin-based passive tension observed in diseased hearts.
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3938-Plat
Insulin Signaling Regulates Cardiac Titin Isoform Composition in Development and Diabetic Cardiomyopathy
Kamila Babicz, Wolfgang A. Linke, Martina Krueger.
Dept. of Cardiovascular Physiology, Ruhr University, Bochum, Germany.
Isoform switching of the giant elastic protein titin is a main mechanism for adjusting passive myocardial stiffness in perinatal heart development and chronic heart disease. Previous evidence suggested that thyroid hormone (T3) signaling converging onto the phosphoinositol-3-kinase (PI3K)/AKT pathway is an important determinant of the cardiac titin-isoform pattern in developing cardiomyocytes. We hypothesized that other activators of PI3K/AKT, particularly insulin, may similarly alter the titin-isoform composition, thereby modifying titin-based stiffness. Embryonic rat cardiomyocytes were cultured in medium containing 0.5% hormone-reduced serum and were treated with 175 nmol/L insulin for seven days. Analysis of titin-isoform expression by 2% SDS-PAGE showed a significant increase in the mean proportion of the stiff N2B titin isoform (3,000 kDa), from 53% in control cells to 64% in insulin-treated cells, the remainder being the more compliant N2BA isoform (>3,200 kDa). This insulin-dependent titin-isoform shift was blocked in the presence of PI3K-inhibitor, LY294002, suggesting that insulin regulates the cardiac titin-isoform pattern by activating the PI3K/AKT pathway. Whether this mechanism operates in vivo was studied by testing the effect of insulin deficiency on titin-isoform expression in streptozotozin-treated (STZ) rats as a model for diabetes mellitus (type 1). Within four months, STZ rats developed cardiac hypertrophy and mild left ventricular (LV) fibrosis, concomitant with elevated glucose levels. The mean proportion of N2B-titin was significantly decreased from 86% in control LV to 78% in LV of STZ rats. Wormlike chain modeling of titin elasticity suggested that such a change reduces titin-based passive stiffness by ~6%. Results of mechanical measurements on skinned cardiac fiber bundles confirmed minor passive stiffness modifications in STZ myocardium. We conclude that insulin signaling regulates titin-isoform composition in cardiac development and could also contribute to altered diastolic function in diabetic cardiomyopathy.

3939-Plat
The Effect of Stiffness and Beta-Adrenergic Stimulation on Neonatal Cardiomyocyte Calcium-Mediated Contractile Force Dynamics
Anthony G. Rodriguez, Sangyoon Han, Michael Regnier, Nathan Sniadecki.
U. of Washington, Seattle, WA, USA.
Embryonic or induced pluripotent stem cells have great potential to treat multiple cardiopathologies. Current limitations include a lack of understanding how contractility of immature cardiomyocytes is affected by microenvironment mechanical properties and beta-adrenergic stimulation. Inability to apply traditional force assessment techniques to immature cardiomyocytes led us to utilize 6 micrometer spaced arrays of elastomer-based microfabricated post force sensors. Posts act as cantilever springs with tunable constants (kp (nN/micrometer)), deflecting linearly in response to cultured cell's acto-myosin contraction transmitted through focal adhesions formed at their tips. We combined this method with an IonOptix system for real-time post displacement and intracellular Ca++ flux monitoring. We found for neonatal rat cardiomyocytes (NRCs) exposed to nanomolar concentrations of the β-adrenergic stimulant isoproterenol for ~2 minutes Ca++ flux decreased with little effect on flux rates. Maximum contractile force increased by as much as 90 % with 100 nM isoproterenol along with a significant increase in relaxation rate (kr). NRCs cultured on post arrays with an effective modulus (calculated based upon the kp) greater than normal cardiac extracellular matrix (~29.3 kPa vs. 10-20 kPa, respectively) generated the largest force per post (56.0 ± 9.8*! nN) and the fastest kr ($0.44 \pm 0.08 \cong \exists$ nN/ms) compared to post with Ep~10.6 kPa (16.1 ± 2.7!% nN, and 0.16 ± 0.030# nN/ms) and Ep~23.0 kPa (30.6 ± 4.5*% $\cong$ nN, 0.14 ± 0.023#$\exists$ nN/ms). Interestingly, kr for lower Ep values remained unchanged (#pvalue=0.349) while force increased with stiffness. Immunofluorescence staining revealed that myofibril actin and z-disc associated vinculin were more organized into parallel, longer fibers on stiffer post arrays. Increased contractility on stiffer posts also correlated with increased isoproterenol effects. The results indicate stiffness of the microenvironment at NRC's focal adhesiveness play a critical role in determining contractility and β-adrenergic responsiveness. (*,!,%,$\cong$pvalue<0.05, $\pm$SEM) HL061683(MR); NSF CAREER and HL097284(NS)

