Biophysics of Organoids
February 15-17, 2023

Poster Session

1	**Isaac Breinyn**	Princeton University	*Pump it up: bioelectric stimulation controls tissue hydrostatic pressure and water transport via electro-inflation*
2	**Eric Fowler & Evelyn Navarro Salazar**	Princeton University	*Monitoring real-time signaling during formation of alveolar organoids*
3	**Solene Herve**	NIH	*Wnt-driven colorectal cancer onset and progression triggers changes in nuclear shape and architecture*
4	**Aria Huang**	University of Pennsylvania	*Recreating the physical environment to study mechanoresponsive kidney development in vitro*
5	**Dhiraj Indana**	Stanford University	*Actin polymerization drives lumenogenesis in 3D synthetic human epiblasts*
6	**Harold McNamara**	Princeton University	*Recording positional information during gastruloid symmetry breaking*
7	**Christina McNerney**	Johns Hopkins University	*Intrinsic regulation of thyroid hormone signaling in human retinal organoids*
8	**Neha Mohan**	Drexel University	*HDAC6 is a novel therapeutic target for SPAST-based Hereditary Spastic Paraplegia revealed by transgenic mice and hiPSC-derived forebrain organoids*
9	**Salem Mosleh**	Harvard University	*A universal developmental program across avian adaptive radiations*
10	**Alex Plum**	University of California San Diego	*Epiblast area homeostasis in gastrulation*
11	**Louis Prahl**	University of Pennsylvania	*Independent control over cell patterning and adhesion on hydrogel substrates for tissue interface mechanobiology*
12	**Xiaochen Qin**	Lehigh University	*Patient-Derived Non-small Cell Lung Cancer Organoid in Hydrogel Towards 3D Tissue-Engineering Vascular Drug Screening Platform*
13	**Skandha Ramakrishnan**	Drexel University College of Medicine	*The functional level of spastin is essential to axonal regeneration in vertebrate animals.*
14	**Sreejith Santhosh**	University of California, San Diego	*Mechanics and a Turing mechanism pattern the early chick embryo*
15	**Friedhelm Serwane**	LMU	*Mechanical and electrophysiological characterization of neural organoids*
16	**Xiaohuan Sun**	Drexel	*Probing mechanisms for axonal degeneration in primary tauopathy using patient derived cerebral organoids*
17	**Carolina Trenado-Yuste**	Princeton University	*Tumor progression and cell-cell coordination in 3D breast cancer spheroids*
18	**Evan Underhill**	Princeton University	*Characterizing and Directing Signaling Gradients during the Growth of mESC-Derived Gastruloids*
19	**John Viola**	University of Pennsylvania	*Probing the effects of mechanical transients in the nephron progenitor niche using organoid and 3D co-culture models*
20	**Sifan Yin**	Harvard University	*Three-dimensional chiral morphodynamis of chemomechanical active shells*
21	**Muhmmad Sulaiman Yousafzai**	NIH	*Defining the functions of myosin 2 isoforms in epithelia using intestinal organoids*
22	**Chunzi Liu**	Harvard University	*The role of mechanical forces on diseased cell models and morphogenesis process*

1	**Isaac Breinyn**	**Princeton University**	***Pump it up: bioelectric stimulation controls tissue hydrostatic pressure and water transport via electro-inflation***

Epithelial tissues sheath many organs, separating 'outside' from 'inside' and exquisitely regulating ion and water transport via electromechanical pumping to maintain homeostatic balance and tissue hydrostatic pressure. While it is becoming increasingly clear that the ionic microenvironment and external electric stimuli can affect epithelial function and behavior, the coupling between electrical perturbation and tissue form remain unclear. We investigated this by combining electrical stimulation with three-dimensional epithelial tissues with hollow 'lumens'—both kidney cysts and complex intestinal stem cell organoids. Our core finding is that physiological strength electrical stimulation of order 1-3 V/cm (with both direct and alternating currents) can drive powerful and rapid inflation of hollow tissues through a process we call 'electro-inflation', inducing a nearly threefold increase in tissue volume and striking asymmetries in tissue form. Electro-inflation is primarily driven by activation of the CFTR ion channel pumping chloride into the tissue, causing water to osmotically flow. This influx generates hydrostatic pressure, and inflation results from a competition between this pressure and cell cytoskeletal tension. We validated these interpretations with computational models connecting ion dynamics in the Diffuse Double Layer around tissues to tissue mechanics. Electro-inflation is a unique biophysical process to study and control complex tissue function.

