JOINT SYMPOSIUM

BETWEEN

UNIVERSITY OF PENNSYLVANIA
USA

&

MECHANOBIOLOGY INSTITUTE
SINGAPORE

December 9, 2017

Venue:

Mechanobiology Institute
NATIONAL UNIVERSITY OF SINGAPORE

Organizers

***Vivek Shenoy**, University of Pennsylvania, USA*
***G.V.Shivashankar,** Mechanobiology Institute, Singapore*

PROGRAMME
Saturday, December 9, 2017
Venue: MBI Level 5 Seminar Room

Time	Programme
0930-1000am	**Michael Sheetz** ***Mechanobiology Institute, Singapore*** Non-Muscle Sarcomeres Sense Rigidity and Generate High Forces
1000-1030am	**Paul Janmey** ***University of Pennsylvania, USA*** Viscous dissipation in tissues and soft substrates affects focal adhesion formation, cell morphology, and motility
1030-1100am	**Paul Matsudaira** ***Mechanobiology Institute, Singapore*** A Strain Map of Zebrafish Gastrulation Describes the Mechanics of Convergence and Extension and Emergence of Left-Right Symmetry during Epiboly
1100-1130am	Coffee Break
1130-1200am	**Michael Ostap** ***University of Pennsylvania, USA*** Myosin-I motors in membrane transport, deformation, and tubulation using single molecule approaches and engineered cytoskeletal networks
1200-1230pm	**Pakorn (Tony) Kanchanawong** ***Mechanobiology Institute, Singapore*** Visualizing the Molecular Clutch in Cell Adhesions
1230-0100pm	**Guy Genin** ***Washington University in St. Louis, USA*** Microenvironmental remodeling in plant and animal cell mechanobiology
0100-0200pm	Lunch
0200-0230pm	**Rebecca Wells** ***University of Pennsylvania, USA*** Mechanics of normal and fibrotic tissues
0230-0300pm	**Linda Kenney** ***Mechanobiology Institute, Singapore*** *Salmonella* as an anti-tumor agent
0300-0330pm	**Simon Tang** ***Washington University in St Louis, USA*** Effects of Advanced Glycation End-products on the Mechanobiology of the Intervertebral Disc
0330-0400pm	**Lim Chwee Teck** ***Mechanobiology Institute, Singapore*** Mechanobiology of Collective Epithelial Cell Migration
0400-0430pm	Coffee Break
0430-0500pm	**Vivek Shenoy** ***University of Pennsylvania, USA*** Multiscale model predicts increasing focal adhesion size with decreasing stiffness in fibrous Matrices
0500-0530pm	**Alexander Bershadsky** ***Mechanobiology Institute, Singapore*** Integrin cell-matrix adhesions mediate and are shaped by the microtubule-myosin II crosstalk
0530-0600pm	**G.V.Shivashankar** ***Mechanobiology Institute, Singapore & IFOM, Italy*** Geometric control of genome programs

ABSTRACTS

Non-Muscle Sarcomeres Sense Rigidity and Generate High Forces

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A critical issue for normal cells is rigidity sensing since they will grow or die dependent upon matrix rigidity. Recent findings show that the basis of rigidity sensing in non-muscle cells is the contraction of a sarcomere-like complex to a fixed distance; and if the force exceeds 25 pN, then the surface is rigid (Meacci et al., 2016; Wolfenson et al., 2016). In dual stiffness pillar experiments, we find that very rigid (60 pN/nm) pillars are pulled to 60 nm by 110-150 myosin heads in bipolar filaments (>20 pN/head), which is theoretically possible because of the slow velocity (2-3 nm/sec) of the contractions (Lohner, Ruprecht, Prost and Sheetz). On soft surfaces, rigidity-sensing causes apoptosis but the depletion of many cytoskeletal proteins such as tropomyosin 2.1, α-actinin, and myosin IIA will block rigidity-sensing (Meacci et al., 2016; Saxena et al., 2017b; Wolfenson et al., 2016) causing transformed growth. All transformed cancer cells tested lacked rigidity sensing (Yang et al., submitted) and the restoration of normal levels of cytoskeletal proteins restored rigidity sensing and rigidity-dependent growth or anoikis. Thus, the sarcomeric contraction units can sense rigidity and develop very high forces. Their loss results in transformed growth of cancer cells.

