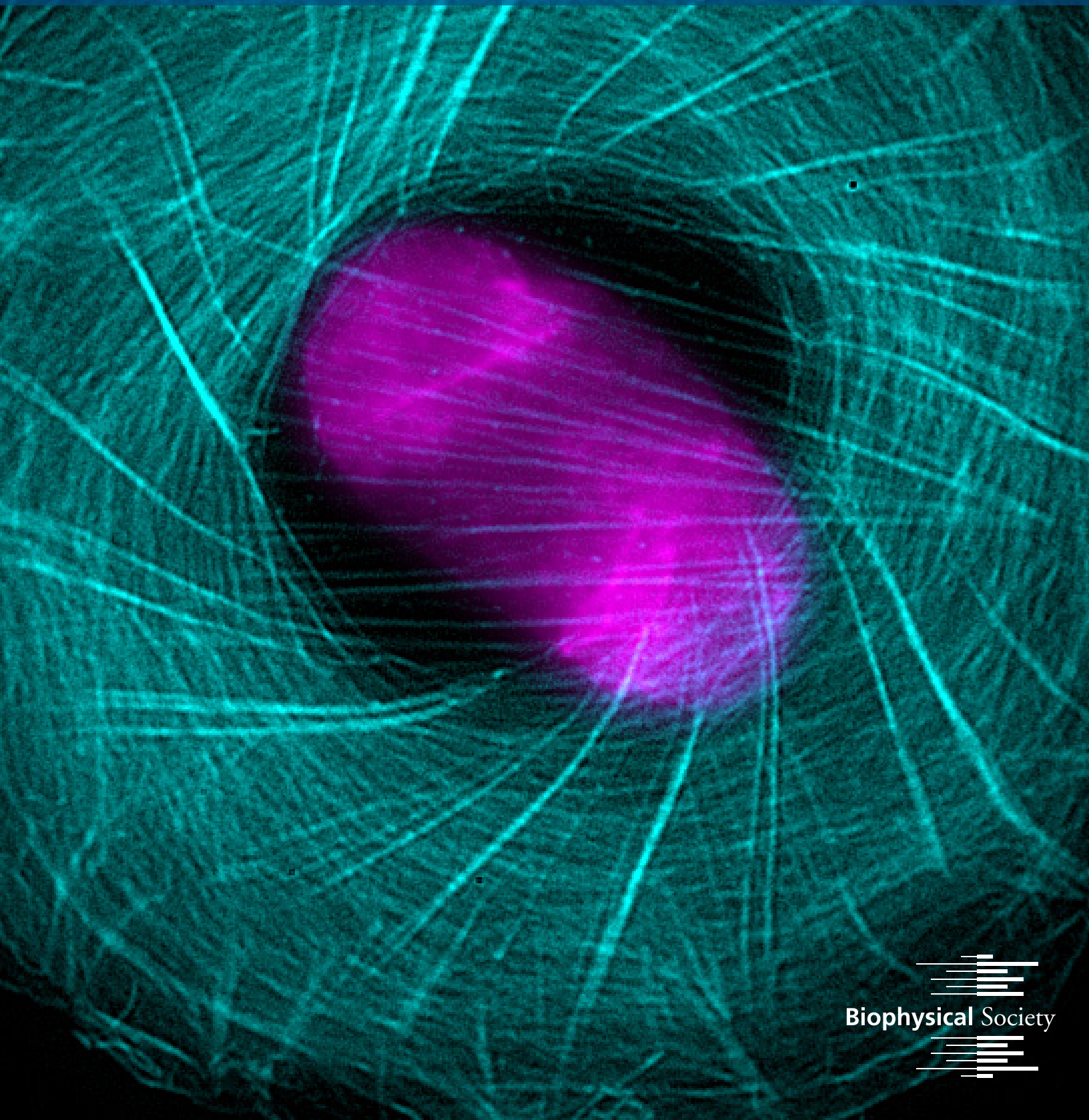
Mechanobiology of Disease

Singapore | September 27–30, 2016



Biophysical Society

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Cover photo by Dr. Tee Yee Han, Mechanobiology Institute
or Dr. Tee Yee Han, Bershadsky Lab, Mechanobiology Institute

Thank You to Our Sponsors

September 2016

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting, *Mechanobiology of Disease*, co-organized by the Mechanobiology Institute (MBI), National University of Singapore.

Over the past few years, mechanobiology has been instrumental in answering fundamental biological questions. Excitingly, many researchers are now applying mechanobiology to revolutionize our understanding of disease pathogenesis. Our understanding of how cells integrate mechanics is now inspiring the design of new therapies and diagnostic tools.

At its core, mechanobiology is a truly interdisciplinary science. Although the core principles lie at the interface between physics and biology, mechanobiology has benefited from contributions from many other disciplines including engineering, developmental biology, virology, and computational science. As a result, this meeting brings together a diverse group of world-leading scientists to provide their international and multidisciplinary perspectives on the mechanobiology of disease.

The scientific program will have around 30 talks and 80 posters, providing a snapshot of the latest research findings in the field. We have also allowed plenty of time for informal discussion and networking during lunches, coffee breaks, and the banquet, and hope that this will enable a lively exchange of ideas and inspire new research collaborations to dissect the mechanobiology of cancer, infectious diseases, and many other disorders.

We would like to thank our sponsors Fisher Scientific, MERCK, Rexadvance, Nanyang Technological University and The Company of Biologists for supporting this thematic meeting.

Finally, we hope you enjoy your visit to tropical Singapore and the unique professional and cultural experiences offered by this little red dot!

Sincerely,

The Organizing Committee

Dino Di Carlo
Jochen Guck
Linda J Kenney
Chwee Teck Lim
Michael Sheetz
G.V. Shivashankar

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All meeting functions will be held in the Theatre at Level 1 of the University Cultural Center (UCC), National University of Singapore unless otherwise noted

GENERAL INFORMATION

Registration Hours/Information Location and Hours

Registration will be located at Level 1 of the Theatre Foyer at University Cultural Centre (UCC), National University of Singapore. Registration hours are as follows:

Tuesday, September 27	9:00 – 16:00
Wednesday, September 28	9:00 – 16:00
Thursday, September 29	9:00 – 16:00
Friday, September 30	9:00 – 13:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be made available in the Theatre. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

1) All poster sessions will be held at Level 2 of the Hall Foyer at UCC

2) A display board measuring 1000mm wide by 2000mm high will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the e-book.

3) There will be formal poster presentations on Tuesday, Wednesday, and Thursday. All posters will be available for viewing during all poster sessions.

4) During the assigned poster presentation sessions, presenters are requested to remain in front of their poster boards to meet with attendees.

5) All posters left uncollected at the end of the meeting will be disposed of.

Meals and Coffee Breaks

The following food functions are included in your registration: morning and afternoon coffee breaks, daily luncheons September 27, 28, and 29 and an evening banquet on Thursday, September 29. The banquet will be held at the Republic of Singapore Yacht Club. Transportation will be provided.

Smoking

Please be advised that smoking is not permitted on University Campus, including the conference venue.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions, and social functions. Please wear your badge throughout the conference.

University Map

Click here for a map of the campus.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from September 27 – September 30 during registration hours.

In case of emergency, you may contact the following:

Lathe Krishnarajpet Shiva
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Dorothy Chaconas
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Mechanobiology of Disease

Singapore
September 27-30, 2016

PROGRAM

Tuesday, September 27, 2016

9:00 – 16:00	**Registration/Information**	**Level 1 Theatre Foyer**
9:20 – 9:30	Michael P. Sheetz, Mechanobiology Institute, Singapore **Welcome**	**Level 1 Theatre**
Session I	Alexander Bershadsky, Mechanobiology Institute, Singapore, Chair	
9:30 – 10:00	Michael P. Sheetz, Mechanobiology Institute, Singapore ***Rigidity Sensing Contractions Inhibit Transformed Growth***	
10:00 – 10:30	Bernhard Wehrle-Haller, University of Geneva, Switzerland ***Integrin-Dependent Mechano-signaling, Switching Integrin Behavior by Alternative Splicing and Posttranslational Modification***	
10:30 – 11:00	Vania Braga, Imperial College London, United Kingdom ***Interplay Between Cortical Tension and Junction Composition and Configuration***	
11:00 – 11:30	**Coffee Break**	**Level 1 Hall Foyer**
Session II	MinWu, Mechanobiology Institute, Singapore, Chair	
11:30 – 12:00	Katharina Gaus, University of New South Wales, Australia ***Receptor Clustering – a New Model for Signal Transduction in T Cells***	
12:00 – 12:30	Gregory Giannone, Interdisciplinary Institute for Neuroscience, France ***Super-resolution Microscopy: a Window for Integrin Spatiotemporal and Mechanical Regulation***	
12:30 – 12:45	Aastha Kapoor, Indian Institute of Technology, India* ***Rho-ROCK-Myosin Based Cellular Contractility Regulates Distinct Modes of Invasion in Paclitaxel and Cisplatin Resistant Ovarian Cancer Cells***	
12:45 – 13:00	Amit Pathak, Washington University in St. Louis, USA* ***Mechanobiology of Epithelial-to-Mesenchymal Transition in Confined Environments***	
13:00 – 14:00	**Lunch**	**Level 1 Hall Foyer**
Session III	Paul Matsudaira, Mechanobiology Institute, Singapore, Chair	
14:00 – 14:30	James R. Sellers, NHLBI, NIH, USA ***Mechanical Properties of Nonmuscle Myosin-2 Filaments***	
14:30 – 15:00	Susan M. Rosenberg, Baylor College of Medicine, USA	

	How Bacteria and Cancer Cells Regulate Mutagenesis and Their Ability to Evolve
15:00 – 15:30	Marco Foiani, IFOM, Italy ***An Integrated ATR, ATM, and mTOR Mechanical Network Controlling Nuclear Plasticity***
15:30 – 15:45	Elisa Caberlotto, L'OREAL, France* ***Effects of a Soft Massaging Device, Based on an Oscillating Torque, upon the Expression of Some Dermal Proteins of Human Skin. Influence of Frequency***
15:45 – 16:00	Rafi Rashid, National University of Singapore, Singapore* ***A Tale of Two Viscosities: Microviscosity More Important Than Macroviscosity in a Crowded Microenvironment***
16:00 – 18:00	**Coffee Break and Posters Session I** **Level 2 Hall Foyer**

Wednesday, September 28, 2016

9:00 – 16:00	**Registration/Information** **Level 1 Theatre Foyer**
Session IV	Jochen Guck, Technische Universität, Dresden, Germany, Chair
9:30 – 10:00	Chwee Teck Lim, Mechanobiology Institute, Singapore ***Mechanobiology of Collective Cell Migration in Health and Disease***
10:00 – 10:30	Amy Rowat, University of California, Los Angeles, USA ***Cell Mechanotype in Cancer***
10:30 – 11:00	Oliver Otto, Dresden University of Technology, Germany ***Feeling for Phenotype: Real-Time Deformability Cytometry for Label-Free Cell Functional Assays***
11:00 – 11:30	**Coffee Break** **Level 1 Hall Foyer**
Session V	Jochen Guck, Technische Universität, Dresden, Germany, Chair
11:30 – 12:00	Shyni Varghese, University of California, San Diego, USA ***Role of Matrix Proteins in Balancing Tissue Stiffness and Inflammation in Fibrosis***
12:00 – 12:30	Henry T. Tse, CytoVale, Inc., USA ***Next-Generation Deformability Cytometry for Rapid Biophysical Phenotyping***
12:30 – 12:45	Natalie Woolger, INMR, Australia* ***Calpains Influence both Cytoskeletal Remodeling and Ca^{2+}-Triggered Vesicle Fusion in the Emergency Response to Repair a Membrane Injury***
12:45 – 13:00	Victor Ma, Emory University, USA* ***Molecular Tension Probes Reveal the Role of Mechanics in T-Cell Recognition***
13:00 – 14:00	**Lunch** **Level 1 Hall Foyer**
Session VI	Dino Di Carlo, University of California, Los Angeles, USA, Chair

14:00 – 14:30	Adam Engler, University of California, San Diego, USA ***Improving iPSC Disease Modeling with Dynamic Matrices***
14:30 – 15:00	Krystyn J. Van Vliet, MIT, USA ***Crawling Toward a Cure: Mechanobiology of Cell Migration and Differentiation in the Disease Microenvironment***
15:00 – 15:30	Yunn Hwen Gan, National University of Singapore, Singapore ***Bacterial Pathogen Induces Host Cell Fusion and Triggers the Type I Interferon response through cGAS and STING***
15:30 – 16:00	Samuel Safran, Weizmann Institute of Science, Israel* ***Self-healing of Holes in the Nuclear Envelope***
16:00 – 16:15	Nicolas Plachta, Institute of Molecular & Cell Biology, Agency for Science, Technology & Research, Singapore ***Imaging How Cells Choose their Fate, Shape, and Position in the Mouse Embryo***
16:15 – 18:15	**Coffee Break and Posters Session II** **Level 2 Hall Foyer**

Thursday, September 29, 2016

9:00 – 16:00	**Registration/Information** **Level 1 Theatre Foyer**
Session VII	Virgile Viasnoff, Mechanobiology Institute, Singapore, Chair
9:30 – 10:00	Thomas Lecuit, Insitut de Biologie du Développement de Marseille, France ***Biomechanical Control of Tissue Morphogenesis***
10:00 – 10:30	Timothy Saunders, Mechanobiology Institute, Singapore ***Muscle Specification in the Zebrafish Myotome***
10:30 – 11:00	Lars Dietrich, Columbia University, USA ***Interplay Between Morphology and Metabolism in Pseudomonas Aeruginosa Biofilms***
11:00 – 11:30	**Coffee Break** **Level 1 Hall Foyer**
Session VIII	Koh Cheng Gee, Mechanobiology Institute, Singapore, Chair
11:30 – 12:00	Carl-Philipp Heisenberg, Institute of Science and Technology Austria, Austria ***The Physical Basis of Coordinated Tissue Spreading in Zebrafish Gastrulation***
12:00 – 12:30	Stuti K. Desai, Mechanobiology Institute, Singapore ***SsrB as a Driver of Lifestyle Changes in Salmonellae***
12:30 – 13:00	Andrew W. Holle, Max Planck Institute of Intelligent Systems, Germany ***Cancer Cells Sense and Respond to Their Mechanical Environment During Confined Invasion***
13:00 – 14:00	**Lunch** **Level 1 Hall Foyer**

Session IX	Ashok Venkitaraman, University of Cambridge, United Kingdom, Chair
14:00 – 14:30	Yusuke Toyama, Mechanobiology Institute, Singapore ***Mechanical Impact of Apoptosis in a Tissue***
14:30 – 15:00	Marvin Whiteley, University of Texas at Austin, USA ***Biogeography of in vivo Microbial Biofilms***
15:00 – 15:30	Alexander Dunn, Stanford University, USA ***Single Molecule Force Measurements in Living Cells Reveal a Minimally Tensioned Integrin State***
15:30 – 15:45	Pere Roca-Cusachs, University of Barcelona, Spain* ***Force Loading Explains How Substrate Rigidity and Ligand Nano-distribution Regulate Cell Response.***
15:45 – 16:00	Priyamvada Chugh, University College London, United Kingdom* ***Cortex Architecture Regulates Cortex Tension during the Cell Cycle***
16:00 – 18:00	**Coffee Break and Posters Session III** **Level 2 Hall Foyer**
18:15	Bus departs UCC for banquet
18:30 – 20:30	**Banquet** **Republic of Singapore Yacht Club**

Friday, September 30, 2016

9:00 – 13:00	**Information** **Level 1 Theatre Foyer**
Session X	Linda J. Kenney, Mechanobiology Institute, Singapore, Chair
9:30 – 10:00	Xavier Trepat, Institute for Bioengineering of Catalonia, Spain* ***A Mechanically Active Heterotypic Adhesion Enables Fibroblasts to Drive Cancer Cell Invasion***
10:00 – 10:30	Kevin Chalut, University of Cambridge, United Kingdom ***Mechanical Signalling in Embryonic Stem Cell Self-renewal and Differentiation***
10:30 – 11:00	G.V. Shivashankar, Mechanobiology Insitute, Singapore ***Nuclear Mechano-genomics and Disease Diagnosis***
Session XI	Linda J. Kenney, Mechanobiology Institute, Singapore, Chair
11:00 – 11:30	**Coffee Break** **Level 1 Hall Foyer**
11:30 – 12:00	Daniela Rhodes, Nanyang Technological University, Singapore ***Telomerase and G-quadruplexes***
12:00 – 12:30	Ashok Venkitaraman, University of Cambridge, United Kingdom ***Cancer Suppression and the Mechanics of Nucleoprotein Assemblies in DNA Recombination***
12:30 – 13:00	Shee Mei Lok, DUKE-NUS Medical School, Singapore ***Near Atomic Resolution CryoEM Structure of the Thermally Stable Zika Virus***

13:00 – 13:15	Zeinab Jahed, University of California, Berkeley, USA* ***Molecular Mechanisms of Mechanotransduction through LINC Complexes***
13:15 – 13:30	Radu Tanasa, University of Cambridge, United Kingdom* ***Mechanics of Embryonic Zebrafish Revealed by Magnetically Applied Local Forces***
13:30 – 13:40	Linda J. Kenney, Mechanobiology Institute, Singapore **Closing Remarks and *Biophysical Journal* Poster Awards**

**Contributed talks selected from among submitted abstracts*

SPEAKER ABSTRACTS

Rigidity Sensing Contractions Inhibit Transformed Growth

Michael P. Sheetz[1,2] Bo Yang[1], Haguy Wolfenson[2], Zi Zhao Lieu[1], Feroz M.Hameed[1], Alexander D. Bershadsky[1]
[1]Mechanobiology Institute, National University of Singapore, Singapore, [2]Department of Biological Sciences, Columbia University, NY, USA

Matrix rigidity is an important physical aspect of cell microenvironments; however, the mechanism by which cells test substrate rigidity is not clear. Submicron pillar studies indicate that cells sense rigidity by measuring the forces required for local standard contractions at the cell periphery (pinching activity) (Ghassemi et al., 2012. PNAS 109:5328). Recent observations show that sarcomere-like units drive step-wise contractions that depend upon tropomyosin to sense rigidity and block growth on soft surfaces (Wolfenson et al., 2016. Nat. Cell Bio. 18:33). In addition, two tyrosine kinases involved in cancer progression are part of the contractile units and control distance and time of contractions to modify rigidity sensing (Yang et al., 2016. Nanoletters. In Press). Thus, we suggest that these tyrosine kinases affect adhesion-dependent mechanosensitivity and consequently metastasis and morphology changes in development through their regulation of local mechanosensory contractions by sarcomere-like units with tropomyosin.

Integrin-dependent Mechano-signaling, Switching Integrin Behavior by Alternative Splicing and Posttranslational Modification

Bernhard Wehrle-Haller.
University of Geneva, Geneva, Switzerland.

ß1-integrin-dependent cell-matrix adhesions provide anchorage to the extracellular matrix, as well as signaling for cell migration, survival and proliferation. On the other hand, the a5ß1 integrin has also a specific role in the deposition and re-organization of fibronectin fibrils in the extracellular space, which is critical for tissue formation, regeneration, but also relevant to pathologies such as fibrosis or cancer. How integrins can switch between the adhesion, signaling and fibronectin remodeling is not understood. ß1-integrin function is controlled allosterically by ligand binding to the extracellular domain and recruitment of cytoskeletal adapter proteins like talin and kindlin to the cytoplasmic tail. Differential recruitment of talin and kindlin isoforms have been linked to different types of cell-matrix adhesions, as well as alternatively spliced cytoplasmic domains, providing a plausible hypothesis that selective adapter recruitment controls and allows switching integrin function. With a fluorescently tagged ß1-integrin, we have analyzed the dynamics and signaling capacities of the commonly expressed ß1A-integrin, as well as the alternatively spliced ß1D-integrin, exclusively expressed in differentiated muscle. Interestingly, the distinct dynamics of these two splice variants is linked to differences in signaling rather than ligand or adapter binding affinities. This concept is also found in ß3-integrins, in which phosphorylation of the cytoplasmic domain is controlling the dynamic of integrin adhesions and thus cell motility. Our data provide a conceptual framework, how posttranslational modifications of the integrin cytoplasmic tail allow switching between integrin functions critical for cell adhesion and mechano-signaling, but also remodeling of the extracellular matrix. Interestingly the latter switch is coupled to the metabolic state of the cell, providing an unexpected regulation of integrin-dependent functions during proliferation and differentiation or pathologies such as fibrosis and cancer.

Interplay Between Cortical Tension and Junction Composition and Configuration

Vania Braga.
National Heart and Lung Institute, Imperial College London, London, United Kingdom.

Cell-cell adhesion plays an essential role in the determination of cell shape and function during development and adult life. Dynamic remodelling of junctions supports the maintenance of tissue integrity, morphogenesis and homeostasis. Conversely, tumour de-differentiation in epithelial tissues is accompanied by disruption of cell-cell contacts and re-writing of signalling to drive uncontrolled proliferation and migration. In epithelia, stabilization of E-cadherin contacts relies on the reorganization of the cortical actin cytoskeleton to sustain mechanical stress and maintain clustered receptors. I will discuss here how cortical tension modulates the way epithelial cells interact with each other and the cytoskeletal responses triggered to counteract mechanical stress.

Receptor Clustering – A New Model for Signal Transduction in T Cells

Katharina Gaus.
University of New South Wales, Sydney, Australia.

Antigen recognition by the T cell receptor (TCR) is a hallmark of the adaptive immune system. When the TCR engages a peptide bound to the restricting major histocompatibility complex molecule (pMHC), it transmits a signal via the associated CD3 complex. How the extracellular antigen recognition event leads to intracellular phosphorylation remains unclear. Here, we used single-molecule localization microscopy to quantify the organization of TCR-CD3 complexes into nanoscale clusters and to distinguish between triggered and non-triggered TCR-CD3 complexes. We found that only TCR-CD3 complexes in dense clusters were phosphorylated and associated with downstream signaling proteins, demonstrating that the molecular density within clusters dictates signal initiation. Both pMHC dose and TCR-pMHC affinity determined the density of TCR-CD3 clusters, which scaled with overall phosphorylation levels. Thus, a new model of TCR triggering has started to emerge in which ligand binding is first translated into TCR-CD3 clustering and receptor clusters in a second step initiate intracellular signaling. The quality of an antigen can thus be measured by its ability to form signaling signaling-competent receptor clusters. We process that this two-step process is required for antigen discrimination.

Super-resolution Microscopy: a Window for Integrin Spatiotemporal and Mechanical Regulation

Gregory Giannone.
Interdisciplinary Institute for Neuroscience, University of Bordeaux, Bordeaux, France.

Super-resolution fluorescence microscopy techniques revolutionized biomolecular imaging in cells by delivering optical images with spatial resolutions below the diffraction limit of light. The direct observation of biomolecules at the single molecule level enables their localization and tracking at the scale of a few tens of nanometers and opens new opportunities to study biological structures at the scale of proteins inside living cells. We are using super-resolution microscopy techniques and single protein tracking (SPT) to study adhesive and protrusive sub-cellular structures, including integrin-dependent adhesion sites and the lamellipodium.
Integrin-mediated cell adhesion to the extracellular matrix and mechano-transduction are involved in critical cellular functions such as migration, proliferation and differentiation, and their deregulation contributes to pathologies such as cancer. Yet the molecular events controlling integrin biochemical and mechanical activation within adhesion sites (FAs) are still not understood. We unravel the key spatiotemporal molecular events leading to integrins activation by their main activators talin and kindlin in mature FAs. We performed SPT combined with PALM (sptPALM) and super-resolution microscopy to study integrins, talin and kindlin displacements and distributions outside versus inside mature FAs. We demonstrated that FAs are specialized platforms priming integrins immobilization and that talin and kindlin use different mechanisms to reach integrins. Using the same experimental strategy, in collaboration with the group of Valerie Weaver (UCSF, USA), we studied how bulky membrane glycoproteins regulate integrin diffusive behavior and activation. Our findings support a model where large glycoproteins act as physical "steric" barriers impeding integrins immobilization and thus funneling integrins clustering into adhesive contacts. Thus control of membrane nano-topology by the glycocalyx could mechanically enhanced integrin activation and could foster metastatic progression.

Rho-ROCK-Myosin Based Cellular Contractility Regulates Distinct Modes of Invasion in Paclitaxel and Cisplatin Resistant Ovarian Cancer Cells

Aastha Kapoor[1], Snehal Gaikwad[2], Alakesh Das[1], Melissa Monteiro[1], Sejal Desai[1], Amirali B. Bukhari[2], Pankaj Mogha[3], Abhijit Majumder[3], Abhijit De[2], Pritha Ray[2], Shamik Sen[1].
[1]Indian Institute of technology, Bombay, Mumbai, India, [2]Tata Memorial Centre, Mumbai, India, [3]Indian Institute of technology, Bombay, Mumbai, India.

