

MECHANOBIOLOGY IN HEALTH AND DISEASE

31st May 2018

Creation Theatre / Level 4 Matrix
Biopolis / Singapore

SYMPOSIUM PROGRAMME

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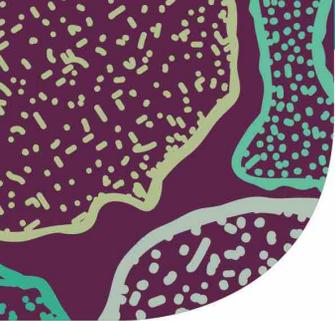
Institute of Medical Biology / A*STAR
Mechanobiology Institute / NUS

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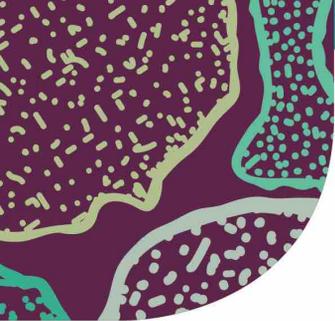
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Welcome Letter

Dear Conference Participants.

It is the great pleasure of the organising committee to welcome you to the 1st Mechanobiology in Health and Disease Symposium, in Biopolis, Singapore.

The symposium is brought to you by the Mechanobiology Institute and the Institute of Medical Biology, to foster collaborations between scientific institutes in Singapore. The symposium has been designed to ignite collaboration, and provide an innovative and comprehensive overview of the latest research developments centred around mechanobiology in health and disease.

The program includes 8 invited talks by group heads and 2 facility talks from researchers associated with MBI and IMB. Additionally, we have selected 12 early career researcher talks from institutes around Singapore. We will finish the event with a networking session, supported by a generous wine donation from David Lane, which we hope you will all attend to discuss and solidify future collaborations.

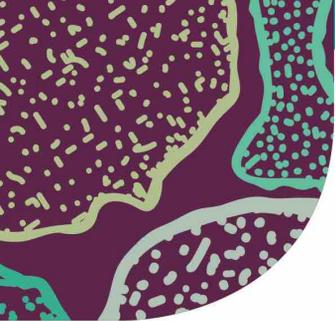
We would also like to express our thanks to our industry sponsors for their generous support, to the ASCB for providing a registration portal and funding, and to the directors Mike Sheetz , Brigit Lane, and Zee Upton for their help, support and advice in planning and arranging this meeting.

We hope that you will enjoy the symposium, and that your interaction with researchers from institutes around Singapore will stimulate a creative exchange of ideas and will be personally rewarding.

Yours sincerely,

Organising Committee:

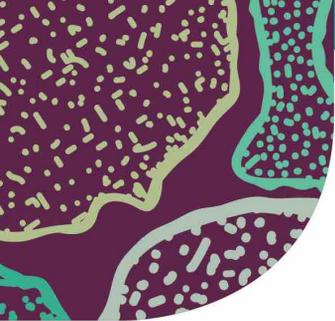
Supatra Marsh	<i>Mechanobiology Institute / NUS</i>
Kim Robinson	<i>Institute Medical Biology / A*STAR</i>
Yin Loon Lee	<i>Institute Medical Biology / A*STAR</i>
Rishita Changede	<i>Mechanobiology Institute / NUS</i>
Shumin Xia	<i>Mechanobiology Institute / NUS</i>
Yue Zhang	<i>Mechanobiology Institute / NUS</i>



Symposium Programme

Mechanobiology in Health and Disease Thursday 31st March 2018 / Morning Sessions

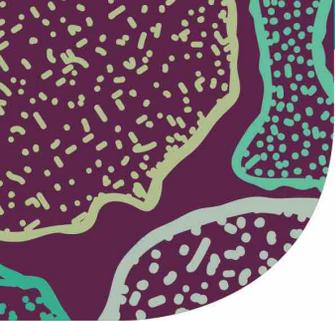
- 08:30 – 09:00 **REGISTRATION**
- 09:00 – 09:30 **CONFERENCE OPENING AND WELCOME**
- Birgit LANE**, Institute of Medical Biology/ A*STAR
Michael SHEETZ, Mechanobiology Institute / NUS
- 09:30 – 10:35 **SESSION 1 / Subcellular Organisation 1**
Chairs: Kim ROBINSON and Supatra MARSH
- 09:30 – 09:50 **Oliver DREESEN**, Institute of Medical Biology / A*STAR
The role of heterochromatin in the accelerated aging syndrome progeria.
- 09:50 – 10:05 **Ai Kia YIP**, Bioinformatics Institute / A*STAR
Keratin cytoskeleton network establishes inter-cellular force transmission.
- 10:05 – 10:20 **Naila ALIEVA**, Mechanobiology Institute / NUS
Force dependence of filopodia adhesion: involvement of myosin II and formins.
- 10:20 – 10:35 **Yin Loon LEE**, Institute of Medical Biology/ A*STAR
Nuclear movement and nuclear envelope microtubule nucleation during muscle development.
- 10:35 – 11:05 **COFFEE BREAK**