Saxena, M., et al. (2017a). "Force induced calpain cleavage of talin is critical for growth, adhesion development and rigidity sensing." Nano Lett. In Press.

Meacci, G., Wolfenson, H., Liu, S., Stachowiak, M.R., Iskratsch, T., Mathur, A., Ghassemi, S., Gauthier, N., Tabdanov, E., Lohner, J., *et al.* (2016). alpha-Actinin links extracellular matrix rigidity-sensing contractile units with periodic cell-edge retractions. Mol Biol Cell *27*, 3471-3479.

Saxena, M., Liu, S., Yang, B., Hajal, C., Changede, R., Hu, J., Wolfenson, H., Hone, J., and Sheetz, M.P. (2017b). EGFR and HER2 activate rigidity sensing only on rigid matrices. Nat Mater *16*, 775-781.

Wolfenson, H., Meacci, G., Liu, S., Stachowiak, M.R., Iskratsch, T., Ghassemi, S., Roca-Cusachs, P., O'Shaughnessy, B., Hone, J., and Sheetz, M.P. (2016). Tropomyosin controls sarcomere-like contractions for rigidity sensing and suppressing growth on soft matrices. Nat Cell Biol *18*, 33-42.

Viscous dissipation in tissues and soft substrates affects focal adhesion formation, cell morphology, and motility

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Soft tissues often exhibit stress relaxation after deformation that changes with aging and disease. Tissues often have loss moduli that are >20% of their elastic moduli and therefore behave as viscoelastic solids on a timescale relevant to mechanical sensing. The response of cells to a time-dependent viscous loss is largely uncharacterized because appropriate viscoelastic materials are lacking for quantitative studies. New soft viscoelastic solids in which the elastic and viscous moduli can be independently tuned to produce gels with viscoelastic properties that closely resemble those of soft tissues show how strongly viscous dissipation alters cytoskeletal assembly and cell function even on stiff substrates. Systematic alteration of the viscous and elastic components in viscoelastic substrates demonstrates the time dependence of cellular mechanical sensing and the influence of viscous dissipation on the mechanical signals to which cells respond.

A Strain Map of Zebrafish Gastrulation Describes the Mechanics of Convergence and Extension and Emergence of Left-Right Symmetry during Epiboly

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During the development of the zebrafish embryo, the major embryonic axis and tissue patterns are organized by morphogen gradients and biomechanical coupling through cell-cell and cell-matrix adhesions. How collective migration and local mechanical stress are coordinated globally over the entire embryo remains unknown. We have adapted the high spatial-temporal resolution of light sheet microscope system for *in toto* imaging and a 2D tissue tectonics algorithm to generate a 3D strain map of zebrafish gastrulation. The resulting strain maps reveal the time-course and location of areas undergoing deformation during well-known morphogenetic movements, such as convergence and extension. In addition to linear strain during gastrulation, we detect a new mechanical signature during epiboly, rotational or curl strain, which suggests that left-right symmetry is mechanically coupled during the earliest stages of development.

Myosin-I motors in membrane transport, deformation, and tubulation using single molecule approaches and engineered cytoskeletal networks

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Many morphological changes in the plasma and intracellular membranes are powered by the actions of cytoskeletal motors. These motors include myosin-Is, which are the widely expressed members of the myosin superfamily that bind directly to membranes, linking them to the actin cytoskeleton. To better understand the molecular roles of myosin-I isoforms, and the mechanisms by which they modulate membrane dynamics, we performed a range of single-molecule biophysical, structural, biochemical, and cell biological experiments to determine force-generating, membrane-binding, and motile properties of these motors. In this talk, I will focus on our work characterizing the attachment of myosin-I to membrane bilayers in the presence and absence of mechanical load, and I will discuss mechanisms by which myosin-I and microtubule motors transport and remodel membrane-bound vesicles. This work includes the use of engineered in vitro cytoskeletal matrices composed of sparse microtubules intersecting regions of dense actin filaments to reveal how molecular motors deform and tubulate membrane vesicles in cooperation with of Bin-Amphiphysin-Rvs-domain (BAR-domain) proteins.

