2023 Taiwanese Physical Society Satellite Meeting: Emerging Behaviors of Collective Cells

Date: January 17 (Tuesday), 2023.

Morning Session: 08:55–10:45
Afternoon Session: 14:00–18:05

Venue: Dept. of Chemistry, 36308 room (化學系館 36308 室)

Venue Map:

Organizing Committee:

Prof. Keng-Hui Lin (林耿慧), Institute of Physics, Academia Sinica

Dr. Fu-Lai Wen (温福來), Intl. Center for Wound Repair and Regeneration, NCKU

Prof. Ming-Jer Tang (湯銘哲), Department of Physiology, NCKU

2023 Taiwanese Physical Society Satellite Meeting Emerging Behaviors of Collective Cells	
08:55–09:00	Opening Remarks **Fu-Lai Wen (溫福來)**
Session 1 **@ 36308 Room, ChemNCKU** **Chair: Grace Pen-Hsiu Chao (趙本秀)**	
09:00–09:30	Ming-Jer Tang (湯銘哲) Department of Physiology, NCKU
09:30–10:00	Tetsuya Hiraiwa Mechanobiology Institute, National University of Singapore
10:00–10:15 (selected)	Chiao-Yu Tseng (曾喬毓) Institute of Physics, Academia Sinica
10:15–10:45	Yuan-nan Young (楊淵能) New Jersey Institute of Technology, USA
10:45–11:10	Tea Time (TPS Program)
11:10–12:00	Plenary Talk– **Steve Quake** (TPS Program)
Lunch Break **(TPS Program)**	
Session 2 **@ 36308 Room, ChemNCKU** **Chair: Chi-Shuo Chen (陳之碩)**	
14:00–14:30	Sheng-Hong Chen (陳昇宏) Institute of Molecular Biology, Academia Sinica
14:30–14:45 (selected)	Lun-Wei Lee (李倫維) Institute of Basic Medical Sciences, NCKU

14:45–15:15	Jean-Cheng Kuo (郭津岑) Institute of Biochemistry and Molecular Biology, NYCU
15:15–15:45	Grace Pen-Hsiu Chao (趙本秀) Institute of Biomedical Engineering, NTU
30-min Break & Free Discussion (with refreshments)	
Session 3 **@ 36308 Room, ChemNCKU** **Chair: Sheng-Hong Chen (陳昇宏)**	
16:15–16:45	Po-Ling Kuo (郭柏齡) Department of Electrical Engineering, NTU
16:45–17:15	Chi-Shuo Chen (陳之碩) Dept. of Biomedical Engineering and Environmental Sciences, NTHU
17:15–17:30 (selected)	Giovanni J. Paylaga Dept. of Physics, Mindanao State University, Philippines
17:30–17:45 (selected)	Cheng-Hsiang Kuo (郭承翔) Intl. Center for Wound Repair and Regeneration, NCKU
17:45–18:00 (selected)	Marco P. De Leon Institute of Cellular and Organismic Biology, Academia Sinica
18:00–18:05	Closing Remarks **Ming-Jer Tang (湯銘哲)**

Ming-Jer Tang (湯銘哲)
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Title: Spatial mechanomics: a new mechanobiological tool for studies of organ fibrosis and keloids pathogenesis