Low survival rates in advanced stage epithelial ovarian cancer patients is attributed to acquisition of drug resistance against widely used chemotherapy drugs cisplatin and paclitaxel. Cellular and sub-cellular differences in drug resistant and normal cancers are responsible for lapse of chemotherapy. In our study, we analysed cellular biophysical differences between drug resistant and drug sensitive ovarian cancer cells to understand their modes of invasion. It is known, that drug sensitive cancers utilize two modes of invasion to spread to secondary locations – amoeboid invasion and mesenchymal invasion. In amoeboid invasion cancer cells utilize the force generated by their cytoskeleton and molecular motors to push through the surrounding extracellular matrix (ECM). In mesenchymal mode on the other hand cancer cells secrete proteases which degrades the surrounding matrix to allow unobstructed movement. While in drug sensitive cancer cells mesenchymal mode is the preferred means of invasion with amoeboid mode coming into play only when former is suppressed, in case of drug resistant cancer cells, we found that, it is drug-type dependent. Cells which have been treated with repetitive doses of paclitaxel, acquire amoeboid mode of invasion while those treated with cisplatin drug retain mesenchymal mode. Surprisingly, even though different drugs impart different modes of invasion to resistant cancer cells, the key regulator of both these mechanisms is common. We have identified cellular contractility as the primary contributor in both these cases, without which mesenchymal cancer cells lose protease secretion, and amoeboid cancer cells their migration and invasion potential. We have also identified Rho-ROCK-Myosin pathway as the key regulator of contractility in both these cases, which raises the exciting possibility of targeting this pathway for treatment of both types of drug-resistant ovarian cancers.

Mechanobiology of Epithelial-to-Mesenchymal Transition in Confined Environments

Amit Pathak.
Washington University in St. Louis, St. Louis, MO, USA.

Epithelial cells disengage from their clusters and become motile by undergoing epithelial-to-mesenchymal transition (EMT), an essential process for fibrosis and tumor metastasis. Growing evidence suggests that high extracellular matrix (ECM) stiffness induces EMT. However, very little is known about how various geometrical parameters of the ECM might influence EMT. We have adapted a hydrogel-microchannels based matrix platform to culture epithelial clusters in ECMs of tunable stiffness and confinement. We report that epithelial clusters undergo EMT to a greater degree in more confined ECM settings. Surprisingly, cell clusters residing in soft ECMs exploit this confinement-sensitive EMT better than those in stiff ECMs. Upon pharmacological inhibition of microtubules, cells lose the ability to polarize their cytoskeleton in response to ECM confinement, which in turn disables the confinement-sensitive EMT. Disruption of cell-ECM adhesions blunts the influence of ECM stiffness on EMT. To gain quantitative insights into relative contributions of subcellular and extracellular features to the ECM-dependent EMT, we simulated our experimental findings through a novel computational model that combines mechanics-based cellular features into a multi-cell network under varied ECM properties. Our model is based on cooperative operation of cell-ECM adhesions, protrusion dynamics, and actomyosin forces, which collectively dictate the state of cell-cell junctions in each cell of a given epithelial cluster. The model also accounts for the stiffness and the geometry of the ECM surrounding the mutli-cell network. Taken together, our experimental and computational results reveal that ECM confinement alone can induce EMT, even in soft tissue contexts that otherwise maintain epithelial integrity in unconfined environments. These findings highlight that topographical structure and mechanical stiffness of the tissue microenvironment can both independently regulate EMT, which brings a fresh perspective to the current understanding of microenvironment-dependent dissemination and invasion of cancer cells through confined spaces around tumor.

Mechanical Properties of Nonmuscle Myosin-2 Filaments

James R. Sellers, Luca Melli, Neil Billington, Yasuharu Takagi, Attila Nagy, Sarah M. Heissler. National Heart, Lung and Blood Institute, NIH, Bethesda, MD, USA.

There are three nonmuscle myosin-2 (NM2) paralogs in humans which participate in many cellular phenomena. Mutations in NM2A are associated with thrombocytopenia, deafness and kidney disease. Mutations in NM2C are associated with deafness. Each NM2 paralog forms 310nm bipolar filaments containing either 30 (NM2A/NM2B) or 16 myosins (NM2C). The three paralogs are slow enzymatically and mechanically compared to other myosins, but have distinct kinetic signatures with NM2B having the highest duty ratio. Neither NM2A or NM2B processively interact with actin in the optical trap as single molecules. In contrast, NM2B bipolar filaments show robust processive movements in "single filament in-vitro motility assays". EM of myosin filaments in the presence of actin and ATP show that multiple motors from a single side of a myosin filament can interact with a single actin filament or with multiple actin filaments. Motors from opposite ends of a bipolar filament can also interact with different actin filaments forming sarcomeric-type attachments. Myosins from a single filament end contact actin subunits covering about 100nm of actin filament length. NM2B molecules co-assemble with headless RFP-myosin rods, reducing the number of motor domains in a bipolar filament. About 5 NM2B motors/half filament are required for processive movements with the run-length, but not the velocity decreasing as the number of motors decrease. Surprisingly, under the same buffer conditions, NM2A filaments do not move processively. Processive movements with NM2A can be achieved by including 0.5% methylcellulose in the assay to increase the viscosity or by forming co-polymeric filaments with NM2B. Myosin filaments can associate laterally to form stacks which can dynamically gain or lose bipolar filament units when interacting with actin filaments in the presence of ATP. In-vitro assays give a model for how myosin stacks might be formed.

How Bacteria and Cancer Cells Regulate Mutagenesis and Their Ability to Evolve

Susan M. Rosenberg.
Baylor College of Medicine, Houston, TX, USA.

Our concept of genomes is changing from one in which the DNA sequence is passed faithfully to future generations to another in which genomes are plastic and responsive to environmental changes. Growing evidence shows that environmental stresses induce mechanisms of genomic instability in bacteria, yeast, and human cancer cells, generating occasional fitter mutants and potentially accelerating evolution including evolution of infectious diseases and cancer. Emerging molecular mechanisms of stress-inducible mutagenesis vary but share common components that highlight the non-randomness of mutation: (1) regulation of mutagenesis in time by cellular stress responses, which promote mutations when cells are poorly adapted to their environments—when stressed; (2) limitation of mutagenesis in genomic space causing mutation hotspots and clusters, which may both target specific genomic regions and allow concerted evolution (evolution requiring multiple mutations). This talk will focus on the molecular mechanism of stress-inducible mutagenic DNA break repair in E. coli as a model for mutations that drive cancer evolution. We consider its regulation by stress responses, demonstrate its formation of mutation hotspots near DNA breaks, and our discovery of a large gene network that underlies mutagenic break repair, most of which functions in stress sensing and signaling. We also show that mutagenesis is induced by the antibiotic ciprofloxacin, causing resistance to other antibiotics, a model of cancer chemotherapeutic type-II topoisomerase inhibitors. We find that cipro-induced mutagenesis occurs by a similar stress-inducible mutagenic break-repair mechanism. Regulation of mutagenesis in time and genomic space may accelerate evolution including evolution of cancers.

An Integrated ATR, ATM, and mTOR Mechanical Network Controlling Nuclear Plasticity

Gururaj Kidiyoor[1], Giulia Bastianello[1], Qingsen Li[1], Martin Kosar[1], Amit Kumar[2,3], Galina V. Beznoussenko[1], Alexandre A. Mironov[1], Dario Parazzoli[1], G.V. Shivashankar[4], Jiri Bartek[5], Michele Mazzanti[6], Giorgio Scita[1,6].**Marco Foiani**[1,6],
[1]IFOM (Fondazione Istituto FIRC di Oncologia Molecolare), Milan, Italy, [2]CSIR-Indian Insttiute of Toxicology Reseearch, Lucknow, India, [3]Academy of Scientific and Innovative Research (AcSIR), Taramani, India, [4]Mechanobiology Institute and Department of Biological Sciences, NUS, Singapore, Singapore, [5]Danish Cancer Society Research Center, Copenhagen, Denmark, [6]Università degli Studi di Milano, Milan, Italy.

ATR and ATM control chromosome integrity, chromatin dynamics and cell cycle events. mTOR exhibits similarities to ATR and ATM and coordinates nutrient sensing pathways and cytoskeleton dynamics.
We recently found (A.Kumar et al. Cell, 2014) that ATR, ATRIP and Chk1 associate to the nuclear envelope during S phase and prophase, and in response to mechanical stimulation of the plasma membrane. The ATR-mediated mechanical response occurs within the range of physiological forces, recovers rapidly, and is not influenced by RPA or DNA damage. ATR defective cells exhibit aberrant chromatin condensation and nuclear envelope breakdown.
We found that this pathway is influence by mTOR, actin dynamics and calcium levels. We used electron microscopy to visualize the nucleus morphology of the nucleus in ATR and CHK1-defective cells and found aberrant condensation events and nuclear envelope anomalies that may contribute to micronuclei formation and chromosome fragmentation. Using mechanobiology approaches we measured the stiffness of wild type, ATR, ATM, CHK1 and mTOR defective cells and found significant differences that influence cell plasticity and interstitial migration. These and other observations implicate ATR, ATM and mTOR in the control of genome integrity, nuclear dynamics and cell plasticity and suggest the existence of an integrated mechanical network involving different PI3-kinases.

Effects of a Soft Massaging Device, Based on an Oscillating Torque, Upon the Expression of Some Dermal Proteins of Human Skin. Influence of Frequency.

Elisa Caberlotto[1], Zane Miller[2], Aaron Poole[2], Laetitia Ruiz[1], Jean-Luc Gennisson[3], Miguel Bernal[3], Mickael Tanter[3], Mickael Poletti[2], Lauri Tadlock[2].
[1]L'Oréal, Paris, France, [2]L'Oréal, Redmond, WA, USA, [3]Institut Langevin, Paris, France.

Different biological models have shown how mechanical stimulations may induce physiological response(s) from solicited cells, tissues or organs. In models of cultured skin cells, the frequency of the mechanical stress appears a paramount parameter, generating a biological response(s) of some cells, particularly from dermal fibroblasts.
Our objective was to explore, in a full-tissue model (ex-vivo human skin explants) the effect(s) of mild massages provided by a torque test device able to generate cyclic strains at different frequencies (40 to 180Hz) and amplitudes (±3° or ±7°). In collaboration with the Langevin Institute, the propagation of mechanical waves generated by the massage device was initially analyzed using ultrafast ultrasound imaging in vitro (on an elastomer material mimicking skin) and in vivo for designing the best shape of the massaging device. Accordingly, three small teflon bulbs, disposed as summits of an equilateral triangle (2.6cm side) were found convenient. Skin explant samples, maintained in a survival biological state, were twice daily submitted to the massaging device for one minute, for 10 days, at different frequencies and amplitudes. At days 0, 5 and 10, samples were processed by immuno-histological procedures, allowing some structural dermal proteins to being semi-quantified (fluorescence).
As compared to non-massaged skin explant samples, the massaging procedure clearly led some dermal proteins (Decorin, Fibrillin, Tropoelastin) to being over-expressed. Modulations of these expressions were found frequency-dependent, the highest at 75Hz frequency, for a ±3° amplitude.
In conclusion, the ex-vivo human skin explant model used here describes, for the first time, the profound biological/structural effects induced onto the human skin by a superficial and defined oscillating strain. This model appears promising for studies that deal with the precise mechanisms of mechano-transduction.

A Tale of Two Viscosities: Microviscosity More Important Than Macroviscosity in a Crowded Microenvironment

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Macromolecular crowders enhance the in vitro differentiation of human mesenchymal stem cells (hMSCs). The fractional volume occupancy (FVO) is a measure of a polymer's "excluded volume": the higher the FVO, the greater the crowding effects. Based on the FVO of blood plasma and interstitial fluid proteins, we calculated the optimum physiological FVO needed for cell culture. However, the existing in vitro FVO (33%) achieved by the carbohydrate polymer, Ficoll, falls short of the physiological FVO (54%). When we deployed the non-carbohydrate polymer, polyvinylpyrrolidone (PVP) as an alternative crowder, we could reach 54% FVO and improve ECM deposition by hMSCs and human fibroblasts. In a collagen fibrillogenesis assay, PVP accelerated the fibrillogenesis rate over 0 - 54% FVO, whereas Ficoll ceased to enhance the rate beyond 9% FVO. Bulk viscosity, or macroviscosity, measurements reveal that PVP is less viscous than Ficoll. Since the rate of a biochemical reaction depends on the positive effect of excluded volume and the negative effect of macroviscosity, we looked more closely at the effect of viscosity on reaction kinetics using an in vitro actin polymerization assay. Against expectations, the actin polymerization rate quadrupled even though the macroviscosity had increased 60 fold. Glycerol, a pure viscogen, suppressed actin polymerization over the same macroviscosity range. As suggested by fluorescence correlation spectroscopy (FCS) measurements, it is the microviscosity, rather than the macroviscosity, that is relevant in a crowded environment. Our results suggest a model in which a crowder's excluded volume increases the reaction rate, but, significantly, the crowder's microviscosity does not increase sufficiently to decrease the rate. As our understanding of crowding effects improves, we will be better able to manipulate in vitro cell culture systems in order to study physiological and pathological processes.

Mechanobiology of Collective Cell Migration in Health and Disease

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Cells migrating in sheets or large cohorts tend to behave very differently from cells migrating individually. Indeed, the 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. Here, we characterized the kinematic behavior of epithelial cell cohorts migrating under well defined geometrical constraints. We also study such collective cell migration over areas without cell adherent proteins to examine the formation of epithelial bridges so as to better wound closure mechanisms. Our results showed that collective cell migration is not only dependent on extent of geometrical constraints as well as size of wound, but also that cell-cell adhesion and acto-myosin contractility can regulate the organization and kinematics of the migrating tissues. We also investigated the collective migration of benign, non-invasive malignant and highly-invasive malignant cancer cells. Benign cancer cells are found to exhibit intact cell-cell adhesion and unidirectional lamellipod formation, and hence produce coordinated migration. On the other hand, the migration of malignant cancer cells is less coordinated due to the altered or defective lamellipodial formation and intercellular adhesion.

Cell Mechanotype in Cancer

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Cell mechanical phenotype, or ‘mechanotype’ can signal a transformation in a cell’s physiological state, such as in malignant transformation. The current paradigm suggests that more invasive cells are more deformable. To develop a deeper understanding of cell mechanotype in cancer progression, we recently invented a mechanotype screening platform that we call Parallel Microfiltration (PMF). We screened panels of ovarian, breast, and pancreatic cancer cells, including those treated with small molecules such as chemotherapy agents or microRNAs. Our results show that we can detect cells based on their status in epithelial-to-mesenchymal transition and chemoresistance; this is enabling us to screen small molecules to identify compounds that have anti-cancer effects. Interestingly, we also discovered that more deformable cancer cells are not always more invasive, suggesting that cell deformability is not sufficient to predict the invasive capacity of tumor cells.

Feeling for Phenotype: Real-Time Deformability Cytometry for Label-Free Cell Functional Assays

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The mechanical properties of cells have long been considered as a label-free, inherent marker of biological function in health and disease. Wide-spread utilization has so far been impeded by the lack of a convenient measurement technique with sufficient throughput, sensitive to cytoskeletal changes. To address this unmet need, we have introduced real-time deformability cytometry (RT-DC) for continuous mechanical single-cell classification of heterogeneous cell populations at rates of several hundred cells per second. Cells are driven through the constriction zone of a microfluidic chip leading to cell deformations due to hydrodynamic stresses only. Our custom-built image processing software performs image acquisition, image analysis and data storage on the fly. The ensuing deformations can be quantified and an analytical model enables the derivation of cell material properties. Performing RT-DC on whole blood we highlight its potential to identify subsets in heterogeneous cell populations without any labelling and extensive sample preparation. We also demonstrate the capability of RT-DC to detect lineage-, source and disease-specific mechanical phenotypes in primary human hematopoietic stem cells and mature blood cells. Finally, we find that different stages of the cell cycle possess a unique mechanical fingerprint allowing the distinction between cells in G2 and M phase, which is not possible using standard flow cytometry approaches. In summary, RT-DC enables marker-free, quantitative phenotyping of heterogeneous cell populations with a throughput comparable to standard flow cytometry for diverse applications in biology, biotechnology and medicine.

Role of Matrix Proteins in Balancing Tissue Stiffness and Inflammation in Fibrosis

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No Abstract

Next-Generation Deformability Cytometry for Rapid Biophysical Phenotyping

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The CytoVale deformability cytometry platform enables rapid, label-free measurements of biophysical changes of single cells. The technology uses microfluidics to deliver cells to a cross junction where the cells are subjected to hydrodynamic deformation forces. In our early proof-of-concept academic studies the technology has shown utility in disease detection of malignant pleural effusions, and characterization of stem cell differentiation[1,2]. To enable real-world applications for biophysical biomarkers, CytoVale has developed a robust instrumentation platform that is focused on ease of use by streamlining sample handling, operation, and data analysis to target operations in challenging environments such as ICUs and triage stations. Our current application efforts are focused on development of a cost-effective diagnostic for detection of early sepsis, allowing aggressive treatment sooner, reducing hospital stay duration, and improving patient outcomes. Early intervention has been shown to be successful in significantly reducing morbidity and mortality from the current rate of 30-50% in addition to realizing health economics savings, yet healthcare providers currently lack a sensitive diagnostic tool that can identify patients early in disease progression with rapid turnaround times. CytoVale is uniquely positioned to improve the sepsis treatment paradigm by offering the first platform to detect the dysregulated host response. Our diagnostic platform will offer rapid, label- free detection of activated white blood cells. The technology has the potential to deliver better patient care while reducing systemic healthcare costs across multiple commercial applications in immunology, oncology, and hematology.

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Calpains Influence both Cytoskeletal Remodeling and Ca^{2+}-Triggered Vesicle Fusion in the Emergency Response to Repair a Membrane Injury

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Repairing membrane lesions is an evolutionary conserved process vital for eukaryotic cells to survive injury from osmotic stress, bacterial infection and parasites as well as mechanical and ischemic insults. Wounded cells survive by mounting an emergency repair response utilizing vesicle fusion to 'patch' membrane tears. The muscular dystrophy protein dysferlin is a Ca^{2+}-regulated vesicle fusion protein that plays a key role in membrane repair. Our research reveals that cells 'sense' and repair membrane injuries through regulated interplay between calpains and dysferlin.

We show that in the unique setting of membrane injury, rapid Ca^{2+}-influx activates calpains that specifically cleave dysferlin, releasing a C-terminal effector fragment termed mini-dysferlin$_{C72}$. 3D structured illumination microscopy (3D-SIM) of primary human myotubes subject to ballistics injury resolved the rapid recruitment of mini-dysferlin$_{C72}$-containing cytoplasmic vesicles to sites of membrane injury. These dysferlin vesicles undergo Ca^{2+}-dependent integration into the plasma membrane; intensely labeling the periphery of the lesion, then form a repair lattice that is eventually 'zippered' together by cytoskeletal motors to repair the injury.

We propose calpains are central regulators of the membrane repair response, acting both to functionally modify the vesicle fusion protein dysferlin and sever plasma membrane tethers facilitating rapid remodeling of cortical actin and microtubule networks for the rapid transport of vesicles and subsequent acto-myosin contraction of the wound site.

We are using murine and cell biology models of dysferlin- and calpain-deficiency to elucidate the respective roles and hierarchy of dysferlin and calpain for the emergency cell survival mechanism of membrane repair. Our molecular understanding of membrane repair will directly inform best practice for emerging calpain-modulatory therapies for recovery from cardiac and brain ischemia-reperfusion injury, and evaluate their application to muscular dystrophy and bacterial infection.

Molecular Tension Probes Reveal the Role of Mechanics in T-Cell Recognition

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T cells protect the body against pathogens and cancer by recognizing specific foreign peptides on the cell surface. Because antigen recognition occurs at the junction between a migrating T cell and an antigen-presenting cell (APC), it is likely that cellular forces are generated and transmitted through T-cell receptor (TCR)-ligand bonds. The objective of the work is investigate the role of mechanics in TCR function. To achieve this goal, we develop a DNA-based nanoparticle tension sensor producing the first molecular maps of TCR-ligand forces during T cell activation. We find that TCR forces are orchestrated in space and time, requiring the participation of CD8 coreceptor and adhesion molecules. Loss or damping of TCR forces results in weakened antigen discrimination, showing that T cells harness mechanics to optimize the specificity of response to ligand (PNAS, 2016).

Improving iPSC Disease Modeling with Dynamic Matrices

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Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) that affect cardiovascular function, and while mechanisms in protein-coding loci are obvious, those in non-coding loci are difficult to determine; even induced pluripotent stem cells (iPSC) from SNP-carrying patients may not exhibit a phenotype. 9p21 is a recently identified locus associated with increased risk of coronary artery disease (CAD) and myocardial infarction. Associations have implicated SNPs in altering smooth muscle and endothelial cell properties but have not identified adverse effects in cardiomyocytes (CMs) despite enhanced disease risk. Using iPSC-derived CMs from patients that are homozygous risk/risk (R/R) and non-risk/non-risk (N/N) for 9p21 SNPs and either CAD positive or negative, we assessed CM function when cultured on dynamic matrices capable of mimicking the fibrotic stiffening associated with disease post-heart attack, i.e. “heart attack-in-a-dish” stiffening from 11 kiloPascals (kPa) to 50 kPa. While all CMs independent of genotype and disease beat synchronously on soft matrices, R/R CMs cultured on dynamically stiffened hydrogels exhibited asynchronous contractions and had significantly lower correlation coefficients versus N/N CMs in the same conditions. Dynamic stiffening reduced connexin 43 expression and gap junction assembly in R/R CMs but not N/N CMs. To eliminate patient-to-patient variability, we created isogenic lines by deleting the 9p21 gene locus from a R/R patient using TALEN-mediated gene editing, i.e. R/R KO. Deletion of the 9p21 locus restored synchronous contractility and organized connexin 43 junctions. As a non-coding locus, 9p21 appears to repress connexin transcription, leading to the phenotypes we observe, but only when the niche is stiffened as in disease. These data are the first to demonstrate that disease-specific niche remodeling, e.g. a “heart attack-in-a-dish” model, can differentially affect iPSC-CM function depending on SNPs within a non-coding locus – something not possible with iPSCs alone.

Crawling Toward a Cure: Mechanobiology of Cell Migration and Differentiation in the Disease Microenvironment

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Several disease contexts include tissue microenvironments that differ substantially in mechanical and chemical properties as compared with healthy tissue. These microenvironment cues are shared among otherwise distinct diseases, ranging from tumors associated with soft-tissue cancers to brain lesions associated with multiple sclerosis and aging. Here, we will discuss how such mechanochemical stimuli affect key biophysical processes and biological responses at the cell-cell and cell-matrix interface. In the context of cancer, local gradients of acidity and stiffness extending from the tumor site can bias cell migration and promote recruitment of vasculature, initiated through changes in binding kinetics of extracellular matrix ligand-receptor. In the context of multiple sclerosis and aging, similar mechanochemical changes at brain lesions can directly affect proliferation and migration of glial stem cells, the differentiation of which is required to maintain or restore myelination of neural axons. We show how computational simulations from the molecular to cellular scale can be integrated with engineered materials and devices to mechanically probe and manipulate cell response, both for basic research of the disease states and for screening of therapeutics that target mechanotransductive pathways. Together, these biophysical mechanisms and engineering approaches provide increased understanding of and new perspectives to manage and treat such diseases.

Bacterial Pathogen Induces Host Cell Fusion and Triggers the Type I Interferon Response through cGAS and STING

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The bacterial pathogen Burkholderia pseudomallei (Bp) is the causative agent of melioidosis, an endemic disease in Southeast Asia, Northern Australia and many areas in the tropics. It is unique among bacterial pathogens in its ability to induce Multinucleated Giant Cell (MNGC) formation through one of its Type Six Secretion Systems (T6SS-1, also known as T6SS-5). After the pathogen enters the host cell and escapes into the cytosol, it turns on T6SS-1 through the sensing of glutathione present abundantly in the cytosol. The induction of cell fusion is important for facilitating cell-to-cell spread by the pathogen. Based on work conducted on Bp and the surrogate model organism, Burkholderia thailandensis, we discovered that late kinetics of Type I interferon (IFN) relied on a functional T6SS-1 and correlated with the extent and time of MNGC formation. Further investigations revealed the involvement of stimulator of interferon genes (STING), an ER-residing pattern recognition receptor, as well as cGAS, which resides in the cytosol and binds double stranded DNA. Engagement of cGAS leads to STING activation and the downstream Type I IFN response. I will discuss our efforts in trying to decipher the relationship between T6SS, cell fusion and the cytosolic DNA response. This represents an attempt by the host to respond to the danger signals of a pathogen invading the cytosol and causing massive remodelling of the host cell membrane.