Visualizing the Molecular Clutch in Cell Adhesions

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Cell adhesion structures such as the integrin-based focal adhesions and cadherin-based cell-cell junctions are multi-protein complexes known to transmit, sustain, sense, and respond to mechanical forces. These force-dependent processes are important for coordinating motion at the cellular and tissue scales, supporting important physiological programmes ranging from cell migration to developmental morphogenesis. The roles of the adhesions with respect to these forces are commonly conceptualized in terms of the 'Molecular Clutch', whereby the motion generated within the actomyosin networks, such as actin retrograde flow, is coupled to the traction borne by cell adhesion receptors with a highly regulated spatiotemporal specificity. Since these cell adhesions are supramolecular complexes self-organized from a diverse ensemble of 'building-block' proteins, the knowledge of their molecular-scale spatial organization is essential for insights into how the Molecular Clutch functions. Here, interference-based techniques in superresolution microscopy have been particularly useful for achieving sub 20-nm resolution important for deciphering protein organization. I will discuss our recent studies whereby we elucidated the nanoscale architecture of cell adhesion complexes and probed the molecular orientation and conformational transitions of key mechanotransducer proteins, providing structural frameworks for understanding how mechanical forces and biochemical signals may be integrated at cell adhesion sites.

Microenvironmental remodeling in plant and animal cell Mechanobiology

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The mechanisms by which plant and animal cells adapt their local mechanical environments are central to the ways that they feel, respond to, and remember mechanical forces. Our group focuses on integrated mechanical modeling and experimental analysis of active cellular remodeling of the plant and animal cell microenvironment. In plant cells, relatively rapid reconfigurations of the extracellular space are possible in response to mechanically induced calcium fluxes. In animal cells, microenvironmental modification is dominated by the actions of filopodia. This presentation will summarize the models and experimental tools that our group is developing to characterize and manipulate these microscale mechanisms that plant and animal cells use to manipulate their microenvironments.

Mechanics of normal and fibrotic tissues

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Fibrotic tissues are markedly stiffer than normal tissues. This is often assumed to be secondary to collagen, which increases as fibrosis progresses. Detailed mechanical studies show, however, that the relationship between stiffness and collagen content is not linear and that collagen cross-linking is a significant determinant of stiffness. Additionally, tissues demonstrate marked stiffening under physiological levels of compression and the degree of this compression stiffening increases as fibrosis progresses. The use of disintegrins (which block integrin-matrix attachments) and either hyaluronidase or amylase (which remove some glycosaminoglycan residues) significantly decreases compression stiffening, suggesting that both non-collagenous components of the matrix and the presence of highly-charged glycosaminoglycans contribute to complex tissue mechanics.

Salmonella as an anti-tumor agent

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Spontaneous tumor regression following a severe microbial infection has been observed for the last >150 years or more, inspiring the earliest cancer therapies. Studies in animal models have demonstrated that bacteria such as *Clostridium*, *Salmonella* and *Bifidobacterium* can colonize solid tumors. These species are now being engineered to provide therapeutics to combat tumor growth. In the case of *Salmonella*, its colonization of the host also promotes a host anti-tumor response in mice, although clinical trials in humans failed. It is not yet clear whether colonization of the tumor is responsible for the anti-tumor response, or whether these two events are separable. Nothing is known mechanistically or at the molecular level regarding how bacteria target tumors. Two opposing models are proposed for how *Salmonella* exerts its anti-tumor response. Both of these opposing mechanisms employ the *Salmonella* regulator SsrB, a regulator long-studied in our laboratory. SsrB drives biofilm formation in the absence of its kinase or in its presence, promotes intracellular survival. Our research is aimed at reconciling these two opposing views and focuses on a mechanistic understanding of tumor targeting and the anti-tumor response of *Salmonella*.