2	**Eric Fowler & Evelyn Navarro Salazar**	**Princeton University**	***Monitoring real-time signaling during formation of alveolar organoids***

The critical function of the mammalian lung, gas exchange, occurs at the alveolus. The epithelial cell types that comprise the alveolus (alveolar type 1 and 2 cells, aka AT1 andAT2) have distinct functional roles. AT1 cells are thin and flat and promote gas exchange, while AT2 cells are cuboidal and produce the surfactant necessary to keep the alveolus open. As the site of major lung diseases, great focus has been paid to producing an ex-vivo model of the alveolus. However, our current understanding of alveolar differentiation has not permitted researchers to construct ex-vivo models with the appropriate AT1/AT2 ratios or spatial patterning. Here we monitored the spatio-temporal activity of signaling pathways involved in the differentiation of AT1 (YAP) and AT2 (ERK) cells using real-time biosensors. To increase the robustness of our study, we characterized the differentiation of alveolar progenitors generated from induced pluripotent stem cells (iPSCs) and those isolated directly from embryonic mouse lungs. We report that alveolar progenitors differentiate towards an AT1 fate in response to elevated substratum stiffness, a condition known to activate YAP signaling. In contrast, we find that supplementing media with the ERK activators FGF7/10 increases the dynamics of ERK signaling as well as differentiation towards an AT2 fate. With an increased understanding of the YAP/ERK activity profiles exhibited by differentiating alveolar epithelium, we hope to replicate these signaling dynamics to engineer alveolar organoids with ratios and spatial patterning of AT1/AT2 cells that mimic alveoli in vivo.

3	**Solene Herve**	**NIH**	***Wnt-driven colorectal cancer onset and progression triggers changes in nuclear shape and architecture***

Nuclear architecture is a critical regulator of coordinated patterns of gene expression and thus cell identity and state. Nuclear shape and architecture are fundamentally altered in most cancers. As a consequence, nuclear shape abnormalities are routinely utilized by clinicians to diagnose cancer and its aggressiveness. However, the causes and direct consequences of nuclear shape alterations have remained unclear and are the focus of this study. Nuclear shape is defined by the nuclear lamina, a meshwork of intermediate filaments at the basis of the inner nuclear membrane, and its associated heterochromatin. Besides nuclear shape, the lamina regulates chromatin organization, transcription, nuclear integrity and nuclear mechanics. A-type lamins are made of two isoforms, lamin A and lamin C, that only differ in their carboxy-terminal tail, leading to their frequent study as a single entity and a lack of knowledge on isoform-specific functions. Nevertheless, a few studies have found a reduction in lamin A but not lamin C expression in several cancer cell types, including colon adenocarcinoma. We used CRISPR-engineered human colon organoids that recapitulate colorectal cancer progression from adenoma to carcinoma to define the relationship between oncogenic mutations, cancer progression and nuclear architecture. We show that the first oncogenic hit - constitutive Wnt activation by APC loss of function - directly impacts lamin A to lamin C ratio. By modifying this ratio with anti-sense oligonucleotides or modulation of the Wnt pathway, we investigate the role of these isoforms on nuclear mechanics and organization and their contribution to Wnt oncogenic signaling in colon. This work will uncover the link between nuclear shape abnormalities, A-type lamin isoforms levels and colorectal cancer onset and progression.