Symposium Programme

Mechanobiology in Health and Disease Thursday 31st March 2018 / Morning Sessions

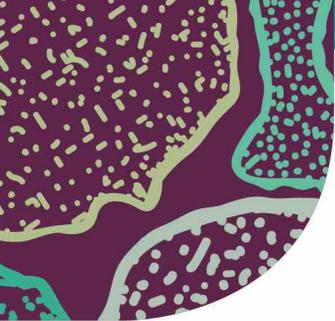
- 11:05 – 11:55 **SESSION 2 / Subcellular Organisation 2**
Chairs: Kim ROBINSON and Supatra MARSH
- 11:05 – 11:25 **Tony KANCHANAWONG**, Mechanobiology Institute / NUS
Probing the actin cortex of embryonic stem cells by super-resolution microscopy.
- 11:25 – 11:40 **Kapish GUPTA**, Mechanobiology Institute / NUS
Regulation of bile canaliculi dynamics in physiological and cholestatic conditions.
- 11:40 – 11:55 **Thuan Beng SAW**, Department of Biomedical Engineering / NUS
Mechanics of epithelial cell extrusion.
- 11:55 – 12:50 **SESSION 3 / Infection and Immunity**
Chair: Shumin XIA
- 11:55 – 12:15 **Norman PAVELKA**, Singapore Immunology Network / A*STAR
Advanced methods to investigate the bacteria and fungi inhabiting the human gut.
- 12:15 – 12:30 **Joanne KU**, Department of Biochemistry / NUS
Pathogen induced cell fusion triggering an innate immune response and genome instability.
- 12:30 – 12:50 **Linda KENNEY**, Mechanobiology Institute / NUS
Imaging Salmonella Infections
- 12:50 – 14:10 **LUNCH BREAK / Poster Viewing**



Symposium Programme

Mechanobiology in Health and Disease Thursday 31st March 2018 / Afternoon Sessions

- 14:10 – 15:35 **SESSION 4 / 3D Microenvironment**
Chair: Minnah THOMAS
- 14:10 – 14:30 **Virgile VIASNOFF**, Mechanobiology Institute / NUS
Artificial microniches induce single cell polarization.
- 14:30 – 14:45 **Supatra MARSH**, Mechanobiology Institute / NUS
Bioengineering in vitro 3D skin models for live-cell imaging.
- 14:45 – 15:00 **Xiaoman LUO**, Institute of Medical Biology/ A*STAR
Osteochondral repair using in situ tissue engineering.
- 15:00 – 15:15 **Yuri DANCIK**, Institute of Medical Biology/ A*STAR
Confocal Raman spectroscopy: a powerful tool for non-invasive analysis of 3D human skin equivalents.
- 15:15 – 15:35 **David LEAVESLEY**, Institute of Medical Biology/ A*STAR
The role of the extracellular microenvironment in facilitating wound healing
- 15:35 – 15:55 **CORE FACILITIES**
Chair: Rishita CHANGEDE
- 15:35 – 15:45 **Gianluca GRENCI**, Mechanobiology Institute / NUS
Nano and Microfabrication Facility.
- 15:45 – 15:55 **David LIEBL**, Institute of Medical Biology/ A*STAR
IMB Microscopy Unit.
- 15:55 – 16:25 **COFFEE BREAK**



Symposium Programme

Mechanobiology in Health and Disease Thursday 31st March 2018 / Afternoon Sessions

- 16:25 – 17:50 **SESSION 5 / Developmental and Genetic Biology**
Chair: Rishita CHANGEDE
- 16:25 – 16:45 **Yusuke TOYAMA**, Mechanobiology Institute / NUS
Mechanobiology of apoptosis in tissue.
- 16:45 – 17:00 **Priti AGARWAL**, Mechanobiology Institute / NUS
Syncytial architecture of the germline is maintained by actomyosin contractility of an inner corset.
- 17:00 – 17:15 **Carine BONNARD**, Institute of Medical Biology/ A*STAR
Role of NUA2 in actomyosin dynamics during brain development.
- 17:15 – 17:30 **Geetika SAHNI**, Department of Biomedical Engineering / NUS
Spatio-temporally patterned neuroectoderm tissues recapitulate neural morphogenesis and pathogenesis.
- 17:30 – 17:50 **Colin STEWART**, Institute of Medical Biology/ A*STAR
Nuclear architecture and disease.
- 17:50 **NETWORKING at Bodacious**

Priti AGARWAL

P. Agarwal and R. Zaidel-Bar
Mechanobiology Institute / NUS

Syncytial architecture of the germline is maintained by actomyosin contractility of an inner corset.

Syncytial architecture is an evolutionary-conserved feature of the germline that plays a crucial role in organism fertility. During gametogenesis, germ cells are connected to each other through stable intercellular bridges sharing a common cytoplasm. Interestingly, these bridges are enriched in actomyosin regulators. Recent studies suggested their significance in the stabilization of germ cell intercellular bridges, however the role of contractility in the maintenance of germline structure remains poorly understood.

Here, we have used the *Caenorhabditis elegans* gonad as a model to study mechanobiological properties of the syncytium. Three-dimensional image analysis of the gonad showed that along with the presence of actomyosin network at the rachis bridge, they form an inner corset enclosing the central cytoplasmic core known as rachis. Laser microsurgery experiment demonstrated that this inner corset has high contractility, which depends on myosin activity. Genetic and pharmacological perturbations of several actomyosin regulators coupled with quantitative image analysis revealed that contractility is vital for the maintenance of proper germ cell membrane length, rachis diameter and germ cell opening. Taken together, our findings identify a novel multi-cellular actomyosin structure within the syncytial germline, map the balance of forces in the germline, and establish the critical role of contractility in the preservation of a functional germline structure.

Naila ALIEVA

N.O. Alieva, A.K. Efremov, D. Oh, Z. Chen, M. Natarajan, J.T. Groves, M.P. Sheetz, J. Yan and A.D. Bershadsky
Mechanobiology Institute / NUS

Force dependence of filopodia adhesion: involvement of myosin II and formins.