Abstract: TGF-β-induced myofibroblast activation plays very important role in the development of tissue fibrosis. However, the mechanobiological mechanisms whereby TGF-β triggers myofibroblast activation has not been understood. We employed live-actin stained NRK-49F cells (renal fibroblast) cultured on FITC-labelled collagen gel and stimulated with TGF-β as the model of myofibroblast activation. To monitor the force generated within the cells as well as the force exerted by the cells during myofibroblast activation, we established an *in vitro* real-time monitoring system with fluorescent confocal microscope co-axis with atomic force microscope (AFM) to assess the elasticity of the cell and single collagen fiber. Our result showed that collagen fibers near NRK-49F cell were stiffer than distant region. TGF-β stimulation increased the rigidity of NRK-49F cells, as well as collagen fibers, but did not alter diameter of collagen fibers or the angle between collagen fibers and NRK-49F cells. Blebbistatin treatment and tropomyosin knockdown significantly alleviated the TGF-β-induced increase in stiffness of NRK-49F cells and collagen fibers. Collectively, we have demonstrated that co-axis confocal microscope and AFM provides high resolution images and precise mechanical properties for collagen fibers during the activation of myofibroblast. Such technological breakthrough shall facilitate our studies in depicting the mechanobiological landscape of soft organ and fibrotic organ *in situ* or *ex vivo*. In light of such technological advancement in mechanobiology, we plan to develop spatial mechanomics which harbors collective information of the mechanical property (i.e. stiffness) of the live cell and ECM in the tissue microenvironment. Such advancement may facilitate the future research on theranostics of organ fibrosis and regenerative medicine.

Tetsuya Hiraiwa
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Title: Dynamic self-organization of migrating cells through cell-cell contact communication

Abstract: Dynamic self-organization, or emergence of dynamic structures and coherent dynamics, is one of the key processes for living systems to acquire complex structures and functions. For example, living cells can form varieties of multicellular assemblies with coherent dynamics by relying on their complex intercellular communication. Studying such cellular dynamic self-organization processes may pave the new way toward understanding mechanisms underlying morphogenesis and functional processes of living systems.

Migratory behavior is a ubiquitous kind of eukaryotic cell dynamics. Some cells migrate on a substrate according to intracellular signals that localize at their front or back, even without extracellular cues. We have been working on theoretical modeling and numerical simulations of such cell migration with intracellular polarity [1,2] and, by applying it to the multicellular case, their cooperated behavior [3-6].

When migrating cells communicate with each other and act in union, they can exhibit varieties of dynamic patterns and coherent motion. In this presentation, we mainly discuss what forms of dynamic self-organization of migrating cells are caused through contact communication between cells theoretically [5]. The concept of our theoretical model based on an individual cell dynamics-based model in which migrating cells perform two ubiquitous types of contact communication, so-called contact following (CF) and contact inhibition of locomotion (CIL), is explained. Our numerical simulations show that (i) tuning strengths of CF and CIL causes varieties of dynamic self-organization patterns, and (ii) this includes a novel form of collective migration, which we named snake-like dynamic assembly [5]. Some results are compared with experimental observations of dynamic patterns shown by some living cells (social amoeba) [4]. If time allows, how such dynamic self-organization can play roles for functional behaviors, like accurate directional migration, is also explained [3,5,6].

[1] T. Hiraiwa et al., Physical Biology 11, 056002 (2014).
[2] T. Hiraiwa et al. Euro. Phys. J. E 36, 32 (2013).
[3] T. Hiraiwa, Physical Review E 99, 012614 (2019).
[4] M. Hayakawa, T. Hiraiwa et al., eLife 9, e53609 (2020).
[5] T. Hiraiwa, Physical Review Letters 125, 268104 (2020).
[6] T. Hiraiwa, Euro. Phys. J. E 45, 1 (2022).

Chiao-Yu Tseng (曾喬毓)
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Title: Precursor State of Apicobasal Polarity in Single Epithelial Cells

Abstract: The function of biological systems often requires programmed organization of cells and tissues. How these units coordinate their topology into functional architectures, such as epithelial polarization, has been of great interest to physicists and biologists for its close links to development and diseases, which are two sides of the same coin. For decades, research has been devoted to investigating how interacting chemicals/molecules form spatiotemporal patterns to guide epithelial polarization. Conversely, much less attention is given to whether the topology of polarity can spontaneously emerge and in turn lead to the development of chemicals/molecules patterning. We study the cytoskeleton dynamics of single epithelial cells and find that these cells, in the absence of direct cell-cell contact, can exhibit various features of polarity, which we name as a precursor state. The single cells in this state share several structural similarities as in the mature epithelium. Namely, the molecules of interest show specific patterns, which are generally considered the hallmarks of polarity. Further, the experimental evidence shows that this state can coordinate the formation of apical-basal polarity when cells encounter each other. Moreover, combining fast live-cell imaging and genetic or pharmaceutical manipulation, we study the mechanism underlying the formation of the precursor state and find several key molecules contributing greatly in this process.