Effects of Advanced Glycation End-products on the Mechanobiology of the Intervertebral Disc

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INTRODUCTION: Diabetes mellitus is a significant risk factor for IVD degeneration [2,3], and diabetics are susceptible to the accumulation of advanced glycation end-products (AGEs). AGEs diminish the mechanical performance of skeletal tissues including bone and cartilage [4,5]. We have previously shown that AGEs and the upregulation of Receptor for AGEs (RAGE) signaling by High Motility Group Box 1 (HMGB1) increases expression of proteases and inflammatory cytokines, and deteriorates the structure and mechanics of the murine intervertebral disc [6, 7]. Here, we investigate the necessity of RAGE signaling in the AGEs- and HMGB1- mediated degeneration of the intervertebral disc using a global deletion of RAGE in nine-week old mice.

METHODS: All animal experiments were done with approval from the Washing University Animal Studies Committee approval. Mice with a global deletion of the RAGE (RAGE KO) [8] and their WT littermates were allowed to age to nine weeks of age (n = 6). The mice were euthanized and 3 lumbar functional spine units (FSU), containing an intact vertebrae-disc-vertebrae structure, from each animal were dissected under aseptic conditions per IACUC approval. The FSU samples were cultured in 2mL of DMEM:F12 supplemented with 20% fetal bovine serum and 1% penicillin-streptomycin. Media was changed every 48 hours. After an initial 48-hour conditioning period, the FSUs from both groups (RAGE KO or WT) are then split further into three groups treated with 100nM of HMGB1 administered once at day 1, 20 g/mL of AGEs, or control media throughout the culture period. These samples were then cultured for 21 days. Disc height was measured using a laser micrometer, proteoglycan were measured using biochemical fluorescent assays, and mechanical properties were determined using cyclical dynamic compression [9]. All quantitative analyses were normalized to either the RAGE KO or the WT control groups. Finally, samples were processed for histology and stained for Safranin-O with a Fast Green counter stain.

RESULTS:
A: Compositional analyses of the IVDs showed that RAGE KO animals significantly blunts the AGEs- and HMGB1- loss of proteoglycans in the IVD ($p < 0.001$; $p < 0.001$). **B:** Likewise, the disc heights in RAGE KO animals for either groups were significantly less than the AGEs- and HMGB1- treated IVDs ($p < 0.001$; $p < 0.001$). **C&D:** Mechanical analyses showed that RAGE KO IVDs had improved stiffness and Tan Delta values at 5% strain levels compared to the WT treated groups ($p < 0.05$ for all comparisons). **E-G:** Histological analyses show that the RAGE KO IVDs remain structurally intact despite the AGEs- and HMGB-1 treatment.

DISCUSSION:
Diabetes increases AGEs and impairs the mechanical behavior of IVD tissues [10,11]. In this study, the deletion of RAGE appears to have mitigated the adverse effects of AGEs and HMGB1 treatment. We have previously shown that AGEs accumulation and RAGE signaling is elevated in the IVDs of diabetic animals [7]. Furthermore, the AGEs rich environment can stimulate a milieu of inflammatory cytokines including elevated TNFa, NFkB, IL1, as well as depressed TIMPs and increased MMPs that may contribution to inflammation and pain in the IVD. The inhibition of the RAGE pathway may be a viable therapeutic strategy to arrest degeneration and alleviating inflammation relating to pain.

SIGNIFICANCE:
The deletion of RAGE blunts the AGEs- and HMGB1- mediated degeneration of the IVD and may be a viable therapeutic strategy in diabetes related low back pain and disc degeneration.

Mechanobiology of Collective Epithelial Cell Migration

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Epithelial cells migrating in sheets or large cohorts tend to behave very differently from cells migrating individually. Such distinctive behavior of cells migrating in a collective manner underlies several important biological processes such as wound closure, maintenance of intestinal epithelium, developmental processes and even cancer metastasis. As such, they can also provide important insights towards better tissue repair and regenerative medicine. Here, we characterized the kinematic behavior of epithelial cell cohorts migrating under well defined geometrical constraints. We also studied collective cell migration over areas without cell adherent proteins to examine the formation of epithelial bridges so as to better wound closure mechanisms. Finally, we investigated the emergent patterns of collective cell migration on curved surfaces and under tubular confinement.