Filopodia are dynamic membrane protrusions driven by polymerization of an actin filament core, mediated by formin molecules at the filopodia tips. Filopodia are involved in numerous processes of embryonic development, as well as in cell migration in adult organisms. Augmented filopodia activity is a hallmark of tumor cells, which use them in the processes of invasion and metastasis. Filopodia can adhere to the extracellular matrix and experience both external and cell generated pulling forces. The role of such forces in filopodia adhesion is however insufficiently understood. Here, we induced sustained growth of filopodia by applying pulling force to their tips via attached fibronectin-coated beads trapped by optical tweezers. Strikingly, pharmacological inhibition or knockdown of myosin IIA, which localized to the base of filopodia, resulted in weakening of filopodia adherence strength. Inhibition of formins, which caused detachment of actin filaments from formin molecules, produced similar effect. Thus, myosin IIA-generated centripetal force transmitted to the filopodia tips through interactions between formins and actin filaments are required for filopodia adhesion. Force-dependent adhesion led to preferential attachment of filopodia to rigid versus fluid substrates, which may underlie processes of cell orientation, polarization and cancer progression.

Yuri DANCIK

G. Sriram, M. Alberti, Y. Dancik, et al.
Institute of Medical Biology / A*STAR

Confocal Raman spectroscopy: a powerful tool for non-invasive analysis of 3D human skin equivalents.

Confocal Raman spectroscopy (CRS) is used to analyze skin in vivo. We show that it can be used to assess the effect of different culture conditions on physical and compositional aspects of viable, unfixed 3D full-thickness human skin equivalents (HSEs). We compared: 1) HSEs cultured under medium perfusion in a microfluidic skin-on-chip platform vs. in conventional static culture inserts; 2) HSEs cultured using different air-lift durations and control vs. interleukin-supplemented media. Significant differences in skin layer thicknesses were obtained as a consequence of dynamic medium perfusion, and due to differences in air-lift duration and culture medium. CRS further revealed differential effects on ceramide, cholesterol and protein composition as a function of depth in the stratum corneum. Measurements are rapid and reproducible, with apparently minimum damage to the HSEs. CRS is an effective methodology for non-invasive, depth-dependent characterization of key physical and compositional parameters that impact the HSEs' barrier function.

Oliver DREESEN

O.Dreesen
Institute of Medical Biology / A*STAR

The role of heterochromatin in the accelerated aging syndrome progeria.

Hutchinson-Gilford Progeria (HGPS) is a rare premature ageing syndrome, caused by a mutated form of lamin A, called progerin. Our goal is to elucidate the molecular mechanism(s) that trigger premature ageing in progeria and to understand its relevance to normal ageing. We and others previously showed that progerin expression causes DNA damage, heterochromatin loss, impaired proliferation and premature senescence, which are prevented by ectopic expression of telomerase or LAP2 α (lamina-associated polypeptide α). However, it remains unclear how progerin causes these disease-associated phenotypes, how they are causally linked – and how both telomerase and LAP2 α can prevent them. To address these questions, we developed a doxycycline-inducible system to regulate the expression of lamin A and mutant progerin. This system, in conjunction with single-cell immunofluorescence microscopy, enables us to delineate the temporal chain of events that occur upon progerin-expression, and ultimately result in premature senescence. Taken together, our results provide evidence for a mechanistic link between the nuclear lamina, chromatin structure and telomeres that is disrupted in progeria. These findings are relevant to normal ageing as alternative splice forms of lamin A and chromatin structure abnormalities accumulate during chronological ageing; thus, providing a link between progeria and normal human ageing.

Kapish GUPTA

K. Gupta, Z Song, E. Li, S. Fong and H. Yu
Mechanobiology Institute / NUS

Regulation of bile canaliculi dynamics in physiological and cholestatic conditions.

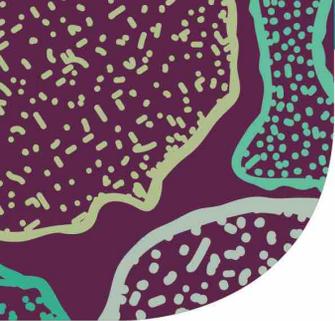
Hepatocytes have the ability to form tubular compartment known as bile canaliculi (BC). It is known that these BC expand and contract to propel bile, however, the mechanism of these expansions and contractions is unknown. We investigated the molecular mechanism of the canalicular dynamics and found that dynamic nature of bile canaliculi is an interplay of canalicular pressure and pericanalicular actomyosin cortex. Additionally, calcium toggles in and out of BC to facilitate periodic contractions. As a homeostatic response to increased canalicular pressure, pericanalicular actin breaks locally leading to the formation of membrane protrusion. Under physiological conditions, actomyosin network can repair these protrusions to give rise to inward bleb-like structures. However, beyond a certain canalicular pressure threshold (or in destabilized actomyosin network), these inward blebs completely bud off the canalicular membrane to regurgitate bile. This phenomenon was further confirmed in bile duct ligation model in lifeact-GFP mice to mimic obstructive cholestasis.

Tony KANCHANAWONG

T. Kanchanawong
Mechanobiology Institute / NUS

Probing the actin cortex of embryonic stem cells by super-resolution microscopy.

Mechanical cues influence pluripotent stem cell differentiation but the underlying mechanisms are not well understood. Mouse embryonic stem cells (mESCs) exhibit unusual cytomolecular properties including low cell stiffness and attenuated responses to substrate rigidity, but the structural basis remains obscure. Here we investigated the integrin-based focal adhesions (FAs) and cortical actin cytoskeleton in mESCs using super-resolution microscopy. We observed that mESC FAs exhibited a multi-layer nanoscale architecture comparable to FAs of differentiated cells, but that the mESC cortex adopted a remarkably sparse architecture that largely exclude myosin II. Combining structural and mechanical measurements with molecular perturbation, our results suggested that mutual competition between formins, Arp2/3, and actin capping protein, CapZ, governed cortical structure and mechanics, in part through transient aster-like intermediate structures. This generated low network density that physically excluded myosin II from the cortex. The distinctive actin cytoskeletal organization in mESCs thus prescribes their unusual cell mechanical properties.