Yuan-nan Young (楊淵能)
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Title: Biophysical modeling of spindle dynamics in *Caenorhabditis elegans* embryos

Abstract: In eukaryotic cells, the mitotic spindle forms during cell division and ultimately separates the chromosomes into the daughter cells. The position and orientation of the division plane, which is of fundamental importance for proper growth and development, are regulated by the spindle's position and orientation. Despite extensive knowledge about the molecular basis of spindle positioning and dynamics, the underlying force mechanisms remain elusive. Here, we developed a coarse-grained model of the spindle, which accounts for the dynamics of microtubules nucleating from centrosomes and their interactions with motor proteins localized on the cell cortex. We show that pulling forces from these motor proteins are enough to explain spindle positioning and elongation dynamics. Beyond a certain number of motors, the model exhibits an oscillatory behavior for the spindle, where the spindle axis rotates periodically. Our model quantitatively explains observed spindle dynamics in *C. elegans* embryos, such as elongation, asymmetric positioning, and oscillation. It also quantitatively predicts the scaling of these traits with cell size.

Sheng-Hong Chen (陳昇宏)
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Title: Trigger waves of cell death oriented by self-organized patterns of spindle-shaped cells

Abstract: Cell death has recently been implicated to spread across cells and cause large-scale tissue damages in various human pathological conditions, yet a clear systems-level understanding of cell death propagation had been lacking. Here, we harness mathematical modeling, time-lapse imaging, and chemical/genetic perturbations to reveal how metabolic stress quantitatively modulate cellular state allowing bistability of reactive oxygen species (ROS), in turn, causing trigger waves of cell death. Intriguingly, these cell death trigger waves (i.e., its initiation, direction and speed) can be oriented by the emergent cellular patterns in a cell population. These cellular patterns dictate cell density and cell-cell alignment that prime cells with heterogeneous sensitivity to metabolic stress. Our findings show how cell death propagation is directed in a cell population via self-organized cellular patterns, featuring how collective cellular behavior in tissues and organs may influence cellular vulnerability to metabolic stress.

Lun-Wei Lee (李倫維)
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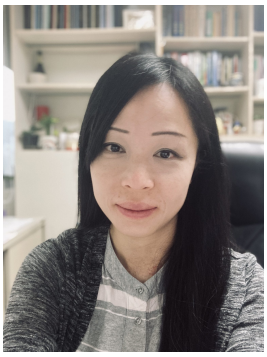
Title: Mechanobiological mechanism of cyclic stretch-induced cell columnarization

Abstract: While epithelium is constantly perturbed by time-varying mechanical forces from the surroundings, epithelial cells are capable of maintaining their integrity required for execution of physiological functions. To understand how epithelial cells respond to a dynamic mechanical stimulus, we tested MDCK cells with cyclic stretch (CS) and found that cells transformed from cuboidal to columnar morphology with increased cell height, revealing columnarization morphogenesis. In particular, CS-induced cell columnarization is associated with reorganization of intracellular actin and microtubule networks but not with cell proliferation. The cytoskeleton reorganization depends on cell-substrate focal adhesion (FA) and the cell-cell adhesion of tight junction (TJ) and adherens junction (AJ). We found that CS decreased FA activity via downregulation of pFAK. Meanwhile, TJ-related protein ZO-1 remained adhered to the apical cell surface, whereas AJ-related protein E-cadherin accumulated in the apical cytosol. We depleted ZO-1 to perturb the formation of TJ and found that the formation of CS-induced cell columnarization was inhibited. Additionally, overexpression of Caveolin-1, which induces modulation of FA activity and TJ formation similar to those of CS, was found to result in columnar cell shape even in the absence of CS, suggesting that CS triggers modulation of FA and TJ that induces cell columnarization. The impact of cytoskeleton reorganization on cell mechanical properties was examined by atomic force microscopy (AFM), which showed that the stiffness of apical cell-cell contacts, but not the medio-apical region, was increased after CS. Such modulation of apical mechanics, together with prediction on the basal mechanics change by a simple mathematical model, pinpoint the mechanical foundation of epithelial cell columnarization. Taken together, our findings support the hypothesis that the mechanical stimulus of CS can induce downregulation of FA activity and maintenance of TJ leading to the development of columnar epithelium.