1. Saw et al., Topological defects in epithelia govern cell death and extrusion, Nature, 2017.
2. Xi et al., Emergent patterns of collective cell migration under tubular confinement, Nature Communications, 2017.
3. Vedula et al., Mechanics of epithelial closure over non-adherent environments, Nature Communications, 2015.
4. Vedula et al., Epithelial bridges maintain tissue integrity during collective cell migration, Nature Materials, 2014.
5. Vedula et al., Emerging modes of collective cell migration induced by geometrical constraints, PNAS, 2012.

Biography

Professor Lim is the inaugural NUSS Professor at the Department of Biomedical Engineering and Mechanobiology Institute at NUS. He has authored more than 320 journal papers and delivered more than 310 invited talks. He is an elected Fellow of both the AIMBE and IAMBE. He is also an elected council member of the World Council of Biomechanics. He currently sits on the editorial boards of 20 international journals. Prof Lim has co-founded five startups which are commercializing technologies developed in his lab. He and his team have garnered more than 80 research awards and honors including the International Precision Medicine Conference Prize 2017, Asian Scientist 100 2016, ASEAN Outstanding Engineering Achievement Award 2016, President's Technology Award 2011 and the IES Prestigious Engineering Achievement Award 2010, 2016 among others.

Multiscale model predicts increasing focal adhesion size with decreasing stiffness in fibrous matrices

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We describe a multiscale model that incorporates force-dependent mechanical plasticity induced by interfiber cross-link breakage and stiffness-dependent cellular contractility to predict focal adhesion (FA) growth and mechanosensing in fibrous extracellular matrices (ECMs). The model predicts that FA size depends on both the stiffness of ECM and the density of ligands available to form adhesions. Although these two quantities are independent in commonly used hydrogels, contractile cells break cross-links in soft fibrous matrices leading to recruitment of fibers, which increases the ligand density in the vicinity of cells. Consequently, although the size of focal adhesions increases with ECM stiffness in nonfibrous and elastic hydrogels, plasticity of fibrous networks leads to a departure from the well-described positive correlation between stiffness and FA size. We predict a phase diagram that describes nonmonotonic behavior of FA in the space spanned by ECM stiffness and recruitment index, which describes the ability of cells to break cross-links and recruit fibers. The predicted decrease in FA size with increasing ECM stiffness is in excellent agreement with recent observations of cell spreading on electrospun fiber networks with tunable cross-link strengths and mechanics. Our model provides a framework to analyze cell mechanosensing in nonlinear and inelastic ECMs.

Integrin cell-matrix adhesions mediate and are shaped by the microtubule-myosin II crosstalk

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Abstract

The interrelationship between microtubules and the actin cytoskeleton in mechanoregulation of integrin-mediated adhesions is poorly understood. Here, we show that uncoupling of microtubules from integrin adhesions by depletion or displacement of KANK family proteins connecting the adhesion protein talin with microtubule tips led to disruption of podosomes and augmentation of focal adhesions, similarly to total disruption of microtubules. Both microtubule uncoupling from adhesions and total microtubule disruption bring about a massive assembly of myosin-IIA filaments, whilst a burst of microtubule polymerization led to a transient disassembly of myosin-IIA filaments. Myosin-IIA filaments are indispensable for microtubule-dependent regulation of integrin-mediated adhesions. The myosin-IIA filament assembly depends on Rho activation by RhoGEF, GEF-H1, which is trapped by microtubules connected with integrin adhesions but released after their disconnection. Thus, microtubule capturing by integrin-mediated adhesions modulates the effect of microtubules on the actomyosin cytoskeleton. The actomyosin reorganization then remodels the adhesions, closing the regulatory loop.

Geometric control of genome programs

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Cells integrate both biochemical and physical signals within the tissue microenvironment to maintain homeostasis. However, cell geometric constraints are highly heterogeneous and it is unclear how such heterogeneity in their shapes (and thus mechanics) affect the micro environmental regulation of genome programs. To address this we use micro patterned substrates to sculpt cell geometry and study its role in integrating micro environmental signals such as cytokines and tissue compression. In this talk, I will discuss an important layer of genome regulation resulting from the coupling between cell geometry and 3D organization of chromosomes. Collectively, our results show that geometric states of cells dictate micro environmental control of gene expression and sustained growth of cells on such geometrically confined substrates result in novel routes to nuclear reprogramming.