Self-healing of Holes in the Nuclear Envelope

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During migration of cells in vivo, both in pathological processes such as cancer metastasis [1] or physiological events such as immune cells migration through tissue [2], the cells must move through narrow interstitial spaces which can be smaller than the nucleus. This can induce extensive deformation of the nucleus which, according to recent experiments, results in hole formation in the nuclear envelope that can lead to cell death [3] if not prevented or healed within an appropriate time. The nuclear envelope, which can be modeled as a thin, viscoelastic gel whose elasticity and viscosity primarily depend on the lamin composition [4] may utilize self-healing mechanisms that allow the hole to be sealed after the stresses that created it are removed. Here, we present a viscoelastic model of the nuclear envelope and quantify the conditions under which the self-healing of existing holes can be related to the viscoelastic properties and to the ratio of lamin A/C and B in the nuclear envelope. Disease states in which the lamin compositions are flawed can result in nuclear envelope holes that do not self-repair, leading to the expulsion of chromatin to the cytoplasm. [1] C. M. Denais et al. Nuclear envelope rupture and repair during cancer cell migration. Science, in press, 2016. [2] M. Raab, et al. Escrt iii repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. Science, in press, 2016. [3] T. Harada, et al. Nuclear lamin stiffnessis a barrier to 3d migration, but softness can limit survival. The Journal of Cell Biology, 204(5):669-682,2014. [4] J. Swift, et al. Nuclear lamin-a scales with tissue stiffness and enhances matrix-directed differentiation. Science, 341(6149):1240104, 2013.

Imaging How Cells Choose Their Fate, Shape and Position in the Mouse Embryo

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No Abstract

Biomechanical Control of Tissue Morphogenesis

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Epithelial tissues exhibit a remarkable dual property of robustness and fluidity. This operates on different time scales and relies on unique mechanical properties of the cell cortex and on adhesive interactions between cells. We seek to understand the fundamental molecular mechanisms responsible for this property.
To that end we develop a range of approaches, from the genetic and pharmacological perturbations of molecular components, the quantitative imaging of proteins using a variety of photonic methods, probing of the physical properties of cells within intact tissues, and computational modelling of morphogenesis at different scales (molecular to tissue scales).
I will present our recent progress in understanding how polarization of cortical tension underlies the dynamic cell shape changes and tissue morphogenesis. I will report recent findings delineating a novel GPCR signalling pathway responsible for the spatial regulation of cortical tension by the Rho1 pathway during tissue invagination and tissue extension. Evidence of mechanical feedbacks will be reported and discussed.

Muscle Specification in the Zebrafish Myotome

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One of the central questions in developmental biology is how cells from an equivalent group commit to different cell fate. Here, we explore cell fate differentiation in the developing zebrafish myotome, where different muscle types (muscle pioneers, slow muscle fibers and fast muscle fibers) are differentiated from adaxial cells. Sonic hedgehog (SHH), BMP and FGF have all been proposed as signaling molecules playing a role in specifying muscle cell fate. Combining live imaging of zebrafish myogenesis with cell tracking and lineage analysis, we develop four-dimensional maps of the developing somite. Using these maps, we explore the specification and migration of different muscle fibres. In particular, we focus on the interaction between different cell types and how this effects the timing of cell specification. Our results suggest that robust cell fate specification in the myotome is effectively dependent on biomechanical processes as well as chemical signaling.

Interplay between Morphology and Metabolism in Pseudomonas Aeruginosa Biofilms

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The relationship between structure and function is a fundamental theme in biology. For communities of cells, overall structure influences access to resources and therefore the metabolisms that can support survival for individuals within. On the other hand, resident cells can control the overall community structure and thereby modulate resource availability. We study the roles of endogenous electron shuttling compounds in the biofilm physiology of Pseudomonas aeruginosa, a bacterial pathogen. These compounds, called phenazines, can act as electron acceptors for P. aeruginosa metabolism when oxygen is not available. While wild-type colony biofilms are relatively smooth, phenazine-null mutant biofilms are wrinkled. Initiation of wrinkling coincides with a maximally reduced intracellular redox state, suggesting that wrinkling is a mechanism for coping with electron acceptor limitation. Mutational analyses and in situ expression profiling have revealed roles for PAS-domain and other redox-sensing regulatory proteins, as well as genes involved in motility and matrix production, in colony morphogenesis. To characterize endogenous electron acceptor production, we have developed a chip that serves as a growth support for biofilms and allows electrochemical detection and spatiotemporal resolution of phenazine production in situ. We are further developing this chip for detection of various redox-active metabolites. Through these diverse approaches, we are developing a broad picture of the mechanisms and metabolites that exert an integrated influence over redox homeostasis and thereby biofilm morphogenesis in P. aeruginosa.

The Physical Basis of Coordinated Tissue Spreading in Zebrafish Gastrulation

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Embryo morphogenesis relies on highly coordinated movements of different tissues. Yet, remarkably little is known about how tissues coordinate their movements to shape the embryo. In zebrafish embryogenesis, coordinated tissue movements become first apparent during ‘doming’ when the blastoderm begins to spread over the yolk sac, a process involving coordinated epithelial surface cell layer expansion and mesenchymal deep cell intercalations. Here, we find that active surface cell expansion represents the key process coordinating tissue movements during doming. By using a combination of theory and experiments, we show that epithelial surface cells not only trigger blastoderm expansion by reducing tissue surface tension, but also drive blastoderm thinning by inducing tissue contraction through radial deep cell intercalations. Thus, coordinated tissue expansion and thinning during doming relies on surface cells simultaneously controlling tissue surface tension and radial tissue contraction.

SsrB as a Driver of Lifestyle Changes in Salmonellae

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SsrA/B is a two-component signaling system in Salmonella enterica that is encoded on one of the horizontally acquired AT-rich segments of the genome called Salmonella Pathogenecity Island-2 (SPI-2). It is essential for the successful existence of serovars Typhi and Typhimurium inside host cells and is absent in the nearest phylogenetic neighbor, S. bongori. In response to environmental stimuli such as changes in pH and osmolality, transcriptional activation of SPI-2 by SsrB~P regulates the intracellular lifestyle of Salmonella. However, for successful pathogenesis in terms of carriage and persistence, Salmonella exists as multicellular communities. We recently found that this sessile lifestyle was also regulated by SsrB. SsrB activated the expression of the master regulator of biofilm formation, csgD (agfD), in the absence of any phosphate donors, including SsrA. This was achieved by relieving transcriptional silencing by H-NS at the csgD regulatory region. Atomic force microscopy revealed that the full-length unphosphorylated SsrB was bound to the upstream regulatory region of csgD, in agreement with our genetic and biochemical results. This binding and subsequent changes in the local DNA topology was sufficient to partially drive off H-NS and activate csgD expression. In contrast, SsrB~P regulates expression of the SPI-2 regulon by both direct transcriptional activation and anti-silencing. Our findings unravelled a novel role for unphosphorylated SsrB in regulating gene expression and established the mechanism by which anti-silencing occured. Therefore, depending on its phosphorylation state, SsrB, assists Salmonella Typhimurium to decide its lifestyle choice: intracellular versus the carrier state or biofilms. Building on this paradigm, we are now studying the larger role of SsrB and SsrB~P in regulating enivronmentally sensitive genes as Salmonella alternates between the two lifestyles. Deciphering the SsrB versus SsrB~P regulons in Typhimurium will also help to understand the carrier state of Typhi.

Cancer Cells Sense and Respond to Their Mechanical Environment during Confined Invasion

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Cancer metastasis is dependent upon individual cancer cells to invade through physical tissue barriers when escaping the tumor microenvironment. This process requires traction force generation, mechanotransduction, and subsequent cytoskeletal rearrangement. Two distinct modes of cancer cell invasion, mesenchymal and amoeboid, have been identified, and plasticity of invasion mode can limit the effectiveness of targeted chemotherapy. Microchannels with widths between 3 and 10 μm and lengths over 150 μm were fabricated, necessitating complete movement of the cell into the channel. Nine different cancer cell lines from three different tissue origins were observed interacting with the channels. Of these lines, five were found to successfully permeate from one side of the channel to the other. In four of these lines, migration was faster in narrow 3 μm channels than in wide 10 μm channels. Furthermore, cells navigating narrow channels exhibited blebs and had smooth leading edge profiles, suggesting a transition from mesenchymal invasion to amoeboid invasion. To better understand the role of the cytoskeleton in this process, highly invasive MDA MB-231 breast cancer cells were analyzed individually. Consistent with the hypothesis of a mesenchymal-to-amoeboid transition, a reduction in focal adhesion protein localization was observed in narrow channels, and cells were found to permeate narrow channels even in the absence of cell-binding ECM proteins. Chemical inhibition of the Rho/ROCK and Rac pathways, which underlie amoeboid and mesenchymal invasion respectively, revealed that amoeboid invasion through confined environments may rely on both pathways in a time-dependent manner. SiR-Actin live cell labeling was used to reveal distinct patterns of cytoskeletal organization inside wide and narrow channels. A mechanosensing period was identified in which the cell determines the channel to be too narrow for mesenchymal-based migration, reorganizes its cytoskeleton, and proceeds using an amoeboid phenotype.

Mechanical Impact of Apoptosis in a Tissue

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Apoptosis, or programmed cell death, is the most common mechanism of eliminating damaged or unnecessary cells during embryonic development, tissue homeostasis, and certain pathological conditions. It has been well known that apoptotic cell is expelled from a tissue mainly by the formation and contraction of the actomyosin cables in the dying and the neighboring non-dying cells. However, this was not been correlated with the dynamics of adherens junction (AJ) and the temporal changes in tissue mechanical tension. We explored the extrusion process of apoptotic cell in developing Drosophila pupae. Here we show that in the middle of cell extrusion, AJs between apoptotic and non-dying cells become defective, with a reduction in the levels of AJ components, including E-cadherin. Concurrently, tissue tension is transiently released. Formation and contraction of a supra-cellular actomyosin cable forms in neighboring cells, brings the non-dying cells together and further reshapes tissue tension toward the completion of extrusion. The extrusion-associated mechanical force deforms not only the nearest-neighbor cells but also the surrounding tissue and contributes to large-scale tissue dynamics. All together, we conclude that a mechanical coordination between adhesion remodeling and tissue tension reshaping represents a mechanism of apoptotic cell extrusion.

Biogeography of in vivo Microbial Biofilms

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Biogeography is the study of the spatial distribution of species within an ecosystem across space and time. The field of microbial ecology has long focused on the micron-scale biogeography and its consequences in polymicrobial communities. For example, studies of the leaf-associated microbiota of plants show that the arrangement of single cells in structured polymicrobial communities is responsible for desiccation tolerance, persistence, and resistance to invading species. The biogeography of human-associated polymicrobial communities, including those in disease, has not been studied to similar depth. While it is now widely accepted that most polymicrobial communities living in natural environments, including the human body, form spatially structured consortia, the mechanisms used by microbes to form these communities is not understood. Here I will discuss the use of a microscopic three-dimensional printing strategy in combination with the micro-scale analytical technique scanning electrochemical microscopy to elucidate these mechanisms. This experimental framework has allowed for the assessment of the role of micro-scale spatial structure on bacterial interactions as well as important clinical phenotypes including antibiotic resistance.

Single Molecule Force Measurements in Living Cells Reveal a Minimally Tensioned Integrin State

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Integrins mediate cell adhesion to the extracellular matrix and enable the construction of complex, multicellular organisms, yet fundamental aspects of integrin-based adhesion remain poorly understood. Notably, the magnitude of the mechanical load experienced by individual integrins within living cells is unclear, due principally to limitations inherent in existing techniques. Here we use FRET-based molecular tension sensors (MTSs) to directly measure the distribution of loads experienced by individual integrins in primary dermal fibroblasts. We find that the majority of integrins transmit relatively modest forces of less than 11 pN, and that a large minority bear loads of less than 3 pN. Our data also indicate that interactions with the fibronectin synergy site, a secondary binding site specifically for α5β1 integrin, lead to increased recruitment of α5β1 integrin to adhesions but not to an increase in overall cellular traction generation. Engagement with the synergy site does, however, increase cells' resistance to detachment by externally applied loads. In total, these observations suggest that a substantial population of bound but minimally tensioned integrins may provide cells and tissues with physical resiliency in the presence of widely varying mechanical loads.

Force Loading Explains How Substrate Rigidity and Ligand Nano-Distribution Regulate Cell Response.

Roger Oria[1,2], Tina Wiegand[3,4], Jorge Escribano[5], Alberto Elosegui-Artola[1], Juan José Uriarte[2], Daniel Navajas[1,2], Xavier Trepat[1,6], Jose Manuel Garcia-Aznar[5], Elisabetta Ada Cavalcanti-Adam[3,4], **Pere Roca-Cusachs**[1,2].
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Processes in development, cancer, and wound healing are determined by the rigidity and ligand density of the extracellular matrix (ECM). ECM rigidity and ligand density are first probed and detected via integrins, transmembrane proteins that link the ECM to the actin cytoskeleton. Current understanding establishes an upper limit of 70nm spacing between integrins bound to ligands on glass surfaces for appropriate clustering and subsequent formation of focal adhesions (FAs). However, the mechanism behind this limit, and its regulation by rigidity, remain largely unknown. Here, we developed a tunable rigidity substrate with controllable ligand spacing and distribution at the nanometer scale. In response to rigidity, we counterintuitively found that FA growth in breast myoepithelial cells was favored as ligand spacing increased from 50 to 100nm. In addition, disordering the distribution of ligands while keeping their density and average spacing constant triggered FA growth at lower rigidities and drastically increased their length. Further, we found that FAs collapse by decreasing their length above a rigidity threshold. Combined with measurements of traction forces and actin flows, these results match qualitatively with a molecular clutch model. This model predicts that substrate rigidity and ligand density affect adhesion formation by regulating integrin-ECM bond force loading, which in turn controls ensuing mechanosensing events. Taken together, our findings suggest a force-dependent mechanism which explains FA formation, growth and collapse in response to rigidity and ligand density. Such a mechanism may mediate cell response to the changes in ECM density and rigidity that occur during the progression of breast cancer.

Cortex Architecture Regulates Cortex Tension during the Cell Cycle

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Cell shape regulation is key to a number of fundamental biological processes, including cell migration and division. In animal cells, cell morphology is controlled primarily by the cortex, a thin actomyosin network underlying the plasma membrane. The cortex determines global physical properties of the cell, such as tension. Previous studies have shown that spatial and temporal changes in cortical tension drive shape changes during the cell cycle, such as mitotic rounding and cytokinetic furrow ingression. However, the changes in cortical architecture and composition driving changes in cortical tension remain unclear. We are investigating this question using a combination of cell biology experiments, quantitative imaging and modeling. As the cortex dimensions are below the resolution limit of conventional light microscopy, we have developed a dual-color localization method to investigate the spatial organization of the cortex. This method is based on estimating the relative localization of cortex components with respect to one another by labeling them with chromatically different fluorophores. Using our method, we quantified cortex thickness and compared the localization of key actin binding proteins in interphase and mitosis. We showed that cortex thickness decreases in mitosis, indicating a reorganization of the cortical network. Using targeted siRNA knockdowns, we identified key regulators of cortex thickness. Interestingly, proteins controlling cortex thickness also affect cortical tension, measured using atomic force microscopy. Agent based simulations of the cortex shed light on how network spatial organization controls cortex tension. Our systematic analysis will help uncover the mechanisms by which cortical structure and organization regulate cortical mechanics, thereby driving cellular morphogenesis.

A Mechanically Active Heterotypic Adhesion Enables Fibroblasts to Drive Cancer Cell Invasion

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Cancer Associated Fibroblasts (CAFs) promote tumor invasion and metastasis. Here we show that CAFs exert a physical force on cancer cells that enables their collective invasion. Force transmission is mediated by a heterophilic adhesion involving N-cadherin at the CAF membrane and E-cadherin at the cancer cell membrane. This adhesion is mechanically active; when subjected to force it triggers β-catenin recruitment and adhesion reinforcement. Impairment of E-cadherin/N-cadherin adhesion abrogates the ability of CAFs to guide collective cell migration in 2D and blocks cancer cell invasion in 3D. Further, N-cadherin mediates not only adhesion and invasion but also repolarization of the CAFs away from the cancer cells. E-cadherin/N-cadherin junctions between CAFs and cancer cells are observed in cells derived from human epidermal carcinoma and human non-small lung cell carcinoma. Together, our findings show that a mechanically active heterophilic adhesion between CAFs and cancer cells enables cooperative tumor invasion.

Mechanical Signalling in Embryonic Stem Cell Self-renewal and Differentiation

Kevin Chalut.
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Stem cell culture has been characterised using soluble signals on tissue culture plastic, providing a biochemical foundation for self-renewal and differentiation. Nonetheless, most previous stem cell research has overlooked the role of the extracellular matrix (ECM) and mechanical signalling, despite increasing evidence that they both mediate self-renewal and differentiation. To investigate the role of ECM and mechanical signalling, we have developed a novel hydrogel protocol that can be mechanically tuned, ranging from embryo stiffness to skeletal stiffness, while maintaining control of ECM density. We can now present any combination of ECM molecules to cells with independent control over matrix density and stiffness. With our hydrogels, we have explored mechanical and ECM signaling in both pluripotent stem cells and oligodendrocyte progenitor cells (OPCs). We have shown, in both mouse and human, that we can maintain optimal naïve pluripotency using soft substrates with high fibronectin density, while forcing heterogeneity and differentiation on stiff substrates. We have also shown that we can reverse the loss of function associated with ageing and neurodegeneration in OPCs using soft substrates with high laminin density. We will present a number of functional studies to support these conclusions, and I hope to present sequencing data revealing a quantitative analysis of how stiffness drives stem cell identity (samples from both projects are currently being sequenced). Using our hydrogel technology, we have also shown there to be significant actuation of Erk and Stat3 signalling, which are important pathways for self-renewal and differentiation, with mechanical stiffness independent of ECM composition and density. Ultimately, I will advance the hypothesis that mechanical sensing acts as a switch to modulate growth factor signaling to support either self-renewal or differentiation in stem cells.

Nuclear Mechano-genomics and Disease Diagnosis

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No Abstract

Telomerase and G-quadruplexes

Daniela Rhodes
Nanyang Technological University, Singapore

No Abstract

Cancer Suppression and the Mechanics of Nucleoprotein Assemblies in DNA Recombination

Ashok Venkitaraman
The Ursula Zoellner Professor of Cancer Research, University of Cambridge, United Kingdom

No Abstract

Near Atomic Resolution CryoEM Structure of the Thermally Stable Zika Virus

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Zika virus (ZIKV), formerly a neglected pathogen came into the spotlight recently, when it was associated with microcephaly in foetuses, and Guillian-Barré syndrome in adults. Here we present a 3.7Å resolution cryoEM structure of ZIKV. The overall architecture of the virus is similar to that of the other flaviviruses. Sequence and structural comparison of the ZIKV envelope (E) protein with other flaviviruses showed that parts of the E protein are similar to the neurovirulent West Nile and Japanese encephalitis viruses while others to the dengue virus (DENV). However, their contribution to flavivirus pathobiology is currently not understood. The virus particle was observed to be structurally stable even when incubated at 40oC, in sharp contrast to the less thermally stable DENV. This is also reflected in the infectivity of ZIKV compared to DENV2 and DENV4 at different temperatures. The cryoEM structure shows a virus with a more compact surface. This structural stability of the virus may help it to survive in the harsh conditions of semen, saliva and urine. Antibodies or drugs that destabilize the structure may help reduce the disease outcome or limit the spread of the virus.

Molecular Mechanisms of Mechanotransduction through LINC Complexes

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Linkers of the nucleoskeleton and cytoskeleton (LINC complexes) provide a direct physical linkage between the interior of the nucleus and the cytoplasm. The tethering of the extracellular matrix, the cytoskeleton and the nucleoskeleton mediated by these complexes allows for a direct transmission of forces to the nucleus. Transmission of forces through LINC complexes is essential for several biological functions of the cell including polarization, differentiation, division and migration and other processes dependent on nuclear deformation and positioning. Recently, mutations in these complexes have been linked to inherited cardiomyopathy, a major cause of heart disease, and Emery-Driefuss muscular dystrophy, a disorder associated with cardiomyopathy and cardiac conduction defect. Despite the numerous cardiac and skeletal functions related to LINC complex proteins, the underlying mechanisms explaining the role of these complexes in health and disease are yet to be explored. In this study, we combine computational methods along with experimental validation to elucidate the molecular mechanisms of force transmission through LINC complexes at the nuclear envelope. First, we study the force response of the complex by introducing mutations in uncharacterized regions of the complex, and directly applying mechanical forces on the complexes through molecular dynamics simulations. We then experimentally test our computational findings by introducing mutations predicted to be important in force transmission by our molecular dynamics models. We also study the effect of these mutations on the oligomer state of the complex using Z-Scan Fluorescence Fluctuation Spectroscopy. We show that specific, point mutations in components of the LINC complex can destabilize the complex under mechanical forces, or alter the oligomer state of the complex. Our findings can ultimately reveal the molecular mechanisms by which mutations in LINC complexes result in phenotypical variations in cell function that lead to diseases associated with LINC complexes.

Mechanics of Embryonic Zebrafish Revealed by Magnetically Applied Local Forces

Radu Tanasa[1], Julien Dumortier[2], Craig Russell[3], Qian Cheng[1], Richard Adams[2], Alexandre Kabla[1].
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Cambridge, Cambridge, United Kingdom.

The path from a single cell to a complex organism is the result of fine and synchronized movements of cells and tissues, including tissue reshaping like convergence and extension, internalization, collective cell migration. A wealth of studies indicate that this supra cellular remodelling is dependent upon careful control of the physical properties of these tissues. While such properties are easily accessible in cell culture or in explants, it remains a challenge to measure mechanical properties in vivo, within the embryo. Here, we propose a method to probe the physical properties of early zebrafish embryo using grafted superparamagnetic beads. Beads are manipulated with a four-pole electromagnetic tweezers, arranged in a tetrahedron configuration. This geometry secures a very accurate control of generated forces in magnitude (nanoNewton range) and direction. The resultant three-dimensional bead movement and the shapes of surrounding cells are spatially (submicron) and temporally (seconds) resolved by a light sheet fluorescence microscope (SPIM). We interpret tissue response to repeatedly applied forces within the framework of a linear viscoelastic model. To validate our technique, we chose to focus on the pre-gastrulation blastodermal tissue, a relative simple morphogenetic transition, before more complex movements begin, which might complicate the interpretation of the results. Before the onset of gastrulation, the embryo goes from an ellipsoid to a spherical shape. We show that this event is dominated by random cell migration accompanied by a notable change of mechanical properties at both cell and tissue levels. We discuss our results in terms of cell motility, cell adhesion and cell stiffness, which are known to be dependent respectively on Rac1, E-Cadherin and MyosinII activities. We see that mechanical properties, cell migration and morphogenesis are linked and impact each other, even at this simple early stage of embryonic development.

POSTER ABSTRACTS

POSTER SESSION I

Tuesday, September 27, 16:00 – 18:00
Level 2 Hall Foyer

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Tuesday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

An, Steven	**1-POS**	**Board 1**
Babu, Anju	**4-POS**	**Board 4**
Bradbury, Peta	**7-POS**	**Board 7**
Chen, Zhongwen	**10-POS**	**Board 10**
Cognart, Hamizah	**13-POS**	**Board 13**
Danilkiewicz, Joanna	**16-POS**	**Board 16**
Fujii, Yuki	**19-POS**	**Board 19**
Han, Je-Hyun	**22-POS**	**Board 22**
Iqbal, Samir	**25-POS**	**Board 25**
Janakiraman, Narayanan	**28-POS**	**Board 28**
Khalaji, Samira	**31-POS**	**Board 31**
Kittur, Harsha	**34-POS**	**Board 34**
Kubitschke, Hans	**37-POS**	**Board 37**
Kao, Yu Chiu	**40-POS**	**Board 40**
Lee, Chau-Hwang	**43-POS**	**Board 43**
Li, Qingsen	**46-POS**	**Board 46**
Miao, Yansong	**49-POS**	**Board 49**
Morawetz, Erik	**52-POS**	**Board 52**
Nakazawa, Naotaka	**55-POS**	**Board 55**
Padmanabhan, Anup	**58-POS**	**Board 58**
Pivkin, Igor	**61-POS**	**Board 61**
Ravasio, Andrea	**64-POS**	**Board 64**
Sathe, Aneesh	**67-POS**	**Board 67**
Serrano, Elba	**70-POS**	**Board 70**
Thite, Trupti	**73-POS**	**Board 73**
Wang, Yejun	**76-POS**	**Board 76**
Yang, Yi An	**79-POS**	**Board 79**

Posters should be set up in the morning of September 27 and removed by noon September 30.