Speaker Abstracts

Linda KENNEY

L. Kenney
Mechanobiology Institute / NUS

Imaging Salmonella Infections.

After ingestion of Salmonella from contaminated food or water, it transits through the stomach and then catalyzes its uptake across the intestinal epithelium. This process requires pathogen-stimulated changes in host actin and other pathways resulting from activation of genes located on Salmonella pathogenicity island 1 (SPI-1). Salmonella subsequently trafficks across the epithelium, and is phagocytosed by macrophages, where it resides in an acidic vacuole. The Salmonella cytoplasm acidifies to pH 5.6 and this acidification step is an important signal in activating genes on pathogenicity island 2 (SPI-2). SPI-2 encodes a type three secretion system that secretes effectors that modify the vacuole, preventing its degradation as well as endosomal tubulation. Using super-resolution imaging in single bacterial cells, we show that low pH induces expression of the SsrA/B two-component signaling system located on SPI-2. Single particle tracking identifies a pH-dependent stimulation of DNA binding by SsrB. The low level of SPI-2 injectisomes observed in single cells is not due to fluctuating SsrB levels in single cells. Super-resolution imaging enables us to visualize the emergence of Salmonella-secreted effectors into the host cytoplasm and follow the resulting endosomal tubulation. This work highlights the surprising role that acid pH plays in the virulence and intracellular lifestyle of Salmonella, and suggests that modification of acid survival pathways represents a potential target for inhibiting Salmonella. Our studies of infection in heterologous host models allow us to visualize many steps in the entire infection process.

Joanne KU

J. Ku and Y.H. Gan
Department of Biochemistry / NUS

Pathogen induced cell fusion triggering an innate immune response and genome instability.

Burkholderia pseudomallei (Bp), the causative agent of melioidosis, is a facultative intracellular bacterium present naturally in soil and water. The disease is endemic in Southeast Asia and Northern Australia, as well as other tropical areas. The disease is often underreported and misdiagnosed and causes significant mortality of 40% or higher in developing countries such as Thailand. Treatment involves prolonged antibiotic treatment with third or fourth generation of cephalosporins and no vaccines are available.

The bacteria could infect mammalian cells to induce multi-nucleated giant cells (MNGC) that is important for bacterial continued intracellular replication and spreading, without having to encounter the extracellular host defence mechanisms. The ability to induce MNGC formation is a property of the bacterial Type VI Secretion System cluster, which is essential for the virulence and pathogenesis of Bp in mammalian hosts. We found that the T6SS induced MNGC formation triggered the cGAS signaling pathway, known to be responsible for detecting cytosolic DNA. cGAS activation leads to increased host cell death in the MNGCs. Cell fusion also corresponds to the DNA damage response and the formation of micronuclei. Thus, unnatural and catastrophic cell fusion is a danger signal that triggers a DNA damage and innate immune response. This could represent a desperate distress call by the host for help against the pathogen.

David LEAVESLEY

D.I. Leavesley, Z. Upton
Institute of Medical Biology / A*STAR

The role of the extracellular microenvironment in facilitating wound healing

Topical administration of growth factors has displayed some potential in wound healing, but variable efficacy, high doses and costs have hampered their implementation. Moreover, this approach ignores the fact that wound repair is driven by interactions between multiple growth factors and importantly, includes interactions with the extracellular matrix (ECM). Indeed, our team was the first to report that complexes of insulin-like growth factor (IGF) bound to the ECM protein vitronectin significantly enhance cellular functions relevant to wound repair in primary human skin keratinocytes in 2D and 3D in vitro cell models and are active, even in the presence of wound fluid. Moreover, these responses require co-activation of both the IGF receptor and the vitronectin-binding alpha-v integrins. This was confirmed via inhibition studies, as well as gene microarray analysis. Further, we have assessed the complexes as a topical agent in the treatment of deep partial thickness burns in a porcine model, as well as in clinical case studies in patients with chronic non-healing venous ulcers. We then went on to develop a chimeric protein that was able to recapitulate within a single molecular species the biological activity of the multi-protein vitronectin:IGF complex thereby conferring numerous manufacturing and hence cost advantages. This chimeric protein has been manufactured to GMP clinical grade standard and is currently in a Phase 2b clinical trial in patients with non-healing venous ulcers in the USA, with the trial to be unblinded in Q3 2018. This presentation will highlight key aspects of the discovery to clinical trial pathway in the development of this novel growth factor-ECM technology.

Yin Loon LEE

Y.L. Lee
Institute of Medical Biology / A*STAR

Nuclear movement and nuclear envelope microtubule nucleation during muscle development.

The nucleus is the main microtubule-organizing center in skeletal muscle cells due to the accumulation of centrosomal proteins and microtubule (MT) nucleation activity at the nuclear envelope (NE). The relocalization of centrosomal proteins depends on Nesprin-1, an outer nuclear membrane (ONM) protein that connects the nucleus to the cytoskeleton. Nesprins are also involved in recruiting kinesin to the NE and play a role in nuclear positioning in skeletal muscle cells. Using proximity-dependent biotin identification proteomics, we identified Akap450 as a potential Nesprin-1 interactor in myotubes. Cell culture models of muscle differentiation, including patient-derived myotubes, revealed that Akap450 depends on Nesprin-1, but not kinesin-1, for NE localization, and is required for MT nucleation from the myotube NE. Computer simulations and cell culture systems showed that Akap450 is required for proper nuclear positioning in myotubes. These studies revealed a novel role for NE MT nucleation in nuclear positioning, mediated by Nesprin-1 and Akap450. Presently, we are investigating the role of Nesprin-1 in nuclear centration following myoblast fusion via live imaging of nuclear movement in differentiating skeletal muscle cells. Additionally, we are examining the role of NE MT nucleation in cardiac muscle by immunofluorescence microscopy of isolated cardiomyocytes.