Jean-Cheng Kuo (郭津岑)
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Title: Early committed polarization of intracellular tension in response to cell shape determines the osteogenic differentiation of mesenchymal stem cells

Abstract: The vertices in cells of different shapes regulate different degrees of cytoskeletal mechanics in tissue morphogenesis. To explore whether the cell shape and mechanics could be coupled with cell differentiation, we used micropatterned human mesenchymal stem cells (MSCs) to demonstrate that square cells with high-curvature vertices dictated MSC lineage commitment towards osteogenic differentiation. At the high-curvature cell shape regions, focal adhesions (FAs) responded accordingly by changing protein composition and maturation. Mature FAs acted as starting points to connect with the radial fibers, which were concentrated and bridged with the transverse fibers. The contractile force generated by actomyosin along the transverse fibers were transmitted outwards along the gathered radial fibers, to the extracellular matrix through FAs, and inwards to control nuclear-actomyosin force balance, which caused nuclear deformability. This mechanism controlled the nuclear translocation of the transcription co-activator YAP, and in turn modulated the switch in MSC commitment. Using a vertex model, we have demonstrated that the dynamic behavior of FAs responds to geometric cues and plays critical roles in regulating morphodynamic events.

Grace Pen-Hsiu Chao (趙本秀)
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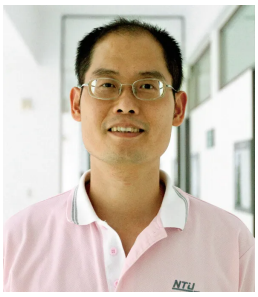
Title: Zyxin and Actin Structure Regulate Anisotropic YAP Mechanotransduction

Abstract: Mechanical stimulation, such as shear stress, compression and tension, are important regulators of cell functions. Abnormal stress or dysregulation of the underlying signaling mechanisms lead to tissue dysfunction and pathology. One aspect of mechanical signal that is seldom studied is the effect of anisotropy. In this study, we investigated the mechanotransduction of axial versus transverse strain on YAP signaling by combining cell patterning and uniaxial stretch. Dynamic stretch parallel to the long axis of the cell activates YAP signaling, but not when stretched in the transverse direction. Looking at the initial cytoskeletal response, parallel stretch leads to actin breakage and repair within the first minute, mediated by zyxin and focal adhesion kinase (FAK). This activation of FAK and zyxin in turn activates YAP nuclear translocation. As these factors control a wide range of mechanical regulation, our findings point to new roles of zyxin and YAP in anisotropic mechanotransduction, and may provide additional perspectives in cellular adaptive responses and tissue homeostasis.