1-POS Board 1

Defining the Mechanical-Phenotype Niche in Cancer through the Lens of Physics

Hong Lam, Jennifer Pluznick, William Isaacs, Kenneth Pienta, **Steven An**.
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A defining hallmark of primary and metastatic cancers is the invasion of malignant cells through surrounding tissues. In this metastatic-invasion framework, the abilities of an individual cancer cell to evade its primitive tumor ecosystem, to emigrate through its local stroma constituting a tortuous extracellular matrix (ECM), and to disseminate to a distant target organ entail, in time and space, mechanical transgression in the cell-cell and cell-matrix interactions. However explicit this mechanical interplay might appear, to date, the rudimentary physics of cancer cell metastasis is not fully explained. It remains equally unclear what cancers are primed to sense and avoid, how these external chemical and/or mechanical cues are assimilated, and to what extent these signals are hardwired to mechanical forces driving local cellular motions to metastatic-invasion of cancers. Here we applied a constellation of enabling engineering platforms to trace the evolution of biophysical events that culminate in cancer metastasis at single-cell resolution. Across the experimental cancer models, we found increased dispersion of metastatic cancer cells on collagen matrix that was universally accompanied by faster cytoskeletal remodeling dynamics and emergent distribution of traction stresses at the cell-matrix interface. In addition, the local tractions were precisely tuned to the surrounding matrix rigidity with the concomitant expression of mechanosensitive integrin receptors. Unexpectedly, in prostate cancer (PCa), we found expression of classical odorant “sensory” receptors belonging to the superfamily of G protein-coupled receptors (GPCRs) and identified a number of olfactory receptors (ORs) that were differentially expressed in localized vs. metastasized PCa. Because ORs are broadly expressed and reported to play diverse homeostatic roles–i.e. sperm chemotaxis and muscle cell migration–we are now studying physiologic and/or pathologic roles for de novo identified PCa ORs and olfactory-like chemosensory signaling in PCa metastasis.

4-POS Board 4

Investigation of the Microstructural Organization and Mechanical Properties of Human Abdominal Aortic Aneurysms

Anju R. Babu[1], Tina U. Cohnert[2], Gerhard A. Holzapfel[1,3].
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Elastin and collagen are the major structural components determining the passive mechanical properties of aortic walls. Aging and different pathological conditions are linked to active remodeling of aortic wall constituents and alteration in mechanical properties. Abdominal aortic aneurysm (AAA) is a local dilation of the aorta associated with changes in arterial wall constituents and properties. Aortic rupture, the main complication of AAA, is a life-threatening emergency situation.
The main goal of this study is to determine the mechanical properties of AAA and understand alteration in aortic wall microstructural contents and morphology. In this study biaxial mechanical experiments are performed on AAA tissue obtained from patients (n=10) undergoing surgical repair at Medical University of Graz, Austria. A combination of optical clearing method and second-harmonic generation (SHG) imaging is used to investigate the collagen organization. SHG signals obtained are processed by Fourier transform and fitted to maximum likelihood estimation distribution function to understand the dispersion of collagen fibers. Alteration in elastin content in the AAA tissue wall is analyzed by immuno-histochemical staining. The material response and structural organization AAA tissues obtained are compared with healthy abdominal aortas (AA).
Biaxial experimental results show a nonlinear mechanical response for AA tissues with a well-organized elastin and collagen architecture. Compared to healthy tissue, AAAs show a stiffer response with alteration in collagen fiber morphology and organization. Aneurysms are associated with degradation of elastin and correspond to a decrease in elastin content, as confirmed from histological analysis. Results from this study show a relationship between the change in the mechanical properties of AAAs and the remodeling of arterial wall constituents. Future work aims at determining the alteration in collagen and elastin crosslinks in abdominal aortic aneurysms.

7-POS Board 7

The p130Cas Focal Adhesion Targeting (FAT) Domain Confers Mechanosensing Function

Peta Bradbury[1,2], Kylie Turner[1], Camilla Mitchell[1], Kaitlyn Griffin[1], Loretta Lau[1], Rebecca Dagg[1], Elena Taran[3], Justin Cooper-White[3], Ben Fabry[4], Geraldine M. O'Neill[1,2].
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The Cas family of focal adhesion docking proteins contain a C-terminal focal adhesion targeting (FAT) domain that is highly conserved both at the primary sequence level and structurally. The FAT domain regulates the molecular exchange of NEDD9 and p130Cas members of the Cas protein family at focal adhesions. However, while p130Cas increases focal adhesion turnover, NEDD9 conversely stabilizes focal adhesions. In order to determine the role of the FAT domain in this differential behaviour we have compared the function of wildtype exogenous NEDD9 with an expression construct in which the NEDD9 FAT domain is exchanged for the p130Cas FAT domain. Strikingly, substitution of the FAT domain amplifies the effects of wildtype NEDD9. Thus, FRAP analysis reveals significantly slowed exchange of the fusion protein at focal adhesions and this correlated with significantly slower 2D migration. Atomic Force Microscopy (AFM) analysis of cell membrane compliance suggests that changes in migration speed are not due to altered cell membrane stiffness. Further, analysis of cell adhesion force using a magnetic tweezer device revealed no difference in adhesion force indicating that slowed migration in cells expressing the p130Cas FAT domain exchanged construct were not due to changes in adhesion. Instead, we find that the p130Cas FAT domain significantly increases the tyrosine phosphorylation of the NEDD9 substrate domain. Phosphorylation of this domain has been previously established to be required for NEDD9 modulation of cell speed. Analysis of cell migration on surfaces of increasing rigidity revealed a striking reduction in cell motility in cells expressing the FAT domain exchanged protein. Collectively our data therefore suggest that the p130Cas FAT domain confers mechanosensing function.

10-POS Board 10

Spatially Controlled Activation of Epha2 by Ephrina1 Increase Contractility and Integrin Adhesion Dynamics

Zhongwen Chen.
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Eph receptors comprise the largest family of receptor tyrosine kinases. Together with their ligand Ephrin which is expressed on the neighboring cells, they form juxtacrine signaling. Specifically, EphA2 receptor is overexpressed in most of malignant breast cancers. There is a long debate about its role in cancer progression and many results seem contradictory, such as if it is pro- or anti-migratory. Recent studies of Eph signaling are beginning to reveal spatio-mechanical aspects of regulation and further resolution of these facets of regulation may explain the complex role of Eph signaling in cancer.
In this study, we developed a spatially patterned hybrid substrate with fluid ephrinA1 ligands presented on supported lipid bilayers patches embedded within regions of immobilized RGD peptides, functioning as both integrin ligands and lipid bilayer barriers. With this, we successfully activate EphA2 receptors in a membrane-membrane configuration while maintaining cell-matrix interactions to activate integrin adhesion formation. This system allows detailed spatially resolved studies of both Eph- and Integrin- signaling activities, and enables direct observation of the influence of Eph signaling on integrin behavior. We found that ligand dependent activation of EphA2 receptors induce local myosin contractions and increase a more global integrin adhesion dynamics, which are in agreement with a migratory phenotype. The increase in integrin adhesion dynamics is dependent on activation of Src in EphA2 receptors, and follows Src/FAK/Paxillin signaling axis. Our results suggest that spatially controlled activation of EphA2 is pro-migratory through crosstalk with integrin.

13-POS Board 13

Study of the Metastatic Process of Circulating Tumour Cells by Organ-on-a-Chip in Vitro Models

Hamizah Cognart[1,2,3], Catherine Villard[1,2,3], Jean-Louis Viovy[1,2,3].
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90% of cancer mortality arises from metastases, due to cells that escape from a primary tumour, circulate in the blood as circulating tumour cells (CTCs), leave blood vessels and nest in distant organs. The processes by which CTCs invade distant organs, remodel their environment to create a "micrometastatic niche", the eventual triggering of a proliferation leading to a macroscopic metastases, are poorly known, mostly because of a lack of experimental models. These events are rare; occur in the body at unknown places and on a microscopic scale.

The loss of cell adhesion of tumour cells detaching from the primary tumour tissues will undergo a transformation phenomenon known as epithelial-to-mesenchymal transition (EMT) leading to the lost of epithelial characteristics with different expression patterns of EMT markers (E-cadherin, N-cadherin, Vimentin, Snail, Twist). EMT is believed to play a key role in metastasis, as it is a morphogenetic transformation in which epithelial tumour cells lose their epithelial characteristics and acquire a mesenchymal-like phenotype to increase cell motility and plasticity allowing dissemination of these tumour cells in blood circulation to distant sites.

Here, we designed and fabricated microfluidic models containing mechanical constrictions in order to mimic the blood microcirculation. Metastatic breast cancer cells, MDA-MB-231, and epithelial Madin-Darby canine kidney (MDCK) cells are subjected and confined to the microfluidic channels using a flow control system. These cells are circulated under optimal culture conditions, and monitored in the channels for the observance of biophysical occurrences from continuous mechanical cellular deformations. We would like to study the biophysical effects of circulation and confinement on tumour cell morphogenesis.

16-POS Board 16

Energy Landscape of RGD Motif and Syndecan-1 Binding to Human Bladder Cells of Different Grades Studied by Dynamic Force Spectroscopy

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Multiple receptors present on cell membrane mediate various interactions with extracellular matrix (ECM). Many processes occurring in metastasis such as cell adhesion, migration, attachment to basal matrix, and invasion involve proteins containing the RGD motif such as fibronectin or vitronectin [1]. Thus, many cellular processes (like cell differentiation, adhesion, migration, proliferation or survival) depend on the ECM properties. The main family of cell surface receptors responsible for the cell–ECM interaction are integrins [2]. They bind to ECM proteins mainly through RGD motif. However, in such proteins as fibronectin, another cell attachment sites i.e. heparin binding sites are present to be specifically recognized by syndecan family acting as integrin co-receptors [3].
In our studies, the unbinding properties were quantified for integrins and syndecans, assuming that they change during the metastasis progression. An AFM-based dynamic force spectroscopy (DFS) was used to carry out measurements of the interactions between the cell surface and the AFM probes functionalized with either the RGD motif or monoclonal antibody against syndecan-1. Human bladder cells originated from non-malignant epithelial cells of ureter (HCV29) and from transitional cell carcinoma (HT1376). Obtained loading rate dependences enabled to re-construct the energy landscapes according to the Bell-Evans model used to characterize the unbinding properties of studied interactions.

References:
[1] Plow et al. J. Biol.Chem. 275, 21785-21788 (2000).
[2] Carey Biochem. J. 327, 1–16 (1997).
[3] Harisi et al. Onco Targets Ther.8, 1387-1398 (2015).
[4] The studies has been financed by the NCN project no UMO-2014/15/B/ST4/04737.

19-POS Board 19

Spatial Mechanical Heterogeneity in Epithelial Cell Sheet : An Atomic Force Microscopy Study

Yuki Fujii, Takaharu Okajima.
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In epithelial sheet, cells are tightly packed and connect the neighboring cells through cell junctions. These cells interact mechanically and migrate collectively by exerting tensile forces between cells and on extracellular matrix [1]. However, it is little known how the cell stiffness is regulated through the cell-cell mechanical interactions. In this study, we investigate the spatial distribution of the elastic modulus of epithelial Mardin-Darby canine kidney (MDCK) cell sheet in a large region by atomic force microscopy (AFM). MDCK cell sheet sample is prepared confluent condition. The AFM force curve measurements were used to map the spatial distribution of MDCK cells.
We observed that the elastic modulus of MDCK cell sheet exhibits a characteristic spatial correlation length that is much longer than the distance between neighboring cells. This means that the elastic modulus of cell increases as the surrounding cells are stiffer whereas the elastic modulus decreases as the surrounding cells are softer. The magnitude of the elastic modulus was drastically reduced as actin filaments were depolymerized, and the spatial correlation of the cell elastic modulus was disappeared by disrupting actin filaments. Moreover, the reduction of the spatial correlation length was clearly observed as E-cadherin-dependent cell-cell adhesion was inhibited by EGTA. Interestingly, the spatial correlation length of the MDCK cells was recovered to that in the control condition as E-cadherin junctions between cells were again stabilized by washing out EGTA. The results suggest that the elastic modulus of epithelial cell sheet sensitively changes depending on the elastic modulus of the surrounding cells and the regulation of the cell elastic modulus is directly driven through the cell junctions.
[1] E, Bazallieres., et al., Nature cell boil 17, 409-420 (2015).

22-POS Board 22

Developing of Artificial Microvasculature Using Coaxial Electrospun Porous Microfibers

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For proper regeneration of severely damaged tissue, tissue engineering focuses on development of transplantable artificial 3D tissues. One of the main issues in the development of artificial 3D tissues is maintaining high viability in the densely packed cells. For this, it would be essential to integrate functional vasculatures in the tissue. Based on these needs, there have been extensive studies on constructing capillary-like structures using 3D printing, MEMS and other micro-manufacture techniques. The coaxial electrospinning would be an efficient way to make micro-sized hollow tubes using soluble core and biocompatible shell polymers. In this study, the overall structure of fibers was fabricated with polycaprolactone (PCL) solution and heavy mineral oil to mimic capillary-like channels. Additionally, to enhance the nutrition exchange between the inside and outside of the fibers, phase separation techniques were utilized using PCL solution and dimethyl sulfoxide (DMSO) mixture to develop nano-sized pores on the surface of the fibers. These fibers were collected using tilted gap aluminum collectors to regulate the spacing between capillary fibers and to constrain the number of fibers. The PCL grip was then printed onto the collected fibers to allow easy stacking of the layers of capillary fibers. Human umbilical vein endothelial cells (HUVECs) were cultured on the collected fibers to be formed artificial capillary-vessel like channels. The viability and functionality of HUVECs were quantified using immunohistochemistry under different fiber morphology and densities. From this study, porous micro-sized channel based vasculature is shown to be utilized as the essential basis of in vitro 3D structures and cell survival for tissue engineering. This work was supported by the NRF grant 2013R1A2A2A01017014 and 2015M3A9B3028685.

25-POS Board 25

Solid-State Micropores for Rapid Investigation of Cancer Cell Mechanical Properties

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Solid-state micropores were used to detect tumor cells to process whole blood and to detect tumor cells without any tagging. The micropore acted as an electromechanical transducer to differentiate tumor cells from the normal cells. The micropore data also distinguished one type of tumor cells from the other types of tumors. The electrical signals were seen to be characteristic of the cell properties. The principle of measurement was like a coulter counter but the micropores were micromachined in a 200 nm thin membrane and the measurement setup could detect changes in ionic current as small as picoamperes. The electrical signals stemmed from the cells' distinct properties (shape, size, elasticity, stiffness, motility, etc.) and thus the translocation profiles of cells that were either small or were more elastic and flexible caused electrical pulses that were shorter in widths and amplitudes. The larger cells or those with lesser elasticity and flexibility resulted in deeper and wider pulses. The investigations have been done on lung cancer cells (non-small cell lung cancer cell lines), metastatic and non-metastatic breast cancer cells, metastatic primary renal cancer cells and primary human glioblastoma cells. The cells translocation through micropores of relevant sizes caused electrical pulses that could clearly distinguish particular cancer cells from others. The transformation of difference in cell properties into differences in electrical profile (i.e. difference in peak amplitude and translocation time) with this micropore electromechanical transducer can be used to differentiate many types of cancer cells before and after treatment. The solid-sate micropore device can process the whole blood sample of cancer patient without any pre-processing requirements and is ideal for point-of-care applications.

28-POS Board 28

Heterogeneity of Elastic Modulus in Metastatic and Non-Metastatic Retinoblastoma(Rb) Cells and Its Correlation with Cluster Size

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Retinoblastoma is the eye cancer in children. The stage dependent mechanical behavior of the tumor cell can alter the course of tumor metastasis. Objective: we aim to understand the mechanical properties of retinoblastoma tumor cells in two different (Metastatic and Non-Metastatic) stages. Method: Young's modulus of WERI-Rb1 (non-metastatic) and RBC NCC 51 (metastatic) retinoblastoma cells were measured using atomic force microscopy (AFM). Measurements were carried out using AFM cantilevers with a bead tip radius of 4.5 micron (and spring constant of 0.02N/m). Results: The Young's moduli of cells were acquired by fitting the force indentation curves with Hertz's contact model. The average young's moduli of metastatic and non-metastatic cells was found to be 128pa±60 (Mean standard deviation) and 149pa±90 (Mean standard deviation) respectively. However, retinoblastoma tumor cells tend to form various size clusters. Singlet, doublet and cluster of more than three cells showed Young's modulus in a decreasing order. Metastatic cluster (more than 7 cells) (NCC) were found to have a lower Young's modulus on average, i.e., 85Pa±11pa (mean ± stand deviation), than non-metastatic cluster cells (WERI-Rb1), i.e., 191Pa±117pa (mean ± standard deviation). Conclusion: The heterogeneity in Young's modulus of cells with different cluster size reveals that the heterogeneous population of tumor cells can have possibility of sub stages where the transition between non-metastatic to metastatic stages can occur.

31-POS Board 31

Young and Senescence Fibroblast Cells Are Different in Mechanical Properties!

Samira Khalaji[1], Fenneke KleinJan[1], Vida Farsam[2], Karin Scharffetter-Kochanek[2], Kay-E Gottschalk[1].
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Biological aging is a complex, multi-dimensional process that makes physiological changes taking place over a long period of time. This process takes up with macroscopic and microscopic scale alterations within individual cells, over transformations in tissues and organs and to changes of the whole organism. Cellular senescence is already characterised based on age associated molecular markers in gene and protein pattern expression such as telomere dysfunction. But there is little information regarding relationship of age and the mechanical properties of cells. Biomechanical properties are vital properties for the cells and tissues of living organisms. Estimation of the mechanical properties of a cell depends on a method of measurement, theoretical model as well as cell state. Hence, we employed a variety of methods such as passive microrheology and atomic force microscopy on primary dermal fibroblasts from male human with CPD 17-35 (young) and CPD 58-59 (senescence) as a model of in vitro replicative senescence. Our primary result shows significant differences in the viscoelastic properties of young and senescence fibroblasts depending on in vitro senescence level and also compared to older studies.

34-POS Board 34

Relative Adhesion Profiler System for Biophysical Interrogation of Malignant Breast Cancer Phenotypes

Harsha M. Kittur[1], Andy Tay[1], Avery Hua[1], Min Yu[2], Dino Di Carlo[1,3,4].
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Breast cancer mortality is linked to metastasis and currently we lack sufficient biomarkers that accurately predict locations of metastases, enabling improved treatment. We aim to complement current genetic biomarkers with physiologically-relevant physical biomarkers associated with cell adhesion and migration. Here, cells are seeded on a PDMS surface patterned with large stripes of eight different extracellular matrix proteins, and are sandwiched from above with another protein-coated surface with the same eight stripes running orthogonally, to interrogate large groups of cells with 64 protein pair combinations. We quantified percent cell transfer to the top surface for each protein pair over five breast cell lines with unique metastatic potential – normal breast cells (hMEC), malignant breast cancer cells (MDA-MB-231) and MDA-MB-231 cells that have tropism to brain, lung, and bone – to generate relative adhesion plots that can distinguish the populations. These heat maps generally reveal transfer of malignant cells toward collagen 1, but also shows transfer away from basement membrane proteins collagen 4 and laminin, regardless of the type of new protein that is introduced. Morphological analyses show that hMEC display epithelial behavior favoring cell-cell contact via amoeboid motions as opposed to adhesion to the new surface. In contrast, MDA-lung cells tend to move with a mesenchymal phenotype as they switch to a new collagen 1 surface. Antibodies to alpha 2 and beta 1, but not with alpha 6 integrins interfere with transfer. The actin cytoskeleton, but not microtubules or myosin II are implicated in the transfer process. Finally, further analyses of select protein pair combinations provide potential to develop protocols that can separate malignant cells from non-cancerous cells, and then further delineate the malignant cells by their tropism to specific secondary sites.

37-POS Board 37

Proteomic Details of Differential Adhesion Hypothesis

Hans Kubitschke[1], Steve Pawlizak[1], Anatol W. Fritsch[2], Steffen Grosser[1], Linda Oswald[1], Lisa Manning[3], Josef Kaes[1].
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We analysed the mechanical properties of three epithelial/mesenchymal cell lines (MCF-10A, MDA-MB-231, MDA-MB-436) associated with properties from benign to metastatic tumours, to quantify the role of cell cohesion in cell sorting and compartmentalization. The analysis included quantitative mass spectroscopy SILAC, of the underlying proteome of the cell lines. We developed a unique set of methods to measure cell – cell adhesiveness, cell stiffness and cell shapes, and compared the results to predictions from cell sorting in mixtures of cell populations. We found that the final sorted state is extremely robust among all three cell lines independent of epithelial or mesenchymal state, suggesting that cell sorting may play an important role in organization and boundary formation in tumours. Furthermore, SILAC mass spectroscopy of these cell lines revealed significant differences in the proteome, especially parts and pathways of it associated with related cellular functions and structures, i.e. adhesion, metabolism, cytoskeleton. SILAC analysis was able to intertwine tumour-associated proteins of cells with their found mechanical properties. We found that surface densities of adhesive molecules fail to correlate with measured cell – cell adhesion, but do correlate with cell shapes, cell stiffness and the rate at which cells sort, in accordance with an extended version of the differential adhesion hypothesis (DAH). SILAC mass spectroscopy reassembles and supports the experimental findings on a proteomic level and bridges the gap from observable macro- and mesoscopic quantities, given above, down to molecular details of cells. Surprisingly, the DAH does not correctly predict the final sorted state. This suggests that these tissues are not behaving as immiscible fluids, cells can be kinetically trapped and that dynamical effects such as directional motility, friction and jamming may play an important role in tissue compartmentalization across the epithelial − mesenchymal transition.

40-POS Board 40

Caveolin-1 Phosphorylation Drives Elevated Hydrostatic Pressure-Induced Invasion of Lung Cancer Cells

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Most solid tumors are characterized by high interstitial fluid pressures (IFPs) that are uniformly distributed throughout the tumor yet the roles of the high IFP on the invasiveness of cancer cells remain unclear. Using three-dimensional transwell assays that were adapted for application of hydrostatic pressure (HP) to the cultured cells to simulate increased IFP conditions ranging from 0 to 20 mmHg, we found that the elevated HPs increased the invasiveness of lung cancer cells CL1-5 and A549. The migration speeds and volumes of the lung cancer cells were also significantly increased in the 20 mmHg HP condition. Biochemical analysis using Western blotting, protein phosphorylation inhibitors, and selected proteins knockdown with siRNA transfection revealed that the high IFP conditions induced caveolin-1 phosphorylation, which in turn promoted phosphorylation of Akt1/2 and subsequently ERK1/2, and the downstream expression of invasiveness markers including Snail, vinculin, aquaporin-1, as well as the phosphorylation of cortactin. Our results disclose a novel pathway relating high IFP to the invasiveness of cancer cells and highlight potential targets to hinder cancer cells spreading.

43-POS Board 43

3D-image-based Assays of Drug Combination Efficacy on Cancer Cell and Fibroblast Co-culture Spheroids

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Objective: We proposed to use cancer cell and fibroblast co-culture spheroids as a platform to evaluate the efficacy of anti-cancer drug combinations. Selective-plane illumination microscopy (SPIM) was employed to provide a 3D perspective of relative positions of the co-cultured cells.

Results: We found that the co-culture of CL1-0 lung cancer cells and MRC-5 fibroblasts could form a spheroid (diameter ~ 200 μm) much easier than the cancer cell alone. Regardless of the seeding sequence, the fibroblasts were enclosed by the cancer cells in a spheroid. In contrast, while the cancer cells were co-cultured with bronchial epithelial cells, the latter did not invade into the cancer cell aggregation. This result implied that fibroblasts could play an essential role in the early stage of tumor development.

Next, we used the co-culture spheroids to test the efficacy of an anti-cancer drug erlotinib in combination with chloroquine, the inhibitor of cellular autophagy. With the SPIM images, we found that the survival rate of cancer cells in the co-culture spheroids was much higher than that of cells in cancer-cell spheroids under the treatment of 10 μM erlotinib. In addition, the combination of 10 μM erlotinib and 20 μM chloroquine showed a high efficacy in killing the cancer cells in the co-culture spheroids. In contrast, in the 2D co-culture of lung cancer cells and fibroblasts, 10 μM erlotinib alone showed a much higher efficacy in killing the cancer cells.

Conclusion: In this work we demonstrated that cancer cell and fibroblast co-culture spheroids combined with 3D imaging could serve as a useful platform to investigate the tumor formation process and to test the drug combination efficacy. The 3D microenvironment could be an important factor that must be included in the evaluation of therapeutic strategies.

46-POS Board 46

Mechanobiology in DNA Damage Response

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IFOM, Milan, Milano, Italy.

The ATR protein kinase controls the DNA damage response (DDR) [1], with ATM, Chk1 and Chk2. DDR genes are often mutated in cancer cells and act as an anti-cancer barrier in response to oncogenic stimuli [2, 3]. ATR is essential and protects the integrity of replicating chromosomes [4, 5], prevents fragile site expression [6, 7] and aberrant condensation events [6, 8]. The Foiani laboratory recently found that ATR associates with the nuclear envelope during S phase and prophase and in response to osmotic or mechanical stress [9]. However, the molecular mechanism and functional relevance of ATR-mediated mechanical response remain unclear. To address these questions, we will employ and develop various multidisciplinary approaches including state-of-the-art Atomic Force Microscopy (AFM), micropatterned protein substrate, novel microfluidic device and accompanied with advanced molecular biology techniques in order to quantitatively and systematically explore the ATR mediated mechanotransduction. Our preliminary results showed that the stiffness of ATR, ATM, CHK1 and mTOR defective cells are significantly different compared to wild type, which influence cell plasticity and interstitial migration. Furthermore, AFM experiments revealed that ATR defective cells have compromised nuclei, which failed to sustain mechanical stress. These and other observations implicate ATR, ATM and mTOR in the control of genome integrity, nuclear dynamics and cell plasticity and suggest the existence of an integrated mechanical network involving different PI3-kinases.