Xiaoman LUO

X. Luo

Institute of Medical Biology / A*STAR

Osteochondral repair using in situ tissue engineering.

Autograft, which contains living cells and growth factors, is the “gold standard” of bony graft yet it is limited in quantity and may cause harvest site morbidity. The search for biomaterial scaffolds for complex musculoskeletal problems such as osteochondral defects remains a significant challenge because synthetic grafts are not as efficient. The reason behind is because synthetic grafts are not alive and in general cannot induce stem cell differentiation. Key to improve this strategy is the development of implantable devices that not only integrate with host tissue but also provide suitable microenvironment that favors cell recruitment and induce stem cell differentiation towards demanded repair. Past studies have shown that osteogenic differentiation of stem cells can be directed by intrinsic features of calcium phosphate implants such as surface morphology without using exogenous stimuli. In addition, the introduction of glycosaminoglycans (such as heparin sulfate, HS) that having high affinity to endogenous proteins, can significantly enhances the pro-chondrogenic activities of resident growth factors found at sites of osteochondral injure. Our preliminary data showed the efficacy of HS in osteochondral repair. Future development merging instructive calcium phosphate scaffolds with heparin sulfate that potentiates endogenous growth factors have the potential to be the next-wave of in-situ tissue engineering constructs that is cell and tissue-free.

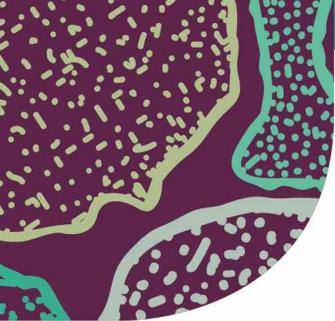
Supatra MARSH

S. Marsh, TB Saw, K Robinson, X Gao, CT Lim

Mechanobiology Institute / NUS

Bioengineering in vitro 3D skin models for live-cell imaging.

In vitro 3D skin models can be used to test drug delivery, toxicology, and to model disease, reducing the need for animal models. The majority of current in vitro skin models rely on complex and time-consuming end-point histological analysis. Here we utilise microfabrication techniques to build a microfluidic device that can support full-thickness 3D skin equivalent growth, compatible with live-cell imaging. Primary keratinocytes, fibroblasts and the N/TERT-1 keratinocyte cell line are used for 3D culture and stable fluorescent labelling of cytokeratin and actin are utilised for live-cell imaging. The microfluidic device can provide dynamic culture media perfusion to the skin model at air/liquid interface and remove waste products. Static full-thickness organotypic cultures of skin are used as a comparative control. Multiple imaging methods of the microfluidic skin culture are presented. Live-cell imaging holds great advantages over traditional histological techniques, as biological processes, such as epidermal homeostasis and disease progression, can be monitored in real time. We hope to apply the methodology developed in this project to investigating such processes in the future.



Speaker Abstracts

Norman PAVELKA

N. Pavelka

Singapore Immunology Network / A*STAR

Advanced methods to investigate the bacteria and fungi inhabiting the human gut.

Sequencing-based microbiome profiling aims at detecting and quantifying individual members of a microbial community in a culture-independent manner. While amplicon-based sequencing (ABS) of bacterial or fungal ribosomal DNA is the most widely used technology due to its low cost, it suffers from PCR amplification biases that hinder accurate representation of microbial population structures. Shotgun metagenomics (SMG) conversely allows unbiased microbiome profiling but requires high sequencing depth. Here we report the development of a meta-total RNA sequencing (MeTRS) method based on shotgun sequencing of total RNA and benchmark it on a human stool sample spiked in with known abundances of bacterial and fungal cells. MeTRS displayed the highest overall sensitivity and linearity for both bacteria and fungi, the greatest reproducibility compared to SMG and ABS, while requiring a ~20-fold lower sequencing depth than SMG. We therefore present MeTRS as a valuable alternative to existing technologies for large-scale profiling of complex microbiomes.

Geetika SAHNI

G. Sahni, S.Y. Chang, J. Teo, C. Meng, K.H. Utami, M.A. Pouladi and Y.C. Toh

Department of Biomedical Engineering / NUS

Spatio-temporally patterned neuroectoderm tissues recapitulate neural morphogenesis and pathogenesis.

Current neural differentiation systems can dictate neuroectoderm (NE) cell fate specification but not their morphogenesis. We report a first instance of directing the formation of a human-specific NE tissue that recapitulates architectural and cellular characteristics of early neural tube morphogenesis. These include having a continuous, polarized epithelium with laminar organization relative to the lateral mesoendoderm (ME) as well as exhibiting invagination-like folding, where the NE cells undergo E-to-N-cadherin switching and apical constriction. This is accomplished by spatio-temporal patterning of ME cells alongside the NE cells to guide their morphogenic folding. We uncovered that TGF beta signaling emanating from endodermal cells is obligatory in tissue folding. Finally, evaluating NE structural dysmorphia uniquely achievable in our model allows for early detection of pathologies mediated by FMR1 silencing in Fragile X Syndrome. This unprecedented degree of control over the NE architecture has significant impact on neurodevelopmental disease modeling and brain organoid organization.

Thuan Beng SAW

T.B. Saw

Department of Biomedical Engineering / NUS

Mechanics of epithelial cell extrusion.