Po-Ling Kuo (郭柏齡)
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Title: Role of increased interstitial hydrostatic pressure in fibroblast migration and myofibroblast activation

Abstract: An emerging trend in biomedical sciences is to elucidate how the mechanical factors perceived by cells in tissue are implicated in their physiological and pathological states. These factors include the pressure and shear stresses exerted by the fluid filled in the interstitial space surrounding the cells, and the elasticity of the filamentous matrix physically connected with the cells. Increased interstitial hydrostatic pressure (IHP) is a hallmark in many pathological and physiological conditions, such as solid tumors and tissue edema resulting from damage or inflammation, whereas the stiffening of extracellular matrix is a late signature in a variety of physiological and pathological states, such as wound healing, tissue fibrosis, and tumor progression. Increased fluid pressure has been shown to significantly relate to changes in cell size, morphology, cytoskeleton organization, differentiation, proliferation, and the invasiveness of cancer cells. Of great importance is to delineate how the increased IHP seen in various pathophysiological conditions potentiates fibroblast migration and myofibroblast activation; both have critical roles in the progress of wound healing and tissue fibrosis. Using cell-culture devices simulating elevated IHP conditions and time-lapse imaging, we showed that exposure to increased IHP significantly accelerated fibroblast migration and changed its migratory characteristics. The increased IHP-induced migration acceleration was dependent on the augmentation of transforming growth factor-β1, and correlated well with the upregulation of filamins, one of the principal proteins responsible for migration initiation, via the activation of p38 mitogen-activated protein kinase kinase. We further developed a 3D culture system that allowed simulation of variable IHP conditions and measurement of matrix elasticity using shear-wave elasticity imaging. We found increased IHP changed fibroblast morphology and promoted stiffening of the cell-populated matrix, whereas the cell-populated matrix exposed to both increased IHP and interstitial flow was significantly stiffer than that solely exposed to increased IHP. Both the increased IHP and interstitial flow conditions upregulated the expression of α-smooth muscle actin in the fibroblasts, indicating that both conditions promoted the transformation of fibroblasts into myofibroblasts, which are more contractile and might potentiate the stiffening of the matrix. The upregulation of α-SMA and matrix stiffening induced by the increased IHP and interstitial flow conditions diminished when a blocker for TGF-β receptor kinase was applied. Our results highlighted the role of changes in IHP associated with various pathophysiological states in the regulation of fibroblast migration and myofibroblast activation.

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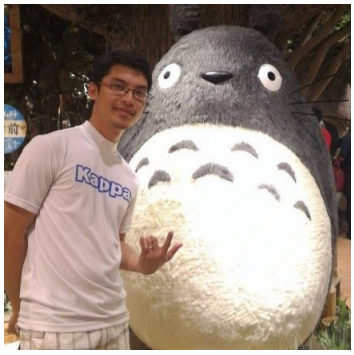
Title: The Broadcast of Mechano-Signaling in Glioma Spheroid

Abstract: Cell mechanics serve essential roles in tissue development and cancer progression; cells sense the mechanical properties of the microenvironment and modulate their physiological functions accordingly. Cellular force signals propagated between cells, however, the influences of cellular force on the mechanical alteration of cell collectives in 3-dimension remains largely underexplored. Considering the critical roles of microglia in glioma progression, using a soft-indentation approach, we studied the impacts of microglia on the mechanical properties of the glioma spheroid (GS) about 300~400 μm in diameter. We noticed a few microglia (~102 cells) attached to the surface of glioma spheroid (~104 cells) can modulate the ensemble rheological characteristics of glioma spheroid; no rheological difference was observed in the paraformaldehyde-treated spheroid, which suggests the dispensable role of cell vitality. In addition to a 2-fold stiffness increase, the results of relaxation measurement suggested that microglia can regulate the viscoelasticity of glioma collectives. By applying the generalized Maxwell model with effective configuration of one elastic element and two Maxwell material constituents in parallel, viscoelastic characteristics GS were analyzed. We further identified the integrity of actin filaments, myosin contractility, and GX43 on the cell membrane are required for the signaling broadcast from the surface to the inner parts of spheroid; the results implied the rheological regulation of microglia on glioma spheroid relies on the transmission of intra/intercellular forces. In summary, we showed that the contacts of a few microglia are sufficient to alter the mechanical properties of the glioma collective, and the cellular forces interconnect the propagation of a signal from the local microglia. Considering the mechanical properties of the tumor microenvironment are critical in therapeutic resistance and cancer metastasis, our findings highlight the critical roles of physical forces in cell collectives and provide an alternative perspective for the regulations of microglia to glioma.