49-POS Board 49

Fimbrin Phosphorylation by Metaphase Cdk1 Regulates Actin Cable Dynamics in Budding Yeast

Yansong Miao[4,3,1], Xuemei Han[2], Liangzhen Zheng[4], Ying Xie[3], Yuguang Mu[4], John Yates[2], David Drubin[1].
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Dynamic assembly of actin cytoskeleton in distinct architectures, including branched and unbranched actin structures, is necessary for many biological processes in diverse eukaryotes. Unbranched actin cable is essential for intracellular membrane trafficking and polarized cell growth. We previously observed that metaphase cells preferentially promote actin cable assembly through cyclin-dependent kinase 1 (Cdk1) activity. However, the relevant metaphase Cdk1 targets were not known. Here, we identified actin bundling protein fimbrin is phosphorylated by metaphase cyclin-dependent kinase (Cdk1) specifically in both in vitro and in vivo. Phosphorylation of fimbrin regulates its affinity to actin filaments. Based on conformational simulations, we suggest that this phosphorylation stabilizes fimbrin's N-terminal domain, and modulates actin filament binding to regulate actin cable assembly and stability in cells. Sac6 is a fimbrin protein that belongs to an evolutionarily conserved protein family, which contains one EF-hand domain and two actin-binding domains for the cross-linking of actin filaments. Overall, this work identified fimbrin as a key target for cell cycle regulation of actin cable assembly in budding yeast, and suggested an underlying mechanism.

52-POS Board 52

E-Cadherin Expression and Localization is Correlated to Cellular Softness in Cancer Development

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The concept of the epithelial mesenchymal transition (EMT) is believed to play a crucial role, not only in beneficial processes like wound healing but also in cancer development. One of its main markers is the down regulation of cadherin CD324, or epithelial cadherin (E-Cad). Before heavy general loss of E-Cad in the cell membrane, a restructuring takes place. This cuts anchoring of the actin and keratin cytoskeleton and increases the amount of mobile E-Cad. It is also strongly suggested, that the malignant transformation of cells is linked to increased softness of the cell body.
To investigate correlations between this two fundamental cellular changes, we use a model system from cell lines, as well as primary human tumor samples. Cells are stained for E-Cad and measured with the Optical Stretcher (OS). In this optical rheometer, cells are deformed non-invasively by a dual beam trap. This can be combined with fluorescent microscopy. Thus, both the softness of a single cell, as well as the corresponding distribution of E-Cad on the cell surface can measured simultaneously.
A well-established model for the EMT in cancer development consists of the cell lines MCF 10A, MDA-MB 436 and MDA-MB 231. Here we show, that the loss of E-Cad expression is linked to softer cell bodies. Primary human tumor samples are provided by the Universitätsklinik Leipzig. Both human mamma and cervix carcinoma are under investigation. The tumor samples are processed into a single cell suspension, depleted of fibroblasts and blood, and measured the same way as the cell line model. We sort the data for cells of high and low E-Cad expression, as well as localization. We show, that this way a primary tumor sample can be sorted into two sub-populations of soft and stiff cells.

55-POS Board 55

Matrix Mechanics Controls FHL2 Movement to the Nucleus to Activate P21 Expression

Naotaka Nakazawa[1], Aneesh R. Sathe[1], G.V. Shivashankar[1,2,3], Michael P. Sheetz[1,2,4]. [1]Mechanobiology Institute, Singapore, Singapore, [2]National University of Singapore, Singapore, Singapore, [3]FIRC Institute of Molecular Oncology, Milan, Italy, [4]Columbia University, New York, NY, USA.

Substrate rigidity affects many physiological processes through mechano-chemical signals from focal adhesion (FA) complexes that subsequently modulate gene expression. We find that shuttling of the LIM domain protein, four and a half LIM domain (FHL2) protein, between focal adhesions (FAs) and the nucleus depends upon matrix mechanics. In particular, on soft surfaces or after the loss of force, FHL2 moves from FAs into the nucleus, and concentrates at RNA Pol II sites, where it acts as a transcriptional co-factor, causing an increase in p21 gene expression that will inhibit growth on soft surfaces. At the molecular level, shuttling requires a specific tyrosine in FHL2 as well as phosphorylation by active focal adhesion kinase (FAK). Thus, we suggest that FHL2 phosphorylation by FAK is a critical, mechanically dependent step in signaling from soft matrices to the nucleus to inhibit cell proliferation by increasing p21 expression.

58-POS Board 58

Non-Junctional Adhesion-independent E-cadherin Clusters Regulate the Actomyosin Cortex in *C.Elegans* Zygote

Anup Padmanabhan[1].Ronen Zaidel-Bar[1,2],
[2]National University of Singapore, Singapore, Singapore.[1]Mechanobiology Institute, Singapore, Singapore,

During cytokinesis in metazoan cells, the furrow ingression is resisted by intercellular adhesion mediated by cell-cell junctions. Although E-cadherins are best known for their essential role in mediating adhesion at cell junctions, a significant amount of E-cadherin on the cell surface is found outside of cell-cell junctions. The cellular function of these non-junctional cadherin clusters is presently not known. Using live imaging and genetics we show that during in early *C. elegans* embryos E-cadherin/HMR-1 formed non-junctional puncta at the cell surface associated with cortical F-actin. Depletion of E-cadherin/HMR-1 puncta in 1-cell stage embryo lacking cell-cell junctions accelerated furrow ingression during the first cell division. At the molecular level we observed E-cadherin/HMR-1 and myosinII/NMY-2 to negatively regulate each other and localize to distinct regions both at the cortex and along the ingression furrow. This antagonistic interaction and spatial segregation of E-cadherin/HMR-1 and NMY-2 was dependent on the formin/CYK-1 polymerized F-actin. Finally, we discovered that the non-junctional E-cadherin/HMR-1 puncta localized at the cell surface helps in holding the cortex and membrane together, a hitherto unknown cellular function of non-junctional E-cadherin/HMR-1.
Our results thus show that surface localized non-junctional E-cadherin/HMR-1 could regulate cytokinesis beyond its canonical role in inter-cellular adhesion by (1) regulating cortical myosin activity and (2) holding the membrane and cortex together thus resisting cortical deformations such as during furrow ingression.

61-POS Board 61

Particle-based Modeling and Simulation of Cells in Flow

Kirill Lykov[1], Yasaman Nematbakhch[2], Chwee Teck Lim[2], **Igor V. Pivkin**[1].
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We developed a computational framework for simulations of red blood cells in fluid domains using particle-based Dissipative Particle Dynamics (DPD) method. The use of DPD provided a unified way to describe fluid, fluid-structure and structure-structure interactions. The framework was employed in simulation of a wide range of blood rheology problems, such as analysis of the flow in the lab-on-a-chip devices and studies of cell transport in the microvascular networks. We recently extended the framework by developing a new model for cells with nucleolus, such as white blood cells and circulating tumor cells. We will present the new cell model and its validation using quantitative data from microfluidic experiments.

64-POS Board 64

Ligand-Activated Epha2 Transport as Marker for Cancer Progression and Population Heterogeneity

Andrea Ravasio[1], Bakya Arasi[1], Myint Z. Myaing[1], Shumei Chia[2], Vin Y. Chung[3], Hui Ting Ong[1], Ruby Yun-Ju Huang[3,4], Ramanuj DasGupta[2], Jay T. Groves[1,5,6], Virgile Viasnoff[1,7,8]. [1]National University of Singapore, Singapore, Singapore, [2]Genome Institute of Singapore, A-STAR, Singapore, Singapore, [3]National University of Singapore, Singapore, Singapore, [4]National University Health System, Singapore, Singapore, [5]University of California, Berkeley, CA, USA, [6]University of California, Berkeley, CA, USA, [7]Centre National de la Recherche Scientifique, Singapore, Singapore, [8]National University of Singapore, Singapore, Singapore.

Evolution of cancer into heterogeneous subclones is due to somatic mutations, epigenetic adaptations and selective pressure, leading to phenotypic differences, that result in different EMT states, migration capacity and, thus metastatic potential. Resistant subclones survive therapeutic intervention giving rise to recurrence of the disease. Here, we employ an assay to access the metastatic potential of cancer cells by their phenotypic ability to cluster EphA2 receptor upon ligand activation. Carcinoma cell lines from different organs, different EMT state and migratory capacity were seeded onto fluid lipid bilayers presenting the ligand - ephrinA1. Following activation of the receptor by the ligand, signalling cascades trigger a cytoskeleton-dependent clustering of the receptor. Using this assay, we could discriminate the degree of receptor clustering in single cancer cells and we found that it correlates with the EMT state and migration potential of the cells. Analysis of population distribution showed that fast migratory mesenchymal-transformed cell lines had a larger spread of the clustering values, suggesting for a greater heterogeneity within their population. To further test the correlation between migration speed and EphA2 clustering, we devised an assay to separate subpopulations of cells for their migration speed and persistence. Fast migratory cells - potentially more invasive – proved to have higher EphA2 clustering as compared to slower ones. Finally, to test the possible application of our assay as screening tool for the invasion potential of cancer cells and their population heterogeneity, we compared cell derived from biopsies from primary and secondary tumours. Cells from secondary tumour had higher EphA2 clustering as compared to primary ones. In summary, we showed that EphA2 receptor clustering proved to be a potential tool to rapidly assess metastatic potential of cancer cells and phenotypic subclonal differences in their population.

67-POS Board 67

Nuclear Mechanical Biomarkers for Cancer Diagnosis

Aneesh R. Sathe[1], Karthik Damodaran[1], Caroline Uhler[2], Shivashankar G.V.[1,3].
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Current diagnosis for cancer employ a number of nuclear morphometric measures. While these approaches have provided insights into late stage disease diagnosis, early diagnosis has been a major challenge. Picking up subtle changes in nuclear morphometrics at an early stage is important for better prognosis and therapeutic intervention. In this study we use single cell imaging methods combined with machine learning to quantitatively detect nuclear morphometric features between normal and cancer cell lines. High throughput widefield images of DAPI stained nuclei are used to extract around 2000 physical and texture features as biomarkers. Linear discriminant analysis (LDA) of these biomarkers was able to discriminate between normal and breast cancer cell lines. In addition, LDA analysis discriminated fibrocystic (MCF 10A) and metastatic (MCF7 and MDA-MB-231) human breast cancer cell lines with high accuracy. Further we describe a compressive loading assay of single cells to amplify the differentiability of these cell types based on structural memory in chromatin condensation states. Finally using sparse LDA we identify the most predictive biomarkers which can be used as diagnostic reporters of altered nuclear architecture. Our methodologies collectively provide a simple and robust platform for potential applications in early disease diagnostics.

70-POS Board 70

Mining Genome Expression Omnibus (GEO) Datasets for Analysis of TRP Channels in Glioma Cell Lines

Taylor M. Nunn, Taylor W. Uselman, **Elba E. Serrano**.
New Mexico State University, Las Cruces, NM, USA.

Gliomas are aggressive primary brain tumors that develop from glial cells and are characterized by a low survival prognosis. Recent literature points to a potential role for TRP channels in glioma proliferation and tumor progression. As a prelude to experiments exploring the role of mechanosensation in glioma cell culture systems, we aimed to determine the prevalence of TRP channels across multiple glioma cell lines using bioinformatics approaches. Our strategy benefited from the emergence of open source metadata repositories that facilitate experimental design which incorporates *in silico* inquiry as a complementary approach to *in vitro* and *in vivo* methodologies. Through literature review we identified a subset of 13 TRP channel genes as candidates for expression analysis in glioma cell lines (TRPA, 1; TRPC, 3; TRPV, 3; TRPM, 5; TRPP, 1). Genes were selected based on their reported role in mechanotransduction and/or evidence that they are involved in glioma brain tumor progression. We queried the GEO repository for glioma high throughput datasets and selected a GEO Dataset Record comprising *GeneChip® Human Genome U133 Plus 2.0*microarray data from 60 cancer cell lines for analysis (GDS4296). Evaluation of normalized expression values for the 13 exploratory TRP channels uncovered the highest expression for TRPC1, TRPM7, and TRPP2 in all six glioma cell lines included in the dataset. Future studies will use qPCR to confirm TRP channel expression in glioma cell lines as part of ongoing experiments that examine glioma proliferation in matrix environnments. We conclude that outcomes from analysis of cell line expression metadata can inform research that investigates the role of TRP channels in brain cancer. Supported by undergraduate research awards: NMSU Discovery Scholars (TMN); NIH R25NS080685 (TWU).

73-POS Board 73

Role and Regulation of Caveolae in Three-dimensional Environment

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Caveolae are 60-80 nm omega shaped structures on the plasma membrane which comprise Caveolin-1 (Cav1) as a major structural protein and are rich in cholesterol and sphingolipids. Caveolae play an important role in cellular signaling, endocytosis and mechanosensing. Apart from these functions, caveolae have been recently emerged as plasma membrane organizers and protectors. Their presence or absence changes the membrane composition, membrane order and membrane tension, further regulating signal transduction in cells.These properties of plasma membrane are either known or expected to be different in cells which are cultured in a three-dimensional (3D) microenvironment as compared to conventional rigid two-dimensional (2D) tissue culture plate. The role and regulation of caveolae, is not known in cells grown in a 3D microenvironment and that is the focus of this study. Our studies show that, in Mouse Embryonic Fibroblasts (MEFs) embedded in a collagen gel, the loss of Cav1, and hence caveolae results in increased mobility of two membrane markers, K-Ras-CAAX-GFP and GPI-GFP. Reconstitution of Cav1-KO MEFs with WT-Cav1 reverses the increased mobility of both the markers. Another aspect of this study shows that Cav1 (but not its phosphorylation) negatively regulates endocytosis of certain cargos in MEFs embedded in 3D. This differential regulation of endocytosis is dependent on collagen matrix concentration. The mechanism of this differential regulation of endocytosis is currently being explored. Cav1 is a known tumor suppressor and the role it has in mediating differential trafficking and endocytosis in cancer cells in 3D matrices is also something we are actively exploring.

76-POS Board 76

Cell Geometry Orients Chromosomes to Regulate Genomic Programs

Yejun Wang[1], Mallika Nagarajan[1], Caroline Uhler[2], Shivashankar G.V.[1,3].
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Extracellular matrix signals from the microenvironment have been found to regulate gene expression patterns and cell behavior. Using a combination of experiments and geometric models we here demonstrate the critical role of 3D organization of chromosome territories in mechanoresponsive genome regulation. Fluorescence in situ hybridization experiments show that micropatterned fibroblasts cultured on large anisotropic versus small isotropic substrates result in rearrangements of specific chromosomes, which contain genes that are differentially regulated in the two cell geometries. Furthermore, specific chromosome pairs are intermingled in a transcription-dependent manner. In addition, experiments combined with ellipsoid packing models reveal that the mechanosensitivity of chromosomes is determined by their orientation in the nucleus. These results suggest that cell geometry modulates 3D chromosome arrangement and gene expression patterns in a predictable manner. This is central to understanding geometric control of genetic programs involved in cellular homeostasis and the associated diseases.

79-POS Board 79

Force Generation in Initial Cell-Matrix Interaction through E-Cadherin Mediated Cell Adhesion

Yi An Yang[1], Emmanuelle Nguyen[1], Felix M. Margadant[1], Shuaimin Liu[2], Benoit Ladoux[1,3], Michael P. Sheetz[1,4].
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In our research we aimed at understanding how cells respond to matrix stiffness through E-cadherin adhesion. Previous research have revealed that cells were able to sense varying extracellular environmental stiffness through cadherin mediated adhesion, but detailed mechanism remained largely obscure. Through utilizing sub-micron size pillar arrays, made with PDMS and surface functionalized with E-cadherin, we were able to mimic continuous surface that allow cell to form cadherin clusters, and record force generated by cell against substrate. We discovered that cells would generate local contraction in their initial contact with pillar substrate, with myosin light chain phosphorylation observed in between pillars, where cells were anchored upon through E-cadherin. Cells pulled pillar through these local contractile units to a constant maximum distance, which was independent of pillar rigidity but dependent of pillar spacing. These results indicated that cells were able to generate force actively based upon E-cadherin mediated adhesion, while contraction length remained constant regardless of stiffness change, allowing differential force input of cell according to substrate stiffness. Such force generation event resembled integrin mediated local contraction in fibroblast that initiated rigidity sensing, and could be involved in cadherin mediated rigidity sensing and adhesion maturation.

POSTER SESSION II

Wednesday, September 28, 16:15 – 18:15
Level 2 Hall Foyer

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Wednesday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Anura, Anji	**2-POS**	**Board 2**
Bertocchi, Cristina	**5-POS**	**Board 5**
Changede, Rishita	**8-POS**	**Board 8**
Chen, Yen-Chih	**11-POS**	**Board 11**
Conway, Daniel	**14-POS**	**Board 14**
Das, Debanjan	**17-POS**	**Board 17**
Galeano-Niño, Jorge	**20-POS**	**Board 20**
Hirata, Hiroaki	**23-POS**	**Board 23**
Iqbal, Samir	**26-POS**	**Board 26**
Jokhun, Doorgesh	**29-POS**	**Board 29**
Kim, Dong-Hwee	**32-POS**	**Board 32**
Ko, Eun Min	**35-POS**	**Board 35**
Kubo, Atsushi	**38-POS**	**Board 38**
Lan, Ganhui	**41-POS**	**Board 41**
Lee, Chia-Wei	**44-POS**	**Board 44**
Luxton, GW Gant	**47-POS**	**Board 47**
Minsky, Burcu	**50-POS**	**Board 50**
Morimatsu, Masatoshi	**53-POS**	**Board 53**
Nishimura, Yukako	**56-POS**	**Board 56**
Park, Eunyoung	**59-POS**	**Board 59**
Qiu, Zhihai	**62-POS**	**Board 62**
Roy, Bibhas	**65-POS**	**Board 65**
Saw, Thuan	**68-POS**	**Board 68**
Shah, Mrinal	**71-POS**	**Board 71**
Venkatachalapathy, Saradha	**74-POS**	**Board 74**
Xi, Wang	**77-POS**	**Board 77**
Yang, Jennifer	**80-POS**	**Board 80**

Posters should be set up in the morning of September 27 and removed by noon September 30.

2-POS Board 2

Unrevealing Nanomechanical Signatures of Epithelial and Connective Tissue of Oral Submucous Fibrosis

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Deciphering altered biomechanical profiles in oral cancer and precancer is important for detailed understanding of malignancy which could have implications in diagnosis and treatment. In this work PeakForce quantitative nanomechanicsatomic force microscopy (PF-QNM-AFM) was performed to investigate nano-mechanical attributes as well as ultra-structural changes in epithelium and connective tissue of normal oral mucosa, submucous fibrosis. The topography obtained from normal mucosa demonstrated irregular and loosely arranged collagen fibres with regular bandwidth and periodicity (67 nm) in lamina propria. However, in oral submucous fibrosis, collagen fibres were arranged in bundles with uneven thickness and irregular periodicity indicating abnormal collagen synthesis. The nano-mechanical analysis indicated that OSF had higher Young's modulus (20-40 kPa)than normal oral tissue (1-20 kPa). Moreover, connective tissue of OSF showed a high adhesion force and reduced deformation indicating high stiffness due to compact collagen I deposition in the extracelluar matrix as confirmed from immunohistochemical and RT-PCR study. The epithelium of oral submucous fibrosis was found to have high Young's modulus(3-10 kPa) , stiffness, adhesion force and reduced deformation to that of normal oral epithelium (0.5- 4 kPa) which might be attributed to associated epithelial atrophy. Thus, the present study elucidates altered mechanobiological attributes of oral precancer which are corroborated with ultrastructural changes and relevant gene expression. In the context of integrative mechanotyping in cancer study, the present study will be contributory in holistic understanding of oral precancer pathology, its relation to malignant transformation and providing essential insight for developing therapeutic measures.

5-POS Board 5

Nanoscale Architecture of Cadherin-Mediated Adhesion.

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Cadherin-mediated adhesions are highly dynamic complexes formed at sites of cell-cell contacts, essential for tissue homeostasis and multicellularity. Perturbations in their expression or function result in loss of intercellular adhesion with possible cell transformation and tumor progression. Recently, progress has been made in understanding the interaction between different components of these complexes and how they are deregulated in cancer cells. However, despite the central importance of such supramolecular complexes, their molecular organization remains unknown. Deciphering the molecular-scale organization of cadherin-adhesion-complexes requires determination of 3D distribution of specific proteins with accuracy matching their nanometer-length scale. While superresolution microscopy offers a potential avenue for dissecting nanoscale organization within these complexes, the inherently 3D geometry of the native cell-cell contacts pose limitations to achieving sub-20 nm resolution required for inferring molecular orientation. To overcome this issue, we utilized a biomimetic cadherin-coated substrate with which cells form cadherin-mediated complexes that recapitulate key attributes of early cell-cell contacts and which are highly amenable to superresolution imaging. Here we mapped the nanoscale organization of cadherin and key proteins of the cadhesome network in cells cultured on such biomimetic surface, observing a well-organized molecular architecture stratified along the z axis. The cadherin-catenin and actin compartments are separated by ~30 nm, interposed by a vinculin-containing interface. Vinculin can undergo a conformational activation, spanning between the cadherin-catenin layer and the actin compartment. Nanoscale positioning of vinculin was determined by alpha-catenin, while vinculin conformational state is controlled by contractility and/or Abl kinase phosphorylation at Y822 and, in turn, modulates the positioning of zyxin and VASP. In conclusion, our measurements reveal a modular nanoscale architecture of cadherin-based adhesions, suggesting a control principle whereby vinculin is a mechanical clutch that integrates mechanical and biochemical signals to differentially engage the cadherin-catenin complexes to the actomyosin contraction machinery.

8-POS Board 8

Nascent Integrin Adhesions Forming on all Matrix Rigidities, Differentially Activate EGFR to Respond to Extracellular Traction Forces.

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Integrin adhesions assemble and mature in response to ligand binding and mechanical factors, but the molecular-level organization is not known. We report that ~100-nm clusters of ~50 β3-activated integrins form very early adhesions under a wide variety of conditions on RGD surfaces. These adhesions form similarly on fluid and rigid substrates, but most adhesions are transient on rigid substrates. Without talin or actin polymerization, few early adhesions form, but expression of either the talin head or rod domain in talin-depleted cells restores early adhesion formation. Mutation of the integrin binding site in the talin rod decreases cluster size. We suggest that the integrin clusters constitute universal early adhesions and that they are the modular units of cell matrix adhesions. They require the association of activated integrins with cytoplasmic proteins, in particular talin and actin, and cytoskeletal contraction on them causes adhesion maturation for cell motility and growth. Force dependent activation of EGFR in the nascent adhesions is required for cell spreading only on rigid substrates and not on compliant substrates, suggesting key differences in the similarly laid out nascent adhesions.

11-POS Board 11

The Effect of Fluid Shear Stress on the Migration Capability of Tumor Cells in Circulating System

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During cancer metastasis, cancer cells separate from the primary tumor, invade through tissues and penetrate through their basement membranes, enter into the blood vessels, circulate in the blood vessels, exit the circulation system, reach other organs, and then initiating secondary tumor. In general, the migration capability of cancer cells is often strongly correlated with their metastatic potential. In our research, we focus on cancer cells entering the circulatory system and flowing in the bloodstream. In circulating system, fluidic shear stress may play an important role in metastasis of cancer cells. In our study, we designed and used a biomimetic shear flow system with a fluid shear stress ~10 dyne/cm^2, (which is within the range of the typical value of fluid shear stress encountered by cells circulating in the blood vessels) to investigate the effect of shear stress on the migration capability of cancer cells in vitro. We quantified, via would healing assay, the effect of fluid shear stress on the migration capability of two cell lines of head and neck squamous cell, namely, OECM-1 (mesenchymal phenotype) and FaDu (epithelial phenotype), as the potential indicator for cancer metastasis. Our results indicated that fluid shear flow within physiological range (10 dyne/cm^2) significantly enhances the migration capability of OECM-1; in contrast, it has much smaller effect on FaDu. These findings suggest that fluid shear flow may promote metastasis potential of mesenchymal cancer cells.

14-POS Board 14

E-cadherin Force Contributes to Lumen Homeostasis in Epithelial Acini

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Little is known about the effects of protein-level mechanical forces on cell behavior in the context of an organized tissue structure. Epithelial cells cultured in a 3D environment comprising of extracellular matrix proteins form hollow spheroids of polarized cells known as acini or cysts. We hypothesized that forces across cell-cell junctions, specifically E-cadherin, are important regulators of these structures. Using a FRET-based tension biosensor in MDCK cells grown in Matrigel, we observed that E-cadherin forces were higher in 3D acini as compared to 2D monolayers. Using forskolin and a CFTR inhibitor to increase and decrease acini lumen pressure, respectively, we observed that E-cadherin force was affected by lumen pressure. Additionally, we were able to show that increased lumen pressure induces cell proliferation, requiring cytoskeletal-connected E-cadherin. Thus, changes in lumen pressure can regulate epithelial proliferation through E-cadherin forces, similar to prior work showing E-cadherin forces mediate stretch-induced proliferation in 2D. Next, we observed that cells expressing low- and zero-force mutants of E-cadherin had impaired lumen formation (multiluminated or complete absence of a lumen). In the case of the low-force mutant we were able to increase E-cadherin force and rescue acini to a single lumen using forskolin, further substantiating the role of E-cadherin force in acini homeostasis. Finally, we observed that E-cadherin force was reduced during the process of TGFß-induced EMT. Pre-treatment with forskolin was sufficient to block E-cadherin force changes, EMT, and lumen filling in TGFß treated cells. We are currently investigating if TGFß reduces E-cadherin force by altering lumen pressure, and also to determine if forskolin prevents EMT is through lumen pressure and/or E-cadherin forces. The major conclusion of these studies is that E-Cadherin tensile forces, which are modulated by lumen pressure, regulate the formation and homeostasis of epithelial acini.