4	**Aria Huang**	**University of Pennsylvania**	***Recreating the physical environment to study mechanoresponsive kidney development in vitro***

Kidney development is a dynamic and highly coordinated dance between branching of the future urinary collecting tubules and induction of nephrons. While genetic and biochemical factors involved in this process have been the primary focus in the past, the role of mechanics is an emergent interest in the field. Our lab recently reported that the mechanical stress generated by jamming of nephron-forming progenitor niches at the tips of tubules may serve as a pace-making cue that instructs nephron commitment. We also showed that pharmacologically inhibiting tension in an iPSC-derived model alters nephron commitment outcomes. To further our understanding of the signaling-level details of mechanotransduction at play here, we are now perturbing the physical

environment during kidney development at the cellular and whole-organ scales. We are determining the effect of substrate stiffness on nephron progenitors collected from mouse embryos or derived from human iPSCs. Preliminary analysis of mechanosensitive YAP localization demonstrates that kidney progenitors are responsive to substrate stiffness at various stages of differentiation. Jumping to the whole-organ level, we recently developed a 3D culture system to mimic the embryonic kidney's in vivo environment. Current thought in the field is that 3D explant culture is impossible due to nutrient or oxygen transport limitations, necessitating flattened culture at air-liquid interfaces. However, our preliminary work shows that embedding kidney explants in a collagen gel supports ex vivo, 3D branching morphogenesis and nephron development for the first time. We are currently determining the role of gel stiffness, adhesion, and viscoelasticity on branching timing and geometry. This work creates a new cell-to-organ-level mechanics perspective that impacts kidney regenerative medicine efforts seeking to address the high burden of congenital and adult kidney disease.

5	**Dhiraj Indana**	**Stanford University**	***Actin polymerization drives lumenogenesis in 3D synthetic human epiblasts***

Embryoids or human induced pluripotent stem cell (hiPSC) models of embryonic development, serve as an excellent tool to uncover mechanisms regulating early morphogenetic events during human embryogenesis. Post-implantation, the pluripotent epiblast in a human embryo forms a central fluid-filled lumen. However, the mechanism of lumen formation in the human epiblast remains unknown. Here, we uncover the biophysical mechanism of de novo lumenogenesis in a 3D hiPSC model of the human epiblast. Previously implicated lumenogenesis mechanisms such as apoptosis and hydrostatic pressure were not found to regulate de novo lumen formation in hiPSCs. Rather, a novel actin polymerization dependent mechanism drove lumenogenesis and once a specific lumen size of ~12 um radius was reached, the mechanism of lumen growth switched to osmotic pressure. Smaller lumens lacked tight junctions and early lumen growth was found to be correlated with an increase in apical surface area of individual hiPSCs. Apically, actin formed a dense mesh-like network and inhibition of actin polymerization and branching via Arp2/3, formins or N-WASP halted lumen formation, implicating that actin polymerization is necessary for lumen formation. Laser ablation of apical actin uncovered quasi-static growth dynamics of the apical actin surface and showed that apical actin polymerization results in increased apical surface area, ultimately causing lumen expansion. Our work has identified a novel actin-driven, pressure-independent mechanism of epiblast lumen formation that switches to pressure-dependent growth following maturation of the apical domain.

6	**Harold McNamara**	**Princeton University**	***Recording positional information during gastruloid symmetry breaking***

Stem cell-based models of embryonic development and organogenesis demonstrate a remarkable capacity to self-organize long range structures in the absence of well-defined spatial cues. 'Gastruloids' recapitulate the establishment of an embryonic coordinate system in vitro. Spherically symmetric aggregates of mouse embryonic stem cells subjected to a spatially homogenous agonist of Wnt signaling activity will spontaneously polarize to generate an anterior-posterior axis. How Wnt signaling dynamics break symmetry to define a body axis was not previously well understood.
We use a combination of live imaging, single cell sequencing, and signal recording genetic circuits to map the emergence of positional information in gastruloids. Observation of patterns of Wnt signaling activity following small molecule activation reveal a heterogenous but well-mixed distribution of signaling activities, which gradually coalesce into locally ordered but global disordered domains before resolving into a single 'node' marking the future posterior. By recording signaling states during defined 'listening windows' with a signaling activity- and drug-gated recombinase circuit, we follow the morphogenic fates of signaling-defined cell populations. Lineage-tracing experiments reveal that the duration of Wnt activity following stimulation encodes positional information along the anterior-posterior axis. Surprisingly, positional information can be measured even before the emergence of a well-defined node, suggested a cell sorting mechanism for axial morphogenesis. Transcriptional analysis suggest that one aspect of sorting may involve a differential adhesion mechanism via Wnt-dependent expression of cadherin superfamily molecules.