Apoptotic extrusion is a process that expels caspase-activated (dead) cells from the epithelium to preserve monolayer integrity and maintain tissue homeostasis. Apoptosis is commonly known to be initiated by UV radiation, low doses of heat, biochemical signaling, and extreme force due to mechanical impact. However, the connections of cell extrusion to the intrinsic mechanics of the epithelium is largely unexplored. Here, by modeling the epithelium as an active, nematic liquid crystal, we found that the topological defects that spontaneously occur due to cell activity (myosin contractility and force generation) are the main drivers of apoptotic extrusion. $+1/2$ defects are comet-like patterns characterized by the misalignment of tens of cells and exhibit unique compressive stress localization that trigger mechanotransductive responses in cells such as YAP (Yes-associated protein) transcription factor activity, caspase-3 mediated cell death, and extrusion. We also found that the significant morphological changes that arise during extrusion progression is driven by different actin-based processes, dependent on the cell density in the tissue. Cell extrusion is driven by a lamellipodia-based cell crawling mechanism at low cell density, while at high cell density, it is driven by a more purse-string based mechanism.

Colin STEWART

Ruth Chai, Tsui Han Loo, Lee Yin Loon, Brian Burke and Colin L. Stewart.

Institute of Medical Biology / A*STAR

Nuclear architecture and disease.

The nuclear envelope (NE) and lamina are the boundary between the nucleus and cytoplasm. Many diseases, including dilated cardiomyopathy (DCM), muscular dystrophies, and progeria (a disease of vascular failure) are caused by mutations in the A-type lamins (LMNA) and other NE proteins. The lamins provide mechanical strength and tether the nucleus to the cytoskeleton through the NE associated LINC complex, so providing a direct physical link between the cell surface/ECM and nucleoplasm.

The N-terminus of the LINC complex protein Sun1 protrudes into the nucleoplasm where it may interact with nuclear proteins. We find that in differentiating myoblasts a muscle specific isoform of SUN1 interacts with the microprocessor enzyme DROSHA. In doing so SUN1 inhibits the processing of a cluster of microRNAs that regulate the translation of RTL1, a protein we show that is required for efficient skeletal muscle regeneration.

Many of the Lmna mutations result in elevated levels of Sun1. Deleting Sun1 in mice that rapidly die from Lmna induced heart failure results in their survival to 1 year, identifying a therapeutic route to treating DCM. We developed a dominant negative Sun1 minigene that on introduction into DCM hearts significantly ameliorates many of the Lmna induced cardiac muscle pathologies.

Yusuke TOYAMA

Y. Toyama

Mechanobiology Institute / NUS

Mechanobiology of apoptosis in tissue.

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. When a cell undergoes apoptosis within a tissue, the apoptotic cell is expelled from its neighboring non- dying cells. It has been shown by many labs, including ours, that this mechanical process is driven by the formation and contraction of the actomyosin cables in the dying and the neighboring cells, and/or by the lamellipodial crawling of the neighboring cells. However, how cell mechanics arises upon apoptotic cell extrusion and feedbacks to cellular and molecular function especially in the neighboring non-dying cells is largely illusive. In this presentation, I will present our current understandings of how mechanical tension and biochemical natures are altered in the neighboring cells as a consequence of apoptosis, and how these two factors are related to each other.

Virgile VIASNOFF

V. Viasnoff

Mechanobiology Institute / NUS

Artificial microniches induce single cell polarization architecture and disease.

In this talk I will present the new technique we developed to create artificial microenvironments that integrate rheological, biochemical and topographical cues in 3D. I will show how these microniches can be scaled from single cell to organoids and how they can be used to perform super-resolution imaging. I will then demonstrate that such systems can induce hepatic polarity hence creating a functional single cell organ.

Ai Kia YIP

A.K. Yip, U. Manivannan, H.N. Huppert, S.S.X Lim, A. Moreb, Y. Toyama, E.B. Lane, G.D. Wright, K.H. Chiam and J.A.E. Common

Bioinformatics Institute / A*STAR

Keratin cytoskeleton network establishes inter-cellular force transmission.

The keratin intermediate filament network provides mechanical strength to epithelial cells, and mutations in keratin genes, KRT5 and KRT14, are associated with skin disorders such as epidermolysis bullosa simplex (EBS), where the skin blisters upon minor mechanical stress. However, the mechanobiology mechanisms relating keratin networks to EBS remain unresolved. We have quantified traction stress generation by keratinocytes in isolation and in colonies and observed no significant difference between single keratinocytes containing wildtype (WT) keratin and keratinocytes overexpressing the dominant EBS-mimicking mutation KRT14-p.R125P (R125P), suggesting that the keratin network does not alter single cell contractility. However, WT keratinocytes in colonies exert higher traction stresses at the leading edge compared with cells centrally located in the colony, in contrast to R125P keratinocytes which exert high traction stresses throughout the colony, indicating that keratin network is required for inter-cellular force transmission. Laser ablation of keratin networks within WT keratinocytes in colonies caused neighbouring cells to recoil, showing that keratin networks are under tension and supporting inter-cellular force transmission. Finally, wound healing assays revealed that inter-cellular force transmission is important for keratinocytes to coordinate cell movements over long distances, which may be crucial for maintaining skin integrity.

Doorgesh S JOKHUN

D.S. Jokhun

Mechanobiology Institute / NUS

Actin dynamics couples extracellular signals to the mobility and molecular stability of telomeres.