17-POS Board 17

AFM based Biomechanical Characterization for Assessment of Cancer Aggressiveness

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Biomechanical characterization is one of the potential markers of onset and progression of cancer and can help for understanding cellular physiology. Therefore, screening the mechanical properties of healthy and cancer cells having different metastatic potentials offers an important indicator to determine cancer aggressiveness. In this study, Peak-Force Quantitative Nanomechanical Atomic Force Microscopy (PF-QNM AFM) has been employed for assessment of cancer aggressiveness by corroborating with the ultrastructural and nanomechanical properties of three cell lines with different degrees of malignancy: HaCaT (healthy), MCF-7 (tumorigenic/noninvasive) and MDA-MB-231(tumorigenic/invasive). Structural topography obtained from AFM reveals that cancer cells are loosely attached to each other with increased cell-cell junction, higher cellular thickness, membrane roughness and membrane folding as compared to normal HaCaT cells. The force indentation curve analysis demonstrated reduced Young's modulus and stiffness for cancer cells (MCF-7: 24.6±2.7kPa, MDA-MB-231: 11.4±3.6kPa) as compared to normal cells (HaCaT: 32.4±4kPa). Altered cellular attachment, spreading, disorganized actin filaments, etc. may induce softer and deformable cytoskeleton of MDA-MB-231 resulting to have higher migration and invasion potency. Further evidence of higher aggressive nature of cancer cells obtained from AFM data shows that migration and metastatic spreading is correlated with low-adhesion force between AFM tip and surface of cells. The lower stiffness and reduced adhesion properties of cancer cells could be related to its ability to grow even in limited anchorage to extra cellular matrix having space constriction and may be considered as a biomechanical signature for identification of cancer cells. Therefore, the present nanomechanical profiling by AFM in association with biochemical study will advance in holistic understanding of complex metastatic process including mechanical compliance, motility, cell–cell and cell-substrate adhesions.

20-POS Board 20

Harnessing Actomyosin Nucleators and Regulators to Control T Cell Migration

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The main immune cells responsible for antitumor activity are Cytotoxic T cells (CTLs). They constitutively patrol organs for cognate antigen and typically migrate using an elongated and polarised shape with a dynamic leading edge and a uropod at the rear. T cells are however able to adopt diverse migration modes depending on both extra- and intracellular cues. The actomyosin cytoskeleton is responsible for the mechanical forces that are involved in the migratory process. The polymerisation of actin filaments, mediated by Arp2/3 complex or formins (actin nucleators), and the contractility of myosin motor proteins, collectively determine the morphology and underpin the migration of cells. However, their functionality during T cell migration remains incompletely understood. Using 3-dimensional migration assays and novel image analysis methods, we found that inhibiting specific components of the actomyosin cortex, CTLs can be forced to adopt different migration modes and extend distinct sets of protrusions. The pharmacological inhibition of formins induces a loss of directionality during CTL scanning. These finding suggest that formins are required for directional maintenance by CTLs and thus their overall scanning efficiency. The inhibition of particular actomyosin nucleators or regulators modifies the migration T cells in a predictable and specific manner, and could thus open up novel therapeutic avenues for controlling both excessive and deficient T cell movement in various pathological contexts ranging from autoimmune diseases to cancers.

23-POS Board 23

Actomyosin Activity is Essential for Contact Inhibition of Keratinocyte Proliferation

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Confluence-dependent inhibition of keratinocyte proliferation, termed contact inhibition, contributes to epidermal homeostasis. Loss of the contact inhibition in keratinocytes is associated with keratinocyte carcinomas, the most common type of cancers in the United States. Whilst both E-cadherin ligation and actomyosin activity have been suggested to regulate proliferation of confluent keratinocytes, it remains elusive how these two cooperate to achieve contact inhibition. Here, we report distinct roles of E-cadherin ligation and actomyosin activity in confluence-dependent regulation of keratinocyte proliferation. Under actomyosin inhibition, cell-cell contact through E-cadherin promoted proliferation of keratinocytes. By contrast, actomyosin activity in confluent keratinocytes inhibited nuclear localization of β-catenin and YAP, and caused attenuation of β-catenin- and YAP-driven cell proliferation. Depending on the actomyosin activity, confluent keratinocytes developed E-cadherin-mediated punctate adhesion complexes, to which radial actin cables were connected, at the apical portions of cell-cell boundaries. Eliminating the actin-to-E-cadherin linkage by depleting α-catenin expression increased proliferation of confluent keratinocytes. External application of pulling force through E-cadherin using magnetic beads, in turn, diminished proliferation of actomyosin-inhibited keratinocytes, suggesting an inhibitory effect of tensile stress at E-cadherin adhesions on keratinocyte proliferation. Our results highlight actomyosin activity as a crucial factor that provokes confluence-dependent inhibition of keratinocyte proliferation.

26-POS Board 26

Identifying Metastasis from Mechanical Behavior of Tumor Cells Captured on Functionalized Substrates

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The metastatic tumor cells invade tissues and spread to other organs. There are a number of chemical and mechanical interactions all the way from when these cells leave primary cancer site, propagate through the circulatory system, and reach other organs. One way to screen for cancer in a person is to look for cancer cells in bloodstream. Detection of such circulating tumor cells (CTCs) in blood can thus be employed for diagnosing cancer at early stages. In this work, we present results of a platform where CTCs from blood were captured on a functionalized substrate. The mechanical behavior of captured cells clearly distinguished not only tumorous cells from normal cells but also showed well-defined behavior difference between metastatic and indolent cancer cells. An anti-EGFR aptamer was used to capture CTCs on glass chips. The breast cancer cells were mixed with rat blood followed by the introduction of red blood cell lysis buffer. The sample then had only WBCs and cancer cells in the serum. The cells captured on anti-EGFR aptamer functionalized substrate were imaged for 15 minutes and analyzed with custom written MATLAB program routines to extract data. It was observed that the CTCs vigorously changed their shapes over time. The mechanical behavior of the cells was measured in terms of convexity, average radial distance, bounding box area and cell compactness. It was clearly evident that captured tumor cells on functionalized surface were distinguishable from WBCs. Furthermore, metastatic tumor cells showed much more “alive and kicking” behavior than indolent tumor cells. This approach can be used as an important modality to screen for cancer and to identify metastatic potential of a given sample.

29-POS Board 29

Cell Mechanics Regulate Nuclear and Chromatin Dynamics by Altering the Cytoskeletal and Nucleoskeletal Organization.

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Conditions like cancers, progeria and fibrosis are often accompanied by changes in the physical landscape of the affected tissue. At the single cell level, this alteration in physical cues modulate the cytoskeletal organization and thus the force being transmitted to the nuclear lamina and chromatin. While the role of these active forces in modulating the prestressed nuclear morphology has been well studied, their effect on nuclear and chromatin dynamics is less understood. To address this, we used distinct fibronectin micropatterns as a means of defining the mechanical landscape of single cells and seeded NIH3T3 fibroblasts transfected with H2B-GFP and TRF1-DsRed to visualize the nuclear and chromatin (telomeric) dynamics respectively. We observed that cells plated on small isotropic patterns lack apical actin stress fibers and have more plastic nuclei compared to cells on large polarized substrates. We showed that this enhanced nuclear plasticity can be attributed to active forces from short actin-myosin-formin structures and a transcriptional downregulation of laminA/C levels in the small isotropic condition. We also showed that the changes in cytoskeletal and nucleoskeletal architecture further regulate chromatin dynamics, as measured by the 3D spatiotemporal tracking of telomeres. In conditions which curb the formation of apical actin stress fibers and the expression of Lamin A/C, the telomeres explore larger volumes, move faster, more independently and more freely. Taken together, our results show that active cytoskeletal forces and rigidity from the lamin A/C nucleoskeleton, as defined by the physical state of the tissue, are important regulators of nuclear and chromatin plasticity in living cells, providing a possible mechanical route for genome regulation.

32-POS Board 32

Cytoskeleton-medicated Nuclear Mechanics : Mechanobiological Approach into the Subcellular Nanomachinery

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Alterations in nuclear morphology are closely associated with essential cell functions and correlate with a wide range of human diseases, including cancer, muscular dystrophy, dilated cardiomyopathy and progeria. However, the mechanics and forces that shape the nucleus are not well understood. Accumulating evidence suggests that the three-dimensional organization of the nucleus regulates gene expression through lamina-chromosome interactions. The nuclear lamina is a thin filamentous meshwork that provides mechanical support to the nucleus and regulates essential cellular processes such as DNA replication, chromatin organization, cell division, and differentiation. Conventional microscopy has long suggested that the nuclear lamina is composed of structurally different intermediate filamentous lamin proteins and nuclear lamin-associated membrane proteins that together form a thin shell largely confined to a narrow region underneath the nuclear envelope. Here we show that both A-type lamins and transcriptionally active chromatins are vertically polarized by the tension exercised by the perinuclear actin cap that is composed of highly contractile actomyosin fibers organized at the apical surface of the nucleus. Furthermore, we first demonstrate that the nucleus undergoes a large volumetric reduction accompanied by a morphological transition from an almost smooth to a heavily folded surface. We develop a mathematical model that systematically analyzes the evolution of nuclear shape and volume. Our analysis suggests that the pressure difference across the nuclear envelope, which is influenced by changes in cell volume and regulated by microtubules and actin filaments, is a major factor determining nuclear morphology. Our results show that physical and chemical properties of the extracellular microenvironment directly influence nuclear morphology and suggest that there is a direct link between the environment and gene regulation. These findings broaden our understanding of 3D nuclear architecture and provide new prospects in laminopathies and cellular mechanotransduction.

35-POS Board 35

Establishment of the Quantification Platform for Cardiomyocyte Differentiation

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Cardiovascular disease is one of the most popular causes of deaths in 2000s. However, current medical procedures cannot completely restore the dysfunctional cardiac tissues. Only the heart transplantation can recover the damaged tissues, but unfortunately there are few donors for the needs. For this reason, it is essential to figure out the roles of differentiation factors for in vitro cardiac tissue. In physiological condition, cardiac tissue influenced by microenvironment like alignment of cells, electrical and biochemical stimulus, and it is known as these microenvironment also effects on cardiomyocyte differentiation. Also the force generated by cardiomyocyte usually used as an indicator of degree of differentiation. In this study, we developed a cardiomyogenic platform that provides these microenvironments to understand the differentiation factors and the mechanisms. We developed platform that implements biochemical factors, uniaxial patterns generated by micro-contact printing (μCP) and measures traction stress of cardiomyocyte by traction force microscopy (TFM). Using our platform, we successfully figured out change of the cellular elongation with width of pattern and direction of traction stress vector ordered as width of pattern narrowed. With the traction stress vector, different position of cells within the pattern made cells influenced by force differently, this made change in degree of differentiation. We also checked distribution of traction force for a pattern change with sequence of differentiation. From the results, we expect to distinguish a degree of differentiation by magnitude and distribution of traction stress and further more combine with electrical stimulus and find the optimal conditions for better differentiation of cadiomyocyte to be used in tissue engineering applications. This work was supported by a National Research Foundation of Korea (NRF) grant (No. 2015R1A2A2A04004469 and No. 2013R1A2A2A01017014) from the Ministry of Science, ICT and Future Planning, Republic of Korea.

38-POS Board 38

A Mechano-transduction in Zebrafish Heart Development

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The physical forces are regarded as new important signals for development and homeostasis. In zebrafish heart, mechanical stresses evoked by blood flow (eg. shear and stretch stresses) play essential roles during morphogenesis of heart (Nature 421, 172, 2003, Nature Communications 4, 1978, 2013). Nonetheless, molecular mechanisms and functional links between mechanical forces and transcriptional control of genes remain largely unknown.
In this study, we focused on the transcriptional co-activator, *mkl2*, since we found that this co-activator plays an important role during mechano-transduction pathway. We carried out an in vitro stretch assay to apply mechanical stresses to cardiac myoblast cells, H9C2. H9C2 cells stably expressing flag-tagged mkl2 were seeded on an elastic chamber and subjected to cyclic stretch. Soon after a short period of stretching, nuclear localization of mkl2 increased at the expense of cytoplasmic localization, indicating a rapid nuclear shuttling of mkl2. When we overexpressed an actin mutant S14C that enhances actin polymerization, again, nuclear accumulation of mkl2 was observed, suggesting that the stretch-induced nuclear translocation of mkl2 is mediated by the actin polymerization.
mkl2-deficient zebrafish embryos generated by Morpholino antisense oligos (MO) or the CRISPR/Cas9 system showed severe heart defects. To analyze relationship between the mechanical forces and mkl2 translocation, we first visualized actin dynamics in the transgenic embryos that express Lifeact-eGFP. At 24 hours post fertilization (hpf), contraction of heart was still very weak, and no polymerized signal of actin was detected. However, at 72 hpf, the heart contracts strongly, and highly polymerized actin meshwork was evident at this time point. This actin polymerization was disappeared when cardiac contraction was arrested by BDM or injection of MO against *tnnt2*. These results indicate that heartbeat promotes polymerization of actin, which is indispensable for nuclear shuttling of mkl2 in zebrafish embryos.

41-POS Board 41

Overcrowding Drives the Collective Motility within Gap-Free Monolayers

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Collective cell motility plays central roles in various biological phenomena such as inflammatory response, wound healing, cancer metastasis and embryogenesis. These are biological demonstrations of the unjamming transition. However, contradictory to the typical density-driven jamming processes in particulate assemblies, cellular systems often get unjammed in highly packed, sometimes overcrowding tissue environments. Here, we combine the Voronoi tessellation model with a Monte Carlo stimulation scheme to investigate the monolayers' collective behaviors when cell number changes under the gap-free constraint. We report that overcrowding can unjam gap-free monolayers through increasing isotropic compression, which spontaneously drives the collective cell migration within the monolayers. We show that the transition boundary is determined by the isotropic compression and the cell-cell adhesion. Furthermore, we explicitly construct the free energy landscape for the T1 topological transition during monolayer rearrangement, and discover that the landscape evolves from single-barrier "W" shape to double-barrier "M" shape upon completion of the unjamming process. We also discover a distributed-to-disordered morphological transition of cells' geometrical properties, coinciding with the unjamming of the monolayer. Our analyses reveal that the overcrowding and adhesion induced unjamming transition reflects the mechanical yielding of the highly deformable monolayer, suggesting an alternative mechanism that cells may robustly gain collective mobility through growth and division in confined environments, which differs from those caused by loosing up a packed particulate assembly. From a mechano-biological coupling viewpoint, the biological cues induce intracellular biomechanical actions that may be in charge of navigating the cell migrations of the cellular assemblies; meanwhile, the overcrowding by cell growth and division, together with the enhanced cell-cell adhesion, may be in charge of removing the barrier of morphological rearrangements of the cells that enables and enhances the collective migrations of the entire cellular assemblies.

44-POS Board 44

Optical Measurements on the Variations of Neuronal Cell Membrane Roughness in Response to External Treatments

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Living cells exhibit many topographic features that depend heavily on membrane properties as well as cytoskeleton configurations. Membrane roughness measured by atomic force microscopy (AFM) was long proposed as a diagnostic parameter for determining the status of a cell under external physical or chemical stimulations. However, the imaging speed of AFM is far below the requirement of high-content screening. In this study, we proposed to use a wide-field optical profiling technique, non-interferometric wide-field optical profilometry (NIWOP), to quantify the membrane roughness of mouse neuroblastoma cells (N2a). The NIWOP technique can acquire membrane roughness of all cells in a field of view within a few seconds. This speed is much more adequate for obtaining statistical results in a reasonable period.

We treated the N2a cells by 10 uM paclitaxel (Taxol), and found that the membrane roughness was significantly decreased. A similar trend was observed on fixed cells by using scanning electron microscopy (SEM). Membrane stiffness increased by Taxol and microtubule translocation might both take parts in this result. We also found that the membrane roughness showed transient increase under the hypertonic condition with 75 mM sucrose in the culture medium. Meanwhile, treatments of oppositely charged gold nanoparticles (AuNPs, 30 nm) were also executed. Positively charged AuNPs decreased the membrane roughness, while negatively charged AuNPs showed insignificant effects. We also confirmed that the adsorption efficiency of positively charged AuNPs on cell membranes was much higher than that of negatively charged AuNPs by using SEM.

In conclusion, we demonstrated that wide-field optical profilometry could acquire the membrane roughness of a group of living cells in a few seconds. The membrane roughness reflected the variations in cytoskeleton configurations, membrane tension, as well as the membrane affinity of nanoparticles.

47-POS Board 47

LINC Complex Assembly Within The Nuclear Envelope Of Living Cells

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LINC (linker of nucleoskeleton and cytoskeleton) complexes enable the direct transmission of mechanical forces across the nuclear envelope and into the nucleoplasm, which is required for wholesale positioning of meiotic chromosomes and nuclei. LINC complexes conserved molecular bridges that span the nuclear envelope and consist of the inner and outer nuclear membrane KASH and SUN proteins, respectively. KASH proteins interact with the cytoskeleton within the cytoplasm, while in the nucleoplasm SUN proteins interact with nuclear lamins and chromatin. Within the PNS (perinuclear space), KASH and SUN proteins directly interact to form the core of the LINC complex. Recent structural studies revealed that SUN2 forms a mushroom-like homo-trimeric structure with a stalk of three coiled-coils and a globular head that recruits three KASH proteins that bind in three deep hydrophobic grooves formed between adjacent SUN2 protomers. Despite this significant structural insight into LINC complex assembly, the in vivo relevance and conservation of SUN homo-trimerization remain unclear. Here, we apply z-scan FFS (fluorescence fluctuation spectroscopy), a biophysical imaging-based approach with single-molecule sensitivity, to quantify interactions within the PNS of living cells by brightness analysis. Consistent with in vitro studies, we demonstrate the existence of SUN2 homo-trimers within the PNS and identify structural requirements for SUN2 homo-trimerization. We then show that within the PNS, SUN1 can form higher-order homo-oligomers than SUN2. Finally, we uncover environment-dependent differences in SUN protein oligomerization suggestive of previously unidentified mechanisms for the regulation of LINC complex assembly and function. Taken together, our results establish z-scan FFS as a powerful tool for studying LINC complex assembly within the nuclear envelope of living cells. This information will be critical for understanding the functional consequences of mutations in KASH and SUN proteins associated with human diseases including cancer and muscular dystrophies.

50-POS Board 50

Investigating the Roles of Hyaluronan in Wound Closure Using Nanostructured PEGDA Hydrogels

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Wound healing is a complex and highly coordinated process that involves dynamic interactions between epidermal and dermal layers. Even though most of the surface wounds could regenerate leading to a minimal scar formation, this orchestrated network of events could be disrupted especially with advanced age or chronic illnesses, such as diabetes. The impairment of this intricate balance can lead to a spectrum of healing process. The processes controlling wound healing are strongly dependent on the spatial and temporal presentation of ECM components, which modulates biochemical signaling events, and structural organization of the matrix, which controls the permeability and stiffness. Hyaluronan, one of the major components of the provisional matrix during wound healing, promotes the healing process. However, the complete understanding of this regulation is largely lacking. The objective of this work is to develop a well-controlled biomaterial platform that enables (1) mechanically suitable environments to guide keratinocyte and fibroblast migration and proliferation, (2) isolating effects of biochemical components, i.e., adhesive ligands and hyaluronan. Therefore, we developed hybrid poly(ethylene glycol) diacrylate (PEGDA) hydrogels including cell adhesive, RGD containing peptide. The advantage of using PEGDA is that the stiffness can easily be tuned by varying the PEG chain length and weight percent to match the stiffness of the wound bed during the healing process. Additionally, these hydrogels are patterned using block copolymer micellar nanolithography (BCML) to immobilize hyaluronan on quasi-hexagonally arranged gold nanoparticles at a controlled distance. The immobilization is achieved by introducing a thiol linker at the reducing end of hyaluronan, which has been proven success to graft hyaluronan on gold surfaces with a high coverage.Furthermore, mechanical properties and biophysical characterizations (mesh sizes and diffusion properties) of the hydrogels are evaluated using well-established techniques.

53-POS Board 53

High Hydrostatic Pressure Induces Signal Transduction of the MAPK Pathway

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Cells sense physical signals and translate them into biological responses via mechanotransduction. In our bodies, pressure also stimulates bone and tooth development in the range of several dozen MPa. However, fundamental aspects of how these cells detect pressure on a molecular level remain poorly understood, in part due to a lack of methods that can quantitatively apply hydrostatic pressure to cells and quantify signal transduction. Here we tested the effect of hydrostatic pressure on activation of the mitogen activated protein kinase (MAPK) pathway. We applied various hydrostatic pressures (0.1 ~ 40 MPa) to fibroblast cells and observed the localization of phosphorylated extracellular signal regulated kinase (phospho-ERK), which leads to cell growth and differentiation. At a pressure level higher than atmospheric (~ 40 MPa), phospho-ERK proteins displayed enhanced localization to the nucleus. Furthermore higher hydrostatic pressure induced the phospholylation of ERK proteins via the MAPK pathway. Our preliminary data suggests higher hydrostatic pressure increases the probability of phospho-ERK translocation to the nucleus. Ongoing work uses the unique capabilities of mechanical hydrostatic pressure control *in vitro* to clarify mechanotransduction mechanisms and induce cell growth.

56-POS Board 56

MT1-MMP Regulates Focal Adhesion Turnover and Cellular Traction Stresses

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Focal adhesions are cellular organelles serving as the mechanical linkages between the cell and the extracellular matrix (ECM). Cells need to control turnover of focal adhesions spatiotemporally for directed migration. In addition, focal adhesions have been shown to be sites for the ECM degradation by matrix metalloproteases (MMPs), key enzymes essential for directed cell migration and tumor-cell invasion. The mechanisms of cross-talk between focal adhesions and MMPs remain, however, poorly understood. Here, we have identified a role of MT1-MMP, one of the major trans-membrane MMPs, in the turnover of focal adhesion. Knockdown of MT1-MMP resulted in increase of focal adhesion size and slowing down their disassembly rate as compared to the focal adhesions in control cells. To test if MT1-MMP affects force exerted by cells on the substrate, we used traction force microscopy. We showed that MT1-MMP knockdown cells developed higher traction stresses than control cells. Live imaging of MT1-MMP has revealed that this enzyme is localized to vesicle-like structures, moving along microtubules and frequently fusing with the plasma membrane in proximity of the focal adhesions visualized by paxillin labeling. Tracking the MT1-MMP vesicles during focal adhesion disassembly revealed a tight spatio-temporal correlation between frequency of MT1-MMP vesicles fusion with the plasma membrane and the disassembly of focal adhesions. Taken together, these data suggest that MT1-MMP is trafficked by microtubules towards focal adhesions and then controls focal adhesions dynamics, as well as mechanical tension applied by focal adhesions to the ECM.

59-POS Board 59

Actomyosin Driven Oscillatory Behavior in Microglial Cells

Eunyoung Park, Young Bin Cho, Unghyun Ko, Jin-Sung Park, Sukyung Park, Jennifer H. Shin. Korea Advanced Institute of Science and Technology, Daejeon, South Korea.

Dynamics of cytoskeletal proteins are critically involved in many cellular processes including polarity establishment, movement, and force generation. Despite the importance of cytoskeletal proteins, identifying their roles in cellular processes is difficult due to the complexities in the combinatorial effects of the involved proteins. In this study, we present microglia, a type of immune cells in the central nervous system, which are known to alter their phenotypes by rearranging the cytoskeletons depending on the microenvironment. Especially, microglia featured oscillatory migration by forming a large lamellipodium at each end. From the physical force measurement with traction force microscopy (TFM), we observed the localized stresses at the both ends and their fluctuations, which are followed by the morphological oscillation. By the correlation analysis, we identified two lagged motion: that the protruding of the lamellipodium was prior to the traction development and nuclear translocation. Based on our observations, we proposed a simple viscoelastic lumped model for oscillating microglia by comprising two masses, two springs and two dampers, which represent the contractility and adhesive drag induced by cytoskeletal proteins. By comparing the obtained coefficients of spring and damping with the previously reported values, we could suggest that our simplistic model is able to capture the dynamic behavior of complex cytoskeletal elements in the oscillating microglia. Moreover, we also investigated the direct role of the cytoskeletal proteins by the inhibition assays, in which the oscillations were shown to be regulated by actomyosin contractility. This work was supported by the National Research Foundation of Korea Grant funded by the Korean government (NRF-2015M3A9B3028685).

