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Substrate Rigidity Modulates the Composition in Cell-Matrix Adhesions

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C. elegans TRNs provide a system in which we not only know the identity of the mechano-electrical transduction channel and many of its interacting partners, but can also explore how MRCs are shaped by the mechanical microenvironment and surrounding tissues. We use *in vivo* whole-cell patch-clamp in combination with a piezoelectric stack-based stimulator and photodiode-based motion detector (Peng et al., *Neuron*, 2013) to probe this system at high (2.5kHz) bandwidth. Consistent with prior work, we find that MRC amplitude increases with displacement and decreases with distance from the cell body. The amplitude and kinetics of MRCs increase with stimulus rate, saturating above a speed of 6mm/s. With sinusoidal stimuli, we confirm that MRC activation is frequency dependent. Lastly, we find that MRCs adapt both to a constant pre-indentation and during continuous sinusoidal stimuli. We are developing a model that integrates the biomechanics of the worm body, the distribution of DEG/ENaC/ASIC channels, and the channels' dependence on both stimulus indentation and velocity to understand these biophysical phenomena. We hope this model will guide genetic dissection of the molecular basis of frequency dependence and both time- and indentation-dependent adaptation.

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100-Plat

The Integration of Mechanical and Chemical Signalling in the Developing Brain

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During development and pathological processes, cells in the central nervous system (CNS) are highly motile. Despite the fact that cell motion is driven by forces, our current understanding of the mechanical interactions between CNS cells and their environment is very limited. We here show how nanometer deformations of CNS tissue caused by piconewton forces exerted by cells contribute to regulating CNS development and pathologies. *In vitro*, growth and migration velocities, directionality, cellular forces as well as neuronal fasciculation and maturation all significantly depended on substrate stiffness. Moreover, when grown on substrates incorporating linear stiffness gradients, glial cells migrated towards stiffer, while axon bundles turned towards softer substrates. *In vivo* atomic force microscopy revealed stiffness gradients in developing brain tissue, which axons followed as well towards soft. Interfering with brain stiffness and mechanosensitive ion channels *in vivo* both led to similar aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Importantly, CNS tissue significantly softened after traumatic injuries. Ultimately, mechanical signals not only directly impacted neuronal growth but also indirectly by regulating neuronal responses to chemical guidance cues, strongly suggesting that neuronal growth is not only controlled by chemical signals – as it is currently widely assumed – but also by the tissue's local physical properties.

101-Plat

Metabolism Modulation of Cancer Cells on Varying Substrate Stiffnesses

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Cancer cells sense their microenvironment and respond to biophysical cues that activate signaling networks through ion channels and integrin proteins. This allows cells to adapt by adjusting their cellular shape and tension that remodels the extracellular matrix (ECM). New evidence shows that these ECM signaling cascades may increase glucose uptake leading to altered metabolic states. The hallmark of metabolic alteration of increase glycolysis, i.e. Warburg effect, in cancer cells together with atypical ECM structure may be responsible for tumor cell aggressiveness and drug resistance. While it is known that tumor cells stiffen the ECM as the tumor progression occurs, a direct relationship between ECM stiffness and altered metabolism has not been explicitly measured. Here we apply the phasor approach to fluorescence lifetime imaging microscopy (FLIM) as a method to measure metabolic as a function of ECM mechanics. We imaged two breast cancer cell lines of high and low aggressiveness (MDA-MB231 and MCF-7, respectively) and compared them to non-cancerous cells. Cells were plated on varying collagen density as measured by image correlation microscopy. Our results show that MDA-MB231 exhibit a decreased fraction of bound NADH (indicative of glycolysis) with increasing substrate stiffness. Inhibition of cell contractility with Y27632 or blebbistatin showed shifts towards a higher free NADH fractional contribution (indicative of oxidative phosphorylation, OXPHOS). All other cell lines showed little change in fraction bound NADH on the varying collagen stiffnesses. We also

conducted metabolism inhibition studies to confirm the shift from OXPHOS to glycolysis and vice-versa on the phasor plot. These results show that the phasor/FLIM approach is a powerful method in monitoring metabolism and mechanics that may improve our understanding in the potential roles it has in cell invasion. This work is supported by the National Institutes of Health grant P41-GM103540.

102-Plat

Cellular Mechanotransduction via Ion Channels at the Cell-Substrate Interface

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Mechanosensitive (MS) ion channels are the fastest mechanotransducers in living cells as they respond to mechanical stimuli on a less than millisecond time scale. They may transduce the mechanical force directly into electrochemical signals. This pathway underlies several physiological processes such as gentle touch and hearing sensation. A number of tools have been developed to measure ionic currents mediated by MS ion channels across a wide variety of cell types. These channels have traditionally been studied by membrane stretch (using high-speed pressure clamp), shear stress (flow) or cellular indentation (using a glass probe). More recently we have established elastomeric pillar arrays as force transducers to apply fine mechanical stimuli directly at the interface between cells and their substrate. One advantage of this technique is that the cell-matrix interface is the proposed site of mechanotransduction in many MS cells, such as sensory neurons and chondrocytes. MS channels such as PIEZO1 and PIEZO2 can be activated by membrane stretch, cell indentation and pillar-deflection. In contrast, TRPV4 is poorly responsive to membrane stretch, non-responsive to indentation but robustly activated by pillar-deflection. What has not been clear is i) how pillar deflection leads to channel activation and ii) if the mechanism of deflection-mediated channel activation is distinct from stretch-activation. We are currently utilising empirical analysis of an array of MS channels combined with light microscopy and finite element modelling. This will enable us to characterize whether MS channels are activated in the cell-substrate interface by changes in membrane tension, and how these membrane tensions relate to those applied using high-speed pressure clamp.

103-Plat

Substrate Rigidity Modulates the Composition in Cell-Matrix Adhesions

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In cell matrix adhesions, integrin receptors and associated proteins provide a dynamic coupling of the extracellular matrix (ECM) to the cytoskeleton. This allows bidirectional transmission of forces between the ECM and the cytoskeleton, which tunes intracellular signaling cascades that control survival, proliferation, differentiation, and motility. The quantitative relationships between recruitment of distinct cell matrix adhesion proteins and local cellular traction forces are not known. Here, we applied quantitative superresolution microscopy to cell matrix adhesions formed on fibronectin-stamped elastomeric pillars and developed an approach to relate the number of talin, vinculin, paxillin, and focal adhesion kinase (FAK) molecules to the local cellular traction force. We find that FAK recruitment does not show an association with traction-force application whereas a ~60 pN force increase is associated with the recruitment of one talin, two vinculin, and two paxillin molecules on a substrate of effective stiffness of 47 kPa. On a substrate with a four-fold lower effective stiffness the stoichiometry of talin:vinculin:paxillin changes to 2:12:6 for the same ~60 pN traction force. The marked relative change in force-related vinculin recruitment indicates a stiffness-dependent switch in vinculin function in cell matrix adhesions. Taken together, our results reveal a substrate-stiffness-dependent modulation of the relation between traction-force and molecular composition of cell-matrix adhesions.

Platform: Sensing In Vivo and In Vitro

104-Plat

Nanofluidic Sensor for Antigen-Antibody Binding Detection

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Nanofluidics is a well established tool for conducting fundamental studies of molecular-scale phenomena. Both the structures nanometric dimension and the size of biomolecules such as DNA or proteins, added to the excellent control on the geometry, give unique features to nanofluidic devices. In particular,