Extracellular signals often need to be transmitted to the nucleus and chromatin in order to regulate genomic processes. In addition to signalling pathways, the actin cytoskeleton serves as an important mechanical connection for the propagation of physical signals to the nucleus. While many studies have investigated the role of the actomyosin network in directly transmitting stresses, in this work, we focussed on the intrinsically dynamic nature of the cytoskeleton as a means of relaying signals. We showed that the cytoskeletal network continuously reorganizes and applies dynamic forces on the nucleus even under static extracellular conditions. This active cytoskeletal reorganization is modulated by the extracellular microenvironment and feeds into the regulation of chromatin organization. Specifically, we demonstrated that this mechanical pathway is used to regulate telomere tethering. We found that the motion of telomeres are correlated in a length-scale dependent manner and that functional LINC complexes as well as a stable nuclear lamina are required for the regulation of their translational dynamics by the cytoskeleton. Finally, we demonstrated that the molecular stability as well as transcription of telomeric chromatin are impinged upon by such mechanotransduction events. Our results highlight a general mechanical pathway which regulates chromatin positional and molecular stability and can potentially incorporate numerous extracellular cues into genome organization.

Anh Phuong LE

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Anisotropic actomyosin cables and the role of cell-cell junction in epithelial extrusion.

Apoptotic extrusion is crucial in maintaining epithelial homeostasis and has implications in diseases pertaining to epithelial tissues like cancer and asthma. Extrusion is mechanically-regulated when the neighboring cells could use either lamellipodia-based protrusion or contractile cable (purse-string) to actively remove the dying cells. Little is known about how much each of these two modes contribute to the overall effectiveness of extrusion, and which cell component determines one mode of extrusion over the other. Here we shown that during extrusion at highly packed tissue, contractile actomyosin cables are formed anisotropically, on the contrary to previous belief that a uniform purse-string is necessary to extrude the dying cell. Concurrently, there were also lamellipodia-based protrusion observed. The existence of these two mechanisms results in an anisotropic force field surrounding extrusion site. By manipulating alpha-Catenin, a mechano-sensing component of adherent junction, we demonstrated that the reduced cell-cell junction could favour lamellipodia protrusion mode. On the other hand, cells with strengthened cell-cell junction formed more actomyosin cable and the contractility was also enhanced to effectively extrude the dying cells when cell-substrate adhesion was removed. Our results highlighted the importance of previously-undermined lamellipodia-based protrusion in extrusion, which could adequately compensate for the absence of contractile actomyosin cables.

Meng PAN

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The role of ACTN4 in stress fiber mechanics, actin–MRTF circuit regulation and rigidity sensing.

Cells respond to substrate stiffness by adapting the dynamics and organization of their actin cytoskeleton. In addition, cytoskeleton tension can play a role in nuclear transcription and gene expression, but specific mechanisms have not been fully understood. Alpha-actinins (ACTNs) are crosslinkers of the actin cytoskeleton, which has also been reported to function in transcriptional regulation. First we find that in rat fibroblast cells (REF-52), knockdown of ACTN4 increases actin bundling and polarization on soft substrates, and leads to higher traction force on stiff substrates measured by a micro-fabricated array of flexible silicon pillars. Myocardin-related transcription factor A (MRTF-A) translocates to nucleus and binds to serum response factor (SRF) when released from binding to G-actin, which leads to downstream transcriptional activities that modulate actin dynamics. We further show that knockdown of ACTN4 increases the ratio of F-actin to G-actin, nuclear translocation of MRTF-A, expression level of α -smooth muscle actin (α -SMA) and incorporation of α -SMA into F-actin fibers. Finally, traction force and α -SMA expression level increased by ACTN4 knockdown can be rescued by MRTF-A knockdown. Collectively, our finding uncovers a previously unrecognized role of ACTN4 in nuclear translocation of MRTF-A, suggesting potential novel pathways where actin dynamics can engage in transcriptional regulation.

Bhimsen ROUT

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Photodynamic therapy for melanoma using MC1-receptor directed peptide-photosensitizer conjugate.

A strategy by covalent conjugation of photosensitizer to the MC1-receptor specific peptide antagonist for the targeted delivery to melanoma cell (MEL) and sequential LED light dosage to reduce melanogenesis was demonstrated. The preferential accumulation of photosensitizer in melanocytes compared to keratinocyte (KER) leads to extensive decline of collateral damage. The substantial reduction of melanogenesis was successfully achieved by tandem irradiation of LED light dosage using wavelengths at near infrared wavelength (660 nm). This technology has potential therapeutic value for Lentigo Maligna Melanoma.

Jun-ichi SAKABE

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Can tensegrity explain the peculiar structure of the human thymic epithelium?

Human thymus is a primary lymphoid organ whose stromal mesenchymal and epithelial cells (TECs) are essential for the production of functional T-cells. Anatomists have noticed long ago that thymus and epidermis share common features despite their different embryonic origin (endodermal versus ectodermal) and their extremely dissimilar organization (fibroblast sponge-like network versus stratified). To further investigate the relation between TECs and epidermis, we have used 3D imaging and serial block-face scanning electron microscopy and demonstrated that human TECs form a highly organized and continuous network originating from the subcapsular region to the Hassall's bodies. Furthermore, we have demonstrated that human thymus of all ages contains a population of EpCAM+ clonogenic TECs with extensive growth capacity when cultured in conditions routinely used for regenerative medicine of epidermis. We have also demonstrated that cultured hTECs balance between molecular programs implicated in Epithelial-Mesenchymal Transition (EMT) and epidermal differentiation while retaining thymus identity and we have identified ZEB1 and miR-200 family members, well-known players of EMT, as key regulators of this balance. We hypothesize that the combination of partial EMT and epidermal differentiation confers tensegrity to the thymic epithelial cell network.

Kriti SETHI

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RHO-1 GEF RHGF-1 promotes actomyosin contractility in C. elegans spermatheca.