Giovanni J. Paylaga
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Title: Single mesenchymal stem cells grown in concave microwells show two distinct morphological patterns

Abstract: When cells adhere to the extracellular matrix they exert traction forces, which in turn regulate their morphology, migration, and mechanotransduction signaling pathways. Most traction force microscopy researches are performed on 2D planar substrates and only a handful of papers show 3D traction force microscopy and the results focus more on the dynamics of a single cell. Our lab pioneered the work to culture cells on concave substrates as 3D cell culture and showed that the curvature affects cellular behaviors such as proliferation, migration, and morphology. In this work, we further characterize cellular mechanical responses such as 3D traction deformation and cell/nucleus volume and shape changes. Interestingly, we found two phenotypes of cells migrating in the microwell: cells that are stretching across the microwell space; and wall-conforming cells. We found that cells exert non-negligible normal traction, ~50% that of the shear traction inside the microwell. The traction forces that are both contractile and protrusive directions, mostly observed on wall conforming cells. The translocation of transcription factor YAP has been shown related to cell morphology in 2D as higher nuclear over cytoplasm (N/C) ratio in highly spread cells. We found that higher N/C ratio in wall-conforming cells as well. In short, we observe new modes of migratory behaviors and traction patterns on curved substrates. Our measurement can provide insight on how curvature affect cellular behaviors.

Keywords: 3D cell culture, substrate curvature, 3D TFM, cell migration, mechanobiology

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Title: Modeling renal tubulogenesis is a dish – focusing on the Mechanoregulation

Abstract: The kidney is composed of multiple tubules derived from 2 major mechanisms: branching morphogenesis of the collecting system and non-branching morphogenesis of proximal tubule elongation. Non-branching morphogenesis of the proximal tubules is the key to nephron generation while less studied due to the lack of a suitable cell system. We have established a cell model that represented the feature of nephrogenesis in vitro utilizing renal proximal tubular epithelial cells cultured on collagen gel. During the timely process of tubulogenesis, renal proximal tubular epithelial cells underwent dynamic self-organization into a tubule-like structure through a cell density- and matrix stiffness-dependent manner. Reduction of matrix stiffness and inhibition of cell contractility promote tubulogenesis, indicating the significant role of decreased extracellular and intracellular force. On the other hand, interfering with the dynamic of F-actin and microtubules completely suppressed tubulogenesis, indicating the significant role of cytoskeleton remodeling. Taken together, our results suggest that microenvironmental mechanoregulation on the cell-cell and cell-matrix communication play an important role in renal tubulogenesis.

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Title: Mechanical waves identify the amputation position during wound healing in the amputated zebrafish tailfin

Abstract: For over 250 years, scientists have been puzzled by how highly regenerative animals regrow lost appendages and why the rate of regrowth is proportional to the amount of appendage loss. This phenomenon prompted us to determine whether the mechanism of wound healing, as the first stage of regeneration, is responsible for discerning amputation position. Recent *in vitro* studies have provided substantial information about the mechanics of the wound-healing process, including the discovery of mechanical waves in collective epithelial cell expansion. These mechanical waves have been speculated to participate in positional sensing. Here, we perform live-cell imaging on adult zebrafish tailfins to monitor the collective migration of basal epithelial cells upon tailfin amputation. Remarkably, we observed a cell density wave propagating away from the amputation edge, with the maximum traveling distance proportional to the amputation level and cell proliferation at later stages. We developed a mechanical model to explain this wave behavior, including the tension-dependent wave speed and amputation-dependent traveling distance. Together, our findings provide the first demonstration of an *in vivo* positional sensing mechanism in regenerative tissue based on a coupling of mechanical signals manifested as a traveling density wave.