62-POS Board 62

Acoustic Mechanogenetics for Controlling Neuron Activity and Signaling

Zhihai Qiu[1], Yaoheng Yang[1], Jinghui Guo[2], Shashwati Kala[1], Hsiao Chang Chan[2], Lei Sun[1]. [1]The Hong Kong Polytechnic University, Hung Hom, Hong Kong, [2]The Chinese University of Hong Kong, Sha Tin, Hong Kong.

Mechanosensitive receptors and ion channels in neuron surface can sense the mechanical properties in their microenvironment and mediate neuronal activity and signaling. However, how these signals integrate with other signals such as chemical and spatial signal to give rise to human thought and plasticity reminds elusive. The challenge is to develop a mechano-tool for controlling the neuron activity and signaling non-invasively with high spatiotemporal resolution. To date, we developed an ultrasonic mechanogenetic tool for quantitative and selective manipulation of the neuron activity and signaling. Nano-gas vesicles (NGV) which can induce highly localized mechano-perturbations in low intensity ultrasound fields, were functionalized with ligands and antibodies to target specific mechanosensitive membrane proteins (e.g. TRPV1 and Piezo 1 channels etc.) on primary neurons. Neuron activities mediated by the targeted oscillating NGV driven by ultrasound were investigated by calcium imaging and patch-clamp, while the neuronal signaling especially calcium related signaling regulation and phosphorylation were tested by Western blot. Our results showed that under low intensity ultrasound irradiation, local ultrasound pressure will be significantly intensified and localized where the oscillating NGV were placed. Furthermore, the intensity of the generated highly localized ultrasound field depending on ultrasound intensity and frequency was able to activate the targeted mechanosensitive proteins followed by inwards ion currents, calcium influx, and PKA up-regulations. It achieves molecular selectivity with subcellular precision. Capable of non-invasive transmission though the tissue with fine focal size to integrate other stimulation in the brain, ultrasonic mechanogenetics is an encouraging means for investigating mechanobiology in brain and a good alternative to existing stimulating strategies for studying brain function with the advantages of non-invasiveness, fine spatial control, and deeper tissue penetration. We also envision that it is an invaluable tool for studying cancer mechanobiology in vivo as well.

65-POS Board 65

Geometric Confinement of Cells Induces Nuclear Reprogramming

Bibhas Roy[1,2], Prasuna Ratna[1].G V. Shivashankar[1,2,3].
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Biochemical factors can help reprogram somatic cells into pluripotent stem cells, yet the role of biophysical factors during reprogramming is unknown. Here, we show that biophysical cues, in the form of cell geometry and associated confinement, can induce nuclear reprogramming and significantly improve its efficiency. Establishing and prolonging geometric confinement of mouse embryonic fibroblast on a fibronectin micropattern of defined shape and size results in change in the physical properties of nucleus including size, shape, orientation, deformability and sptio-temporal organization. These transitions in nuclear dynamics induce pronounced change in lamin A expression, histone acetylation and methylation patterns, which facilitates a stochastic phase of transdifferentiation, specifically mesenchymal to epithelial transition—an early event in nuclear reprogramming. We also show that prolonged exposure of this increasing confinement on these fibroblasts leads to formation of embryonic stem cell like colony and characteristic biomarkers, suggesting the maturation of nuclear reprogramming. Our work not only establish biophysical factors as a key regulator of reprogramming, but also elucidating a new correlation between geometric confinement, epigenetic mechanomodulation and nuclear reprogramming, which have great implication in regenerative medicine.

68-POS Board 68

Distinct Modes of Apoptotic Cell Extrusion Governed by Epithelia Packing Density

Leyla Kocgozlu[1], **Thuan Saw**[1,2], Anh Phuong Le[1,2], Ivan Yow[1], Murat Shagirov[1], Eunice Wong[1], René- Marc Mège[3], Chwee Teck Lim[1,2,4], Yusuke Toyama[1], Benoit Ladoux[1,3]. [1]Mechanobiology Institute (MBI), National University of Singapore (NUS), Singapore, Singapore, [2]National University of Singapore Graduate School of Integrative Sciences and Engineering (NGS), NUS, Singapore, Singapore,[3]Institut Jacques Monod (IJM), CNRS UMR 7592 & Université Paris Diderot, Paris, France, [4]Department of Biomedical Engineering, National University of Singapore (NUS), Singapore, Singapore.

Unnecessary or pathological cells are usually removed from the epithelia by a highly controlled process called cell extrusion, which is important for normal tissue development and homeostasis. In the context of programmed cell death, the consensus is that the dying cell receives certain biochemical trigger, and signals to the neighbor cells to help squeeze (extrude) itself out via a acto-myosin purse-string mechanism. Here we show by confining an in vitro MDCK tissue on a micro-patterned surface that the rate of apoptotic cell extrusion increases with cell density, suggesting that mechanical cues are important for extrusion. By measuring tissue dynamics and using pharmaceutical drug perturbations, we further show that the mode of cell extrusion also switches from a more lamellipodia-driven to a more purse-string based mechanism (as commonly known in the literature) when tissue becomes more packed. Altogether, we propose that these two mechanisms work in tandem to ensure the progression of apoptotic cell extrusion under diverse conditions in vivo.

71-POS Board 71

Role of Formin Fhod1 in Epec Pedestal Formation

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Enteropathogenic E. coli are extracellular pathogens that cause dense puncta of polymerized actin at the site of bacterial attachment referred to as 'actin pedestals'. The polymerization of actin in the pedestal is thought to be solely regulated via the Nck-WASp-Arp2/3 pathway. Recent in vivo studies in mice have shown that Nck-deficient mice can still form actin pedestals, suggesting a redundancy in actin polymerization pathways and the possible involvement of multiple actin nucleators. It was therefore of interest to determine whether additional pathways involving the formins might play a role in the formation of actin pedestals in EPEC. We discovered that pedestal surface area was drastically reduced upon treatment with a small molecule formin inhibitor, SMIFH2, indicating a role for formins in the pedestal formation process. When we examined the localization of several classes of formins, we discovered a dense localization of formins FHOD1 and mDia1 at the base of the pedestal. In agreement with our result with formin inhibitors, we also observed that FHOD1 knockdown resulted in a similar size reduction of pedestals and this effect was restored by complementing FHOD1 on a plasmid. We also see interesting differences in the dynamics of Arp2/3 and FHOD1 in the pedestals, such that very large pedestals show a near absence of Arp3 intensity but very high FHOD1 intensity. This suggests a temporal segregation in the activity of Arp 2/3 and formins. Our findings suggest that EPEC uses multiple pathways in the process of pedestal formation and they probably act at different stages of pedestal the formation. We are now trying to understand the bacterial effectors that could be involved in this differential regulation and also its importance in the pathogenesis.

74-POS Board 74

Nuclear Positioning and Its Translation Dynamics is Regulated by Cell Geometry

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The collective activity of several molecular motors and other active processes generate large forces for directional motion within the cell and a background of fluctuating forces. These processes are vital for a multitude of cellular functions such as migration, division and contraction. In addition, they can also influence the transport and positioning of many cellular organelles by affecting their intracellular dynamics. This creates unique biophysical signatures which are altered in many diseases. In this study, we have used the nucleus as a probe particle to understand the micro-rheological properties of the cytoplasm by using micropatterning techniques to confine cells in two structurally and functionally extreme geometries. We find that nuclear positional dynamics is sensitive to the cytoskeletal organization by studying the effect of actin polymerization, nuclear rigidity, and TNFα cytokine stimulation on the position and diffusive behavior of the nucleus. Taken together, our results suggest that mapping nuclear positional dynamics provides important insights into biophysical properties of the cytoplasm. These biophysical signatures could also be used as an ultrasensitive single-cell assay for early disease diagnostics.

77-POS Board 77

Molecular Insights into Division of Single Human Cancer Cells in On-Chip Transparent Microtubes

Wang Xi[1,2,3], Christine K. Schmidt[4], Samuel Sanchez[2,3], David H. Gracias[5], Rafael E. Carazo-Salas[4], Stephen P. Jackson[4,6], Oliver G. Schmidt[2,7,8].
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In vivo, mammalian cells proliferate within 3D environments consisting of numerous microcavities and channels, which contain a variety of chemical and physical cues. External environments often differ between normal and pathological states, such as the unique spatial constraints that metastasizing cancer cells experience as they circulate the vasculature through arterioles and narrow capillaries, where they can divide and acquire elongated cylindrical shapes. While metastatic tumors cause most cancer deaths, factors impacting early cancer cell proliferation inside the vasculature and those that can promote the formation of secondary tumors remain largely unknown. Prior studies investigating confined mitosis have mainly used 2D cell culture systems. Here, we mimic aspects of metastasizing tumor cells dividing inside blood capillaries by investigating single-cell divisions of living human cancer cells, trapped inside 3D rolled-up, transparent nanomembranes. We assess the molecular effects of tubular confinement on key mitotic features, using optical high- and super-resolution microscopy. Our experiments show that tubular confinement affects the morphology and dynamics of the mitotic spindle, chromosome arrangements, and the organization of the cell cortex. Moreover, we reveal that chromosome segregation errors caused by mitosis can happen in both a transformed and non-transformed human cell line in confined circumstances, especially in tubular 3D microenvironments. Collectively, our study demonstrates the potential of rolled-up nanomembranes for gaining molecular insights into key cellular events occurring in tubular 3D microenvironments *in vivo*.

80-POS Board 80

Nanoscale Extracellular Matrix Properties Influence Chemoresistance

Jennifer L. Young, Sascha Klar, Joachim P. Spatz.
Max Planck Institute for Medical Research, Stuttgart, Germany.

Cancer cell-ECM interactions have been shown to positively influence cancer cell survival and invasion by conferring adhesion-based resistance in response to chemotherapeutic drugs and subsequently upregulating pathways driving metastasis. The frequent inability of chemotherapy to provide a long-term, complete cure may be attributed to this specific, adhesion-mediated resistance of malignant cells to chemotherapeutic interventions. Here, we present a platform designed to identify and perturb protective matrix properties on the nanoscale in a high-throughput manner, allowing for the conversion of chemoresistant cells into chemosensitive ones, and subsequently examining these nanoscale properties in combination with mechanical properties of the ECM. Block copolymer micelle nanolithography (BCML) was utilized to create large, scalable arrays of defined ligand presentation at 50 and 75 nm spacing. Breast cancer cell lines were then treated with chemotherapeutic drugs in order to identify the most protective ligand interactions, as determined by cell survival assays and immunofluorescence. Chemoresistance was found to greatly depend on ligand presentation, e.g. laminin is protective at 75 nm spacing but not 50 nm. By utilizing BCML in conjunction with soft polymer transfer nanolithography, the influence of mechanical matrix properties in parallel with ligand presentation was analyzed on 2D substrates and inside 3D microchannels of 125 μm diameter. In both 2D and 3D systems, tumorigenic stiffness hydrogels (5 kPa) were more effective than softer, healthy breast tissue hydrogels (1 kPa) in upregulating cell area, polarity and motility, which have all been hypothesized to play a role in chemoresistance. Additionally, mechanical properties were shown to influence chemosurvival in conjunction with ligand type and spacing, e.g. survival on laminin was enhanced on 1 kPa vs. 5 kPa hydrogels for 50 nm spacing but not 75 nm. These scalable platforms allow for screening several aspects of ECM-conferred chemoresistance in a highly defined manner.

POSTER SESSION III

Thursday, September 29, 16:00 – 18:00
Level 2 Hall Foyer

All posters are available for viewing during all poster sessions; however, below are the formal presentations for Tursday. The presenters listed below are required to remain in front of their poster boards to meet with attendees.

Asano, Yuka	**3-POS**	**Board 3**
Biswas, Kabir	**6-POS**	**Board 6**
Chaudhuri, Parthiv Kant	**9-POS**	**Board 9**
Cho, Youngbin	**12-POS**	**Board 12**
Damodaran, Karthik	**15-POS**	**Board 15**
Das, Alakesh	**18-POS**	**Board 18**
Gan, Wan Jun	**21-POS**	**Board 21**
Ihalainen, Teemu	**24-POS**	**Board 24**
Jalal, Salma	**27-POS**	**Board 27**
Kalidasan, Kamaladasan	**30-POS**	**Board 30**
Kim, Dong-Hwee	**33-POS**	**Board 33**
Koch, Matthias	**36-POS**	**Board 36**
Kuboki, Thasaneeya	**39-POS**	**Board 39**
Le, Anh Phuong	**42-POS**	**Board 42**
Lekka, Malgorzata	**45-POS**	**Board 45**
Maldonado, Amir	**48-POS**	**Board 48**
Mitra, Aninda	**51-POS**	**Board 51**
Nakayama, Yoshitaka	**54-POS**	**Board 54**
Oh, Dongmyung	**57-POS**	**Board 57**
Sun, Wanxin	**60-POS**	**Board 60**
Raiteri, Roberto	**63-POS**	**Board 63**
Victor, Ma	**66-POS**	**Board 66**
Sellers, James	**69-POS**	**Board 69**
Sun, Zijun	**72-POS**	**Board 72**
Wala, Jyoti	**75-POS**	**Board 75**
Xi, Wang	**78-POS**	**Board 78**

Posters should be set up in the morning of September 27 and removed by noon September 30.

3-POS Board 3

Comprehensive Analyses of Gene Expression Patterns in Early Mouse Early Embryos Experiencing Chemical and Mechanical Stimuli

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In vitro mammalian embryonic development can be enhanced by mimicking physiological conditions, which can provide better infertility treatment. By comprehensive analyses of gene expression, we evaluated differences in gene expression patterns of ICR mouse blastocysts cultured *in vitro* from two-cell embryo stage using different media under a static or dynamic culture system to subject the embryos to mechanical stimuli (MS). To compare the influences of the medium on early mouse embryonic development, we used mW medium (mW-S) and KSOM medium (KSOM-S) under static culture. The influences of MS in mW medium were investigated using the tilting embryo culture system (TECS), which improves blastocyst development. We compared the gene expression patterns between the TECS (mW-T) and mW-S groups. Blastocyst development rates in the mW-S, KSOM-S, and mW-T groups were 62.6%, 94.1%, and 70.8%, respectively ($P < 0.01$). We extracted total RNAs thrice from the three groups, and the total number of blastocysts was approximately $80 \times 9 = 720$. RNA amplification, RNA-Seq library preparation, and RNA sequencing were consigned to Hokkaido System Science Co. Ltd. Upon comparison of the relative gene expression levels between the KSOM-S and mW-S groups, the gene expression level in the trophectoderm (TE), which functions as a source of embryonic nutrition, was improved in culture conditions under KSOM medium compared with that in culture conditions under mW medium. *Tagln2* gene expression as a TE marker increased ($P < 0.01$) under KSOM culture. *Gdf3* gene expression levels related to epiblast (EPI) development in the mW-T group was higher than that in the mW-S group ($P < 0.01$). MS improved EPI development under the same medium conditions. The expression patterns of EPI and TE markers differed between the culture systems with chemical stimuli by medium and MS. We consider that optimized medium components can improve both TE and EPI development and that MS can improve the gene expression of EPI markers. However, the gene expression level induced by MS would be relatively lower in TE than in EPI.

6-POS Board 6

Sustained α-catenin Conformational Activation at E-cadherin Junctions in the Absence of Mechanical Force

Kabir Biswas[1], Kevin L. Hartman[1], Ronen Zaidel-Bar[1,3], Jay T. Groves[1,2,4].
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Mechanotransduction at E-cadherin junctions has been postulated to be mediated, in part, by a force-dependent conformational activation of α-catenin. Activation of α-catenin allows it to interact with vinculin, in addition to F-actin, resulting in strengthening of junctions. Here, using E-cadherin adhesions reconstituted on synthetic, nanopatterned membranes, we show that activation of α-catenin is dependent on E-cadherin clustering, and is sustained in the absence of mechanical force or association with F-actin or vinculin. Adhesions are formed by filopodia-mediated nucleation and micron-scale assembly of E-cadherin clusters, which could be distinguished as either peripheral or central depending on their relative location at the cell-bilayer adhesion. While F-actin, vinculin and phosphorylated myosin light chain associate only with the peripheral assemblies, activated α-catenin is present in both peripheral and central assemblies, and persisted in the central assemblies in the absence of actomyosin tension. Impeding filopodia-mediated nucleation and micron-scale assembly of E-cadherin adhesion complexes, by confining bilayer bound E-cadherin extracellular domain movement on nanopatterned substrates, reduced levels of activated α-catenin. Taken together, although the initial activation of α-catenin requires micron-scale clustering that may allow development of mechanical forces, sustained force is not required for maintaining α-catenin in the active state.

9-POS Board 9

Topography Induces Differential Sensitivity on Cancer Cell Proliferation

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Cancer is presently one of the topmost non-communicable diseases and the microenvironment that surrounds the cancerous cells plays a critical role in determining the metastatic ability of the malignant cells. During the progression of breast cancer, the extracellular matrix (ECM) fibers that surrounds the cancer cells align themselves in a parallel orientation and this helps in the migration of the tumor cells away from the primary tumor. Since cancer cells also possess uncontrolled proliferation ability, here we questioned whether such changes in the ECM topography during tumor progression could also affect the proliferation efficiency of the cancer cells. To answer this question, we used micro fabrication tools to mimic such topographic features *in-vitro* by fabricating micrograting of specific dimensions. We discovered the existence of a Mechanically Induced Dormancy (MID) where the anisotropic topographical cues provided by micrograting could reduce normal healthy cell proliferation; however, the cancer cells could successfully overcome this mechanical barrier and continue uncontrolled proliferation. Further research to understand the molecular mechanism of proliferation reduction led to the identification of higher Rho-ROCK-Myosin based contractility in normal cells that generates mechanical cues to prevent their proliferation. This study reveals a novel mechanism by which normal cells could sense external mechanical cues and restrict their proliferation in a microenvironment that promotes tumor progression.

12-POS Board 12

Physical Characterization of Cellular Transition During Epithelial Monolayer Expansion

Youngbin Cho[1], Bomi Gweon[2], Jacob Notbohm[3], Ung Hyun Ko[1], Hwanseok Jang[4], Yongdoo Park[4], Jennifer H. Shin[1].
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During regeneration, metastasis, and morphogenesis, cells often migrate as a pack in a collective manner in vivo. Within the cell monolayer, the cellular motion is regulated by the forces from their surroundings through physical adhesions to the substrate and the neighboring cells. In highly packed cells, a jammed state, cells show limited intercellular motion due to the stable cell-cell junctions while the cells display relatively unrestrained motion in an unjammed state. When the cells are patterned as a monolayer island, the monolayer expansion accompanies EMT-like phenotypic cell transition, from the migrating edge. Such cellular transition involves dynamic remodeling of physical junctions and stresses. Recent researchers have studied the key molecules and mechanisms regulating the transition of cellular phenotypes but the dynamic correlation between the key junction molecules and physical force redistribution are not fully understood yet. Here, we aimed to understand the correlation between physical stresses and cellular components. We visualized the physical force distribution within the patterned epithelial monolayer using traction force microscopy (TFM) and monolayer stress microscopy (MSM). Real-time force measurement by TFM and MSM revealed the two distinct groups of cells in the monolayer; the 3-4 cell layers' width of the mesenchymal-like band around the monolayer edge and epithelial-like region at the core. Cells in the mesenchymal band feature the developed lamellipodia and high inward traction with fast migration while cells at the core maintain tight cell-cell junctions with limited migration speed. Furthermore, our dynamic adhesion analysis revealed the existence of an intermediate single-celled layer balancing between mesenchymal band and epithelial region, with no biased physical polarity.

15-POS Board 15

Compressive Force Induces Chromatin Compaction in a Reversible Manner by Reducing Actomyosin Contractility and Shuttling HDAC3 to the Nucleus.

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Fibroblasts, one of the major type of cells in connective tissue, experience compressive force (CF) in its local microenvironment. These CFs influence the behavior of fibroblasts by regulating their genomic programs. The genomic programs in cells could be regulated by altering their chromatin compaction states. How CF alters chromatin compaction states in fibroblasts is not well understood. In this study, we show that CF on geometrically well defined mouse fibroblast cells reduces actomyosin contractility and shuttles Histone Deacetylase 3 (HDAC3), into the nucleus. HDAC3 then triggers increase in heterochromatin content by initiating the first step of removing acetylation marks on the histone tails in the euchromatin region. These histone tails are then further modified and recruited into the heterochromatin. In addition to increase in heterochromatin content, there is also enrichment of Lamin A/C at the nuclear periphery. This suggests that, in response to CF, fibroblasts stiffen their nucleus and enter into quiescent state, which is also reflected at the transcription level. This process was found to be reversible suggesting an existence of structural memory in these fibroblasts. This study shows how fibroblasts maintain their homeostasis in response to CF, failing which, has major implications in pathological conditions.

18-POS Board 18

Nuclear and Cellular Softening is Essential for Invasive Cancer Cells to Migrate and Metastasize

Alakesh Das, Sandeep Kumar, Shamik Sen.
IIT Bombay, Mumbai, Maharashtra, India.

Cancer cells metastasis is a multistep complicated process in which cellular biophysical modifications plays one of the very crucial role. During mesenchymal to amoeboidal transition cancer cells undergo certain physical deformations which allows them to migrate through dense 3D matrices. However, it is still not clear how cells induce these modifications and what is the molecular mechanism behind this transformations. Here in our work using highly invasive MDA-MB-231 and HT-1080 cells and less invasive MCF-7 cells we have first demonstrated the existence of an integrin-mediated bi-directional crosstak between MMPs and actomyosin contractility. Further, we have shown that GM6001 treatment induces cellular and nuclear softening. To probe the functional relevance of this biophysical alteration we performed sandwich gel 3D invasion assay and observed that GM6001 treated cells were migration as efficiently as untreated cells. However, addition of divalent cations reversed this softening effects and cells were stuck in their migratory tracks. Further using computational approach we provided the conclusion that both cellular and nuclear plasticity is essential for cells to undergo migration through tight and narrow interfaces.

21-POS Board 21

Control of Insulin Secretion by Basement Membrane Proteins

Wan Jun Gan[1], Elena Kosobrodova[2], Marcela Bilek[2], Peter Thorn[1].
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Cell-based therapies such as islet transplantation or engineering of stem cells, to restore insulin secretion, are promising treatments for type-1 diabetic patients. However, these cells typically secrete less insulin than native beta cells. Our recent work, in intact islets, shows that beta cells are structurally polarised and that insulin secretion is targeted towards the vasculature. This led us to hypothesize that correct orientation of secretory machinery may be important in normal glucose-induced response. We are currently investigating whether signals from the vascular basement membrane provide cues for the establishment and maintenance of beta cell orientation.

We isolate mouse islets and then break them down into single cells. Immunostaining for insulin shows that 85% of the isolated cells are beta cells. When these cells are cultured on gelatin (denatured collagen) coated coverslips, focal adhesion proteins such as talin and synaptic proteins, such as liprin, are selectively enriched at the interface of the beta cells with the coverslip. To investigate secretory function, we use live-cell two photon imaging to identify each insulin granule as it fuses with the cell membrane, in response to 15 mM glucose stimulus. Sequential Z stack imaging (2um/steps, 6 steps/stack), through the cells, enables us to determine the 3D distribution of fusion events. Our data show that 45% of granules fuse in the limited area of the membrane-coverslip interface, indicating enrichment of fusion. Currently experiments are underway to understand the mechanisms that orientate the beta cells and targeting secretion. To this end we are printing micropatterns, of different basement membrane proteins, using plasma immersion ion implantation treated polystyrene to covalently immobilize dense monolayers of protein directly from PDMS stamps.

In conclusion, evidence indicates that beta cells are specifically orientated in the islets and this affects their structure and function.

24-POS Board 24

Mechanical Tension Regulates C-Terminus Accessibility of Lamin A/C

Teemu O. Ihalainen[1,2], Lina Aires[2], Florian A. Herzog[2], Ruth Schwartlander[2], Jens Moeller[2], Viola Vogel[2].
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Cells exploit traction forces to sense the physical characteristics of their microenvironments, which co-regulates a variety of cellular processes. Mechanical forces, rising from the extracellular environment or from the contractile cell cytoskeleton, can be transmitted to the nucleus as the cytoskeleton physically couples the cell periphery to the nuclear envelope. Nuclear lamina plays a key role in the nuclear mechanotransduction, since it resides in the interface between the cytoplasm and the inner nucleus. The nuclear lamina is composed of a 10–60 nm thick protein layer beneath the inner nuclear membrane and its major constituents are A- and B-type lamins. A-type lamins (lamin A, AΔ10, C) are splice variants of a single gene, LMNA, whereas B-type lamins are encoded from two different genes LMNB1 (lamin B1) and LMNB2(lamin B2, B3). Lamins are involved in the regulation of gene expression and control the mechanical stability of the nucleus. However, relatively little is known about mechanoregulation and mechanosensitivity of lamin proteins

Here, we show that at least two N- and C-terminal regions of lamin A/C are not accessible at the basal side of the nuclear envelope under environmental conditions known to upregulate cell contractility. This structural polarization of the lamina is promoted by compressive forces, emerges during cell spreading, and requires lamin A/C multimerization, intact nucleoskeleton–cytoskeleton linkages (LINC), and apical actin-cap assembly. Notably, the identified region in the C-terminus of lamin A/C overlaps with emerin, DNA and histone binding sites, and contains various laminopathy mutation sites.