Tubular tissues in our body are constantly exposed to various stresses and contractile responses from the actomyosin structures within the cells oppose these stresses. However, it remains unknown how molecular pathways resulting in contraction are triggered in response to stresses in vivo. Here, we propose to use the *C. elegans* spermatheca, a flexible pouch like structure, to study contraction of tubular tissues in vivo. We also elucidate a role for a RhoGEF in promoting actomyosin contractility in this tissue.

During each ovulation, an oocyte enters the spermatheca, stretching the myoepithelial cells of the spermatheca. After fertilization, the oocyte is propelled into the uterus as a result of a wave of contractile forces. Contractility is regulated by calcium and Rho signaling and is generated by a circumferential actomyosin network within the spermatheca. We recently identified a RhoGAP protein, SPV-1, which inhibits RHO-1 activity and thus spermathecal contractility. We performed an RNAi screen in an *spv-1* loss-of-function mutant, and identified RHGF-1 depletion to correct for the overcontractile defect of the *spv-1* mutant. Using CRISPR-Cas9, we tagged RHGF-1 with tagRFP and observed its recruitment to actin filaments of spermathecal cells upon oocyte entry. Recruitment of RHGF-1 to actin filaments is required for proper actin network organization and contractile activity of the spermatheca. We are currently working on identifying the cue for activation of RHGF-1.

Aishwarya SRIDHARAN

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Role of polyamines in human skin pigmentation.

Polyamines are a group of small, naturally occurring cationic compounds. Their levels are tightly controlled and are essential for normal growth and development. Although their role has been well studied in skin cancers (melanomas), their involvement in human skin pigmentation under normal physiological conditions has not been analysed. We speculate that polyamines may have a role in the process of pigmentation and dysregulation of their levels may lead to pigmentation disorders. We found that addition of polyamines to human primary melanocytes can increase both the melanin content and the expression of the rate-limiting enzyme, tyrosinase and other melanogenesis related genes/ proteins. When human skin biopsies were treated with polyamines, there was an increase in the pigmentation, which was confirmed both visually and by Fontana-Mason stain. Our data suggests that human melanocytes are sensitive to changes in extracellular polyamine levels and respond by modulating pigment production. Our results disclose an interesting relationship between polyamines and human skin pigmentation. By addressing the mechanism by which the polyamines drive pigmentation could enhance our understanding of not only normal human melanogenesis but also in human skin pigmentation pigmentary disorders.

Gopu SRIRAM

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Skin-on-Chip: Impact of microfluidics on morphology and function of skin.

Skin equivalents (SEs) are gaining importance as an in vitro tool for basic research, and in the skin-care, pharmaceutical and drug delivery research. However, human SEs reconstructed on conventional static culture plates are limited by a weak skin barrier function, probably due to the lack of mechanical forces and dynamic culture conditions that are essential for delivery of mechanistic signals and continuous supply and/or drainage of nutrients and metabolites. Here, we present a biomimetic 'organ-on-chip' system that provides a dynamic perfusion of culture media and air that enables the development of morphologically superior skin architecture and improved skin barrier function. The miniaturized design and use of optically transparent thermoplastic material offers real-time, non-invasive imaging capabilities. In the future, higher throughput and automation of the 'Skin-on-Chip' would deliver low-cost alternative to animal models for applications in dermatology, cosmetic, toxicology and pharmaceutical industries.

Kenneth TAN

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Identifying therapeutic targets for RNA splicing-related cardiomyopathy.

Dilated cardiomyopathy (DCM) is the most common indication for heart transplantation. RNA Binding Motif 20 (RBM20) is a cardiac-specific RNA splicing protein which is mutated in 2-3% of DCM. RBM20 directly binds the RNA transcripts of many cardiomyopathy-associated genes and ensures the production of cardiac-specific protein isoforms. We hypothesize that RBM20 is an attractive target for drug therapy via altering RBM20 splicing activity. We have made multiple RBM20 point mutations and generated epitope tags at the endogenous locus of RBM20 by genome engineering in induced pluripotent stem cells (iPSCs). Our genome engineering techniques allow us to examine an allelic series of highly informative RBM20 mutations in an isogenic background. Since RBM20 is likely to be regulated by interacting with a protein complex, Affinity Purification Mass Spectrometry (AMPS) studies will be ideal to reveal differential protein binding and identifying binding partners that are very likely to be at the core of the RBM20 disease mechanism. These studies will provide foundation of developing a human iPSC-CM based platform to develop new therapeutics and reveal new disease mechanisms that will be targets for therapy. Human RBM20 mutations provide clues to how normal RBM20 contributes to maintaining healthy cardiac muscle. Ultimately, we are hopeful that assays can be built around molecular targets of RBM20 and can be used to find better treatments for patients with cardiomyopathy.

Yu-Hsiu WANG

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Signaling of nuclear lipids in DNA damage response.

Phosphoinositide lipids (PPIs) are enriched in the nucleus and accumulate at DNA damage sites. Here, we show roles of nuclear PPIs in DNA damage response by sequestering PPIs with nuclear-targeted PH domains. They inhibit recruitment of Ataxia telangiectasia and Rad3-related protein (ATR) and reduce activation of Chk1. PPI-binding domains rapidly (<1s) concentrate at damage sites with enrichment of PPIs. Accumulation of PIP3 as a complex with nuclear receptor protein, SF1, occurs at damage sites and requires phosphorylation by inositol polyphosphate multikinase (IPMK). This leads to nuclear actin assembly and ATR recruitment. ATR recruitment/activation is suppressed with Latrunculin A and wortmannin treatment and IPMK or SF1 depletion. Other DNA repair pathways that involve ATM and DNA-PKcs are not affected by PPI sequestration. Thus, nuclear PPI metabolism mediates this early damage response through an IPMK-dependent pathway to specifically recruit ATR.