Platform BG: Nano-Materials

3940-Plat
Bionanoelectronic Devices Based on 1d-Lipid Bilayers on Nanotube and Nanowire Templates
Nipun Misra[1], Julio Martinez[1], Alexander Artyukhin[1], Shih-Chieh Huang[1], Pieter Stroeve[2], Costas Grigoropoulos[3], **Aleksandr Noy[1]**.
[1]Lawrence Livermore National Laboratory, Livermore, CA, USA, [2]UC Davis, Davis, CA, USA, [3]UC Berkeley, Berkeley, CA, USA.
Biological molecules perform sophisticated functions in living systems with complexity often far exceeding most of man-made devices and objects. Direct integration of biological components with electronic circuits could drastically increase their efficiency, complexity, and capabilities and result in novel sensing and signaling architectures. Yet, one of the obstacles for this vision of a bionanoelectronic circuit is the absence of a versatile interface that facilitates communication between biomolecules and electronic materials. We have been building platforms that integrates membrane proteins with one-dimensional inorganic materials such as carbon nanotubes and silicon nanowires. In our devices, a nanotube of nanowire is covered by a lipid bilayer that serves both as a universal membrane protein matrix and an insulating shield. I will discuss the fabrication and properties of these ''shielded'' nanowires and of their use in bionanoelectronic devices that incorporate working membrane proteins in an electronic circuit.

3941-Plat
Molecular Dynamics Study of CNT Nanopores Embedded in Lipid Bilayers
Elizabeth Jayne Wallace, John F. Ryan, Mark S.P. Sansom.
University of Oxford, Oxford, United Kingdom.
There is considerable interest in the use of carbon nanotubes (CNTs) as 'nanosyringes'that span membranes. These nanosyringes form biomimetic pores capable of drug delivery or of the selective transport of ions and water in biosensor devices. To date, a number of different types of simulation system have been used to explore the transport properties of CNT nanopores. These range from isolated CNTs in water, through to CNTs in a bilayer-mimicking 'slab'. However, it has been shown that what lies outside a nanopore may have an important effect on its transport properties, arguing for more realistic membrane models to be used. Up to now, few studies have addressed the transport properties of CNT nanopores embedded in a phospholipid bilayer. This more complex system may capture important effects of the membrane environment on the functional behaviour of the nanopore. Here we use molecular dynamics to simulate CNT nanopores that are embedded in a lipid bilayer. We explore how the size of the nanopore influences both its interactions with the lipid bilayer and the transport properties through the pore.

3942-Plat
Single-Step Coating and Bifunctionalization of Gold Nanoparticles
Valerio Voliani[1], Stefano Luin[2], Fabio Beltram[1].
[1]NEST, Scuola Normale Superiore and IIT Research Unit, Pisa, Italy,
[2]NEST, Scuola Normale Superiore and CNR-INFM, Pisa, Italy.
Metal nanostructures are attracting increasing attention in bio-sciences owing to their versatility and the peculiarities of their optical properties1,2. Their exploitation, however, often demands stable and biocompatible multifunctional surface coating.
We shall present a single-step method to coat and functionalize gold nanoparticles (NPs) with two distinct reactive groups by a properly designed peptide. NPs were prepared by reducing tetrachloro auric acid in water. The peptide we employed bonds to the NP by the N-Cysteine aminoacid and terminates with a C-terminal Lysine. In this way we can produce stable nanospheres