Our findings help to decipher how the physical properties of cellular microenvironments can regulate nuclear events and gene expression via altered lamin A/C structure.

27-POS Board 27

Cytoskeleton Self-organization in Epithelial Cells

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Cytoskeleton self-organization and reorganization is necessary to enable fundamental cellular processes such as extracellular adhesion to matrix and/or other cells, polarization, as well as migration. Remodelling of the actin cytoskeleton is known to play a key role in regulating the process of epithelial-messenchymal transition (EMT), where epithelial cells switch to a more fibroblast-like phenotype and can participate in metastatic cancer progression. However, the current understanding of how actin reorganization regulates EMT is limited by concurrent changes to cell-cell adhesions and cell morphology present in the colony assays used in most studies of the process.

In order to remove the influence of cell-cell adhesions and cell shape changes from our observation of actin dynamics, we use long-term live imaging to observe epithelial cells maintained in standardized conditions. Single cells were geometrically confined on circular matrix or cadherin coated islands. We first compared actin self-organization in keratinocytes (basal epithelial cells) to our prior understanding from fibroblasts, and next look at it in bladder carcinoma cells (simple epithelial cells) before and after growth factor induced EMT.

Preliminary studies show clear differences between the self-organization of actin in keratinocytes when compared to fibroblasts, as well as in the carcinoma cell line before and after EMT. In keratinocytes the actin cytoskeleton self-organizes into a symmetric radial pattern that:-
• Depends on spread area.
• Is not significantly changed upon attachment to cadherin adhesions instead of focal adhesions.
• Does not break symmetry. This is in stark contrast to fibroblasts, where actin self-organizes into an initially symmetric pattern that subsequently becomes asymmetric.
In bladder carcinoma cells plated on fibronectin islands, the actin cytoskeleton organizes into circumferential bundles, membrane protrusions, ventral stress fibres and contractile geodesic-like structures. After EMT induction we have seen radial fibre like structures begin to appear with more prominent geodesic-like structures.

30-POS Board 30

Circular Dorsal Ruffles: A Mode of Steering Cells

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Circular Dorsal Ruffles (CDRs) are actin rich, ring-shaped structures that form across the dorsal surface of fibroblasts upon PDGF stimulation. CDRs have been nominated to play a role in preparing a stationary cell for movement via reorganisation of the actin cytoskeleton and receptor internalisation. When POPX2, a serine/threonine phosphatase known to be upregulated in highly invasive breast cancer cell lines such as MDA-MB-231, is stably expressed in fibroblasts, perpetual CDRs are observed even without PDGF stimulation. Interestingly, it has been shown that POPX2 expression results in loss of directional migration when compared to control fibroblasts. Herein, we investigated the possible link between CDRs and directional migration in stably POPX2 expressing and control fibroblasts. We adopted a strategy that allowed us to track cells on 1D micropatterned substrates, thereby restricting their movement to either a leftward or rightward direction. We then adopted a computational approach to analyse the directional migration persistence in control and stably POPX2 expressing fibroblasts that either exhibited or never exhibited CDRs. We found fibroblasts that were exhibiting CDRs, showing increased directional migration persistence. This suggests that CDRs may have a role in directional migration and may serve as a corrective mechanism in aberrantly migrating cells.

33-POS Board 33

Protection of Nuclear Morphology by the Lamin A/C-Mediated Organization of the Perinuclear Actin Cables

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Recent studies have shown a pronounced correlation between defects in nuclear morphology and the progression of diverse human diseases such as laminopathies, a set of diseases induced by mutations in the LMNA gene encoding nuclear lamin A/C. The perinuclear actin cables, simply actin cap, composed of highly contractile actomyosin lament bundles on top of the nucleus is bridged to lamin A/C of the nuclear lamina through LINC complexes. The distinct spatial organization of the actin cap facilitates rapid communication between extracellular physical stimuli and intracellular responses, including nuclear shaping and mechanotransduction of external forces into biophysical signals. These functions are abrogated in lamin A/C-decient mouse embryonic broblasts, a mouse model of the laminopathies, following disruption of the actin cap. However, how lamin A/C mediates the ability of the actin cap to regulate nuclear morphology in response to external mechanical stimuli remains unclear. Here, we develop a computational model to understand physical interactions between the cytoskeletal network and the nucleus in cellular mechano-responses, where each cellular component is analyzed discretely in a three-dimensional nite element model. The model explores the role of actin-cap-mediated mechanotransduction in maintaining nuclear morphology. Here, we show that lamin A/C harnesses the actin cap to protect nuclear morphology from extracellular physical disturbances.

36-POS Board 36

Force Transmission through the Microtubule Cytoskeleton

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The eukaryotic cytoskeleton is a complex and dynamic network that regulates important cellular functions and is driven by a large variety of forces or mechanical stimuli. Due to their mechanical rigidity, microtubules are able to transport such stimuli, which allows integrating distant regions of a cell nearly instantaneously. This is relevant for the response to pressure, gravity, or osmotic changes and during mechanotransduction, a critical process during many severe diseases such as deafness or cancer.
So far, only equilibrium mechanical properties of single microtubules have been characterized. Since intra- and extracellular forces occur on a brought range of time scales, we fill this void by using an in vitro bottom-up approach to determine the frequency response of single microtubules and small networks thereof that mimic the basic cytoskeletal structure. We combine a label-free darkfield imaging technique with multiple time-shared optical tweezers to flexibly construct and force-probe such networks with a well-defined, user-selected geometry over a broad frequency range.
We report on a length dependent stiffening of individual microtubules above a physiologically relevant transition frequency between 1–30Hz due to the excitation of higher order bending modes. This increased transport efficiency for high frequencies can be regarded as a mechanical high-pass filter with a tunable cutoff frequency, e.g., allowing the cell to react to rapid fluctuations at distant sites. Furthermore, we identify and relate different mechanical responses for different network geometries to different functions inside the cell. Triangular networks, for example, display a comparatively high stiffness even for low frequencies and resemble a load bearing scaffold protecting the nucleus. The mechanistic comparison between basic network geometries, the known cytoskeletal topologies and the general function of different cell lines will substantially strengthen our understanding of the function and structure of the cytoskeleton, both during health and disease.

39-POS Board 39

Mechanotransduction and Redox Signaling in Stem Cells

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Reactive oxygen species (ROS), especially H2O2 that mainly generated from NADPH oxidase (NOX) from mitochondria could function as a second messenger in redox signaling. Oxidative stress, the excess or imbalance of ROS and the antioxidant system of the cells, plays an important role in several pathological diseases such as atherosclerosis, cancer neurodegeneration and aging. Increasing evidences indicated the correlation of mechanical stimuli, redox signaling and pathological diseases. In tissue fibrosis, the NOX 4 in pulmonary fibroblasts was up-regulated. Overexpression of antioxidant enzymes or decreasing ROS provided protective effect whereas the redox imbalance enhanced fibrosis. The mammary epithelial cells on soft polyacrylamide gel decreased the ROS production with inhibition of NOX assembly.
Our study focuses on an investigation of the interplay between mechanotransduction and redox signaling using stem cell as a model study. Emerging evidence suggests that the balance between ROS and antioxidant enzymes could regulate stem cell fate, function and survival. The surface elasticity tunable hydrogel for cell manipulation was fabricated using photocurable styrenated gelatin. We observed the changes in expression of antioxidant enzymes in stem cells on various gel stiffness such as the up-regulation of superoxide dismutase (SOD) and glutathione S-transferase (GST) via proteomic analysis. In a recent study, stem cells cultured on the soft gelatinous gel developed neuron-like shape, increased expression of neurogenic markers and changed the expression of SOD, GST and other redox related genes such as thioredoxin (TRX) and peroxiredoxin (PRX). The cellular redox state such as the NOX/ROS level and antioxidant activity of the cells on various elasticity conditions are now being investigated. Understanding the relationship between mechanotransduction and redox homeostasis of the cells would provide significant implication in cell physiology including the mechanism underlying the development of pathological diseases.

42-POS Board 42

The Mechanotransduction Role of Cell-Cell Junction in Cell Extrusion Context – An Alpha-Catenin Study

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The active role of cell-cell junction (CCJ) during epithelia extrusion has not been extensively discusses in the literature despite a plethora of research on its role in maintaining epithelial integrity. At CCJ, alpha-Catenin (a-Cat) plays a key role as a mechanotransducer at adherent junctions of epithelial cells. In the cadherin adherent complex, a-Cat can interact with F-Actin or via vinculin in a mechano-reponsive manner. Such tension-dependent vinculin recruitment process involves biphasic transition of a-Cat structure from the weak-binding state to the stabilized-binding state to F-actin, and hence, stabilizes the adherent junction. Here we show that by changing CCJ strength via manipulating different forms of a-Cat mutants, the extrusion rate, global dynamics and local traction changes were observed in MDCK monolayer. We transfected two forms of a-Cat mutants into MDCK cells with a-Cat knock-down background and observed the cell extrusion from the confluent monolayer for 24-48 hours. The extrusion rate was decreased and the duration was prolonged in the mutant that binds to vinculin constitutively and vice versa, there was increased rate and duration for the mutant with deficiency of vinculin-binding domain compared to both WT and MDCK with a-Cat knock-down with rescued WT a-cat. Moreover, re-localization of traction force tissue exerted on the substrate was observed with a-Cat mutants, implying different mechanism surrounding extrusion site with different CCJ strength. Instant drop of traction followed by recovery from neighboring cells with weakened CCJ suggests a lamellipodia-forming mechanism to help extrude the cells and close the gaps. Overall, our experimental results demonstrate that cell-cell adhesion via the stabilization of cadherin-catenin-vinculin complex regulates epithelial homeostasis.

45-POS Board 45

Mechanosensitivity of Human Bladder and Prostate Cancerous Cells

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Altered mechanical properties of microenvironment surrounding cells influence various processes such as cellular differentiation, migration, proliferation, and also cell-cell and/or cell-ECM adhesion [1]. There is much evidence showing that on hydrogels substrates, mimicking viscoelastic properties of ECM, cellular response of normal and stem cells is stiffness-depended. In most cases, on highly rigid hydrogels, cells spread extensively, form prominent stress fibres and mature focal adhesions [2]. Understanding the relation between ECM mechanics and cellular response is particularly important in the context of cancer progression, which is typically associated with alterations in rigidity due to local accumulation of a dense, crosslinked proteins network [3]. With the development of new techniques that enable to probe elastic properties of single cells, it is now possible to identify and detect a single, mechanically altered cell [4]. Using this approach, it has been shown that cancerous cells are mostly more deformable (i.e. they are softer) [5]. Simultaneously, cellular elasticity can be used to monitor changes occurring in response to interactions with ECM components and also with neighbouring cells. In our studies, we have focused on mechanosensitive properties of human bladder and prostate cancerous cells originating from various stages of cancer progression. The obtained results showed distinct cellular responses depending on the actin organization, type of ligand and the presence of neighbouring cells. Our results relates changes in cancer cell biomechanics (elasticity) with cellular morphology and metastatic phenotype [6].

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48-POS Board 48

Paclitaxel Affects the Time-Dependent Response of Peripheral Nerves to Uniaxial Tensile Loading in Vitro

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Paclitaxel is a common chemotherapeutic drug for the treatment of cancer; it prevents cell division by stabilizing microtubules and preventing their depolymerization. The systemic delivery of Paclitaxel causes it to accumulate in peripheral nerves leading to debilitating peripheral sensory neuropathy. The mechanisms for abnormal microtubule aggregation and neuropathy after paclitaxel treatment remain unknown. Studies suggest that alterations to microtubules can influence tensile axonal biomechanics and affect nerve function. We hypothesized that changes in the biomechanical properties of peripheral nerves may contribute to paclitaxel-induced neuropathy. In this work we explored the biomechanical changes caused by paclitaxel in mice sural nerves, sensory branches of sciatic nerves, during tensile loading and stress relaxation. Paired sural nerves from each mouse were excised and soaked in Ringer's solution, either with or without paclitaxel. Nerves were secured between a linear actuator and a force transducer. Then, tissues were subjected to uniaxial tensile loading with a relaxation of 180 s per strain. We obtained the tensile moduli from stress vs. strain curves during equilibrium and analyzed the stress relaxation behavior with a viscoelastic model for nerves with and without paclitaxel. Our results suggest that paclitaxel stiffens nerves and reduces their stress relaxation behavior, which can be correlated with the stabilization of microtubules within axons. These data suggest that the response to strain and the time-dependent rate at which nerves can recover is affected by paclitaxel treatment and might contribute to neuropathic progression.

51-POS Board 51

Cell-Geometric-Constraints Differentially Regulate Tnfα-Mediated Gene Expression Programs

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Cells in physiology integrate local soluble and mechanical signals to regulate gene expression programs. While the individual roles of these signals on nuclear mechanotransduction are well studied, the cellular responses to the combined chemical and physical signals are less explored. Here, we investigate the crosstalk between cellular geometric-constraints and its integration to TNFα signaling. We stabilize NIH3T3 fibroblasts in polarized or isotropic states and stimulate them with TNFα to analyze NFκB(p65) nuclear localization and its downstream target gene-expression. We find that cell mechanics influences TNFα-induced actin-depolymerization which enhances IκB degradation and p65 nuclear translocation and sequestration of RNA-polymerase-II foci with p65. This also affects nuclear exit of transcription co-factor MKL in polarized cells. Further, global transcription profile of cells under matrix-TNFα interplay, reveal a geometry-dependent genetic response. Our results provide compelling evidence that cells, depending on their mechanical states, elicit distinct cellular responses for the same cytokine.

54-POS Board 54

Magnetic Control of the Gating of Bacterial Mechanosensitive Channel Mscl for Smart Liposomes

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Liposomal drug delivery systems (LDDSs) are effective tools used for the treatment of diseases especially when highly toxic pharmacological agents are utilized. In order to regulate the drug release from liposomal capsules, remotely controlled nanovalves with a large conductive pore are required. The bacterial mechanosensitive channel of large conductance, MscL, presents an excellent candidate biomolecule for the LDDSs. In this study, we developed superparamagnetic nanoparticles for the activation of the MscL nanovalves by magnetic field. Synthesised $CoFe_2O_4$ nanoparticles with the radius of less than 10 nm were labelled by SH-reactive chemical groups for attachment to M42C MscL mutant. Activation of MscL by magnetic field with the nanoparticles attached was examined by the patch clamp technique showing that the number of activated channels under ramp pressure increased upon application of the magnetic field. In parallel, we have tested and have not observed any cytotoxicity of the nanoparticles in human cultured cells. Our study suggests the possibility of using magnetic nanoparticles as a specific trigger for activation of MscL nanovalves for drug release in LDDSs.

57-POS Board 57

Competition for Grb2 Recruitment between Epha2 and EGFR during Ligand Activation

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Activation of EphA2 and EGFR receptor tyrosine kinases (RTKs) is initiated immediately after binding of their respective ligands, recruiting a variety of downstream signaling proteins and ultimately triggering a diverse range of biological outcomes. Although EphA2 and EGFR respond to distinct ligands (ephrinA1 and EGF, respectively), and trigger distinct responses, they also share key proximal signaling molecules. One such molecule is Grb2, which is an adaptor protein recruited to phosphorylated tyrosine residues and responsible for the recruitment of the Ras activator, SOS. How such receptor triggered signaling activities retain the identity of the triggering receptor and how (or if) different receptors may synergize or compete remains largely unknown. Here monitor Grb2 recruitment to ligand activated receptors in a live cell system in which EphA2 and EGFR are spatially segregated, thus allowing unambiguous distinction of which receptor signaling complex each Grb2 molecule is binding. Results reveal a competitive effect by which one receptor type is able to influence the signaling activity of the other remotely. Detailed analysis of Grb2 membrane recruitment kinetics reveals distinct differences between Grb2 recruitment to activated EphA2 clusters and clusters of activated EGFR. Consequences of this type of molecular competition for adaptor proteins in the overall context of signal transduction will be discussed.

60-POS Board 60

Nanoscale Studies of Cell Mechanics with Atomic Force Microscopy

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Cell biology has seen a surge in mechanobiology-related research directed towards understanding how cells exert and respond to forces. Examining the effects of these forces has a wide-range of applications from understanding disease pathology to tissue engineering. Atomic force microscopy (AFM) not only allows direct examination of the nanoscale structure of cell membrane surfaces, it also provides unique opportunities to measure the nanomechanical properties of cells. In this study we used a combination of AFM force spectroscopy-based modes to investigate both the elastic and viscoelastic behavior of living cells. Using PeakForce QNM mode, we were able to map the modulus across individual mammalian cells. These 2D spatial maps provide both high-resolution and quantitative measurements of cell elasticity. In other PeakForce QNM studies, we successfully detected modulus variations that occur during division of E. coli cells. By integrating PeakForce QNM with fluorescence microscopy we were also able to demonstrate a correlation between changes in elasticity and bacteria viability. However, to fully understand cell mechanics, one must also consider cell response over a wide range of timescales. We used advanced AFM force spectroscopy to examine the creep response of living cells. Through integration with fluorescence microscopy, we also attempted to correlate variations in these properties to subcellular structures with nanoscale resolution. With regards to studying dynamic processes involved with cell mechanics, traditional AFM has been restricted due to the relatively longer image acquisition times. With recent advances in high-speed AFM, we have begun to apply a unique combination of high-resolution and high-speed AFM imaging to investigate the mechanics of cell migration. We were able to directly observe the formation and advancement of individual lamellipodia and fillipodia on living stem cells, as well as reorganization events within the actin cytoskeleton during migration.

63-POS Board 63

Atomic Force Microscopy as Tool to Selectively Investigate the Mehcanical Properties of Different Components of Cytoskeleton in Muscle Fibres in Vitro

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Atomic force microscopy (AFM) allows to measure the transversal stiffness of the sub-sarcolemma region of cells with sub-micrometer lateral and vertical resolution. By AFM nanoindentation measurements we investigated the changes in stiffness of isolated skeletal muscle fibres induced by acute doses of different substances capable to selectively induce changes in the organization of microtubules and intermediate filaments, namely colchicine, taxol, parthenolide, and Withaferin A. Our results confirm that AFM nanoindentation performed at different penetration depths allows to selectively and quantitatively probe the response of different regions of the sarcolemma localized in the first few hundreds of nanometers below the sarcolemma. Such capability represents a powerful tool, complementary to other in vitro and in vivo techniques, to investigate the mechano-transduction at the basis of generation and progression muscle pathologies such as dystrophies.

66-POS Board 66

Nanoscale Optomechanical Actuators for Controlling Mechanotransduction in Living Cells

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Optical approaches for the controlling biological systems are transforming the field of cell biology, as exemplified by caged or photoswitchable molecules and by optogenetic constructs. Similarly, methods to harness light for delivering precise physical inputs to biological systems could potentially transform the study of mechanotransduction. Toward this goal, I will describe our efforts aimed at developing optomechanical actuator nanoparticles to manipulate receptor mechanics with high spatiotemporal resolution using near-infrared illumination (Nature Methods 2016). Nanoparticles are comprised of a gold nanorod coated with a thermoresponsive polymer shell. Illumination leads to local heating, and particle collapse, thus delivering piconewton forces to specific cell surface receptors with high spatial (~micron scale) and temporal resolution (msec timescales). Optomechanical actuators were used to exert forces through the integrin receptors, thus mechanically controlling focal adhesion formation, cell protrusion, and cell migration in living cells. This new approach to controlling mechanotransduction circuits allows for optically controlling cell migration without the use of genetic engineering.

69-POS Board 69

Studying Nonmuscle Myosin-2 Function in Cells: Not as Easy as It Seems!

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We investigate nonmuscle myosin-2 (NM2) filaments using in-vitro approaches to inform studies of myosin function in cells. Here we detail some complexities of studying the function of NM2 in cells. There are three NM2 paralogs which form short bipolar filaments. These myosins co-assemble with each other to form heteropolymeric filaments. A structurally related myosin, myosin-18A, is an enzymatically inactive pseudo-enzyme which also co-polymerizes with NM2. NM2B and NM2C heavy chains are alternatively spliced, giving rise to molecules with altered enzymatic and regulatory features. There are three regulatory light chains (RLC) and two essential light chains that can associate with these myosins. The RLCs are phosphorylated at two the myosin may interact with beta- or gamma-actin which might affect its enzymatic porperties and these actin filaments might be complexed with a variety of tropomyosin isoforms which also differentially affect the activity. Thus one myosin filament may have very different mechanical properties from another seemingly identical filament in the same cell. Overexpression of GFP-RLC which dynamically exchanges into myosin is used for live cell imaging. However RLC binds to at least four myosin classes and fusion of GFP to RLC lowers the enzymatic activity by 50%. Use of phospho-mimetic RLC is not recommended since these mutants do not mimic phosphorylation of S19. Use of disease-associated myosin mutations for "rescue" experiments is also not advised since some of the defects may be related to protein folding which could vary with differing cell types.

72-POS Board 72

Basolateral Protrusion and Apical Contraction Cooperatively Drive Drosophila Germband Elongation

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Robust tissue morphological changes require an integration of cellular mechanics that evolve over time and in three dimensional space. During animal axis elongation, cell intercalation is the key mechanism for tissue convergent extension1, and it is known that the associated polarized apical junction remodelling is driven by myosin-dependent contraction during Drosophila germband extension2-4. However, the contribution of the basolateral cellular mechanics remains poorly understood. Here, we characterize how cells coordinate their shape and movement from the apical to the basal side during rosette pattern formation, a hallmark of cell intercalation. We reveal that there are distinct apical and basolateral mechanisms required for intercalation. As previously reported, the contraction of actomyosin cables formed in a subset of cells (the anterior/posterior (A/P) cells) drives apical rosette formation4. In contrast, basolateral rosette formation is driven by cells mostly located at the dorsal/ventral (D/V) part of the cluster (D/V cells). These cells exhibit wedge-shaped basolateral protrusions and migrate towards each other along the D/V axis. Surprisingly, we find that the formation of basolateral rosettes precedes that of the apical rosettes. Impeding the apical acto-myosin contractility does not prevent the resolution of rosettes on the basal side. This indicates that the establishment of basal rosettes is independent of apical contractility. Instead, by selectively blocking Rac activity, we show that Rac-dependent protrusive motility is required for basal rosette formation. Furthermore, by using an RNAi screen we identify a component regulating basal rosette formation, the inhibition of which leads to abnormal basal intercalation but retains apical rosette formation and eventually results in delayed extension. Our data show that in addition to apical contraction, active cell migration driven by basolateral protrusions plays a pivotal role in body axis elongation.

75-POS Board 75

Effect of Physico-Mechanical Properties of PDMS Substrate on Cell Behavior

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Mechanical properties of cells and associated extracellular matrix play a critical role in several microbiological phenomena such as cell migration, division, differentiation, contraction, wound healing, and cancer propogation etc. Alteration in physico-mechano properties of scaffold helps to understand the changes in bio-mechano-physical properties of cells which could be used as mechanical signature to differentiate cancer and normal cells. In this study, four different Polydimethylsiloxane (PDMS) compositions by varying their base and cross-linker ratios (w/w) (5:1, 10:1, 15:1 and 20:1) were considered as substrates and subsequently, modifying the surface via oxygen plasma for cell culture process. The stiffness and elasticity of substrates increased with increasing base to curing agent ratios for both treated and pristine substrates. The oxygen plasma treated substrates demonstrated hydrophilic surface as confirmed by contact angle and FTIR analysis and AFM analysis showed decrease in roughness for treated substrates. Various substrates compositions and modification technique were used collectively to estimate cellular mechanics. In the present study, HaCaT and 3T3 fibroblast cells were grown over the fabricated substrates. The biological behavior mainly cellular morphology and growth rate were analyzed by SEM and MTT assay. The plasma treated substrates with different compositions showed higher proliferation and spreaded morphology of keratinocytes and fibroblast as compared to pristine substrates and growth characteristic of cells differ for different cells and compositions. However, among the treated scaffolds, composition having 15:1 ratio demonstrated the highest cellular growth rate for HaCaT and 5:1 for 3T3 which may be due to change in mechanical stiffness of substrate. Therefore, different surface modified substrates with enhanced bio-physical properties may be explored for better understanding of differential behavior of normal and cancer cells.

78-POS Board 78

Curvature Effect on Monolayer Cell Migration

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In vivo cell sheets constitute a variety of curved architectures such as lung alveoli, kidney nephron and breast acini. Notably, during certain organ development and wound healing processes, cell monolayers often advanced on a curved surface. While a considerable amount of effort has been made to study monolayer cell migration, most of the studies have primarily used 2D-cell culture systems, which fail to recapitulate the complex geometric nature of tissues and organs. The mechanistic basis of cell behavior on a 3D curvature also remains poorly understood. Here, we investigate monolayer cell migration on various curved surfaces. We study intra-cellular arrangements, cell polarity and cell sheet velocity. Our findings reveal interesting insights into migration of cell monolayer in response to extent of curvature and can assist in better understanding tissue morphogenesis.