7	**Christina McNerney**	**Johns Hopkins University**	***Intrinsic regulation of thyroid hormone signaling in human retinal organoids***

Thyroid hormone (TH) signaling is critical for normal metabolism and development. Endocrine regulation of TH on an organismal scale promotes metabolic health. In contrast, TH levels are regulated in individual developing tissues to control cell fate specification. How TH signaling is regulated locally during human development is poorly understood. To address this question, I studied how dynamic TH signaling specifies cone photoreceptor subtypes in human retinal organoids. TH signaling promotes red/green cone fate and suppresses blue cone fate. My data suggest that DIO3, a TH-degrading enzyme, is expressed in retinal precursor cells (RPCs) to ensure low TH signaling and generate blue cones early. Later, as RPCs differentiate and turn off DIO3, DIO2 (a TH-activating enzyme) is expressed in photoreceptor precursors and blue cones to increase TH signaling and promote red/green cone fate. In addition to these spatiotemporal dynamics, a negative autoregulatory feedback loop controls levels of TH signaling pathway genes within the retina. Genetically or pharmacologically elevating TH levels led to the activation of genes that degrade TH and repression of genes that activate, transport, and mediate signaling of TH. These perturbations of the local TH

negative autoregulatory feedback loop altered the ratios of photoreceptor types and generated mixed photoreceptor fates. These data suggest that, in addition to negative autoregulation of TH levels at the organismal scale, homeostatic regulation of TH levels is required locally in developing tissues for cell fate decisions during human development.

8	Neha Mohan	Drexel University	***HDAC6 is a novel therapeutic target for SPAST-based Hereditary Spastic Paraplegia revealed by transgenic mice and hiPSC-derived forebrain organoids***

Hereditary Spastic Paraplegia (HSP) is a neurodegenerative disorder that manifests as progressive weakness and spasticity of the lower limb. The pathological hallmarks of HSP include swelling and degeneration of the corticospinal tracts (CST) projected from the upper motor neurons of the motor cortex. Mutations in the SPAST gene lead to an autosomal dominant form of HSP, namely HSP-SPG4 (SPAST). SPAST encodes a microtubule (MT)-severing enzyme spastin, which regulates MT behaviors like MT acetylation and other important cellular functions. Consistent with previous findings, we detected hyperactivity of HDAC6, a major tubulin deacetylase, in the neurons of our transgenic mice and human induced pluripotent stem cell (hiPSC) derived forebrain organoids. Here, we tested the effects of a selective HDAC6 inhibitor, Tubastatin A (TubA) in transgenic mouse models, namely the “dHET” (double transgenic heterozygous, hSPAST-C448Y+/-;mSPAST-/+), which encompasses both SPAST-haploinsufficiency and gain-of-toxicity mechanisms of HSP-SPG4 and deep-layer cortical neurons and forebrain organoids derived from CRISPR–Cas9 based isogenic hiPSCs, including those with a disease relevant truncated mutation of SPAST (hiPSC-SPASTS245X). Daily TubA treatment for 3 weeks reduced the hyperactivity of HDAC6 in the dHET mice and was accompanied by a substantial increase of axonal MT acetylation. Catwalk analyses of gait behavior as well as anatomical studies for CST degeneration reveal therapeutic benefit from this treatment regimen. Treatment with TubA also rescued several of the degenerative abnormalities that we documented in the hiPSC-SPASTS245X derived organoids. Finally, subcellular fractionation-based analyses demonstrate enhanced cytoplasmic retention of HDAC6 induced by mutant spastin, supporting the possibility that HDAC6 misregulation may be central to the pathological mechanisms of HSP-SPG4. Collectively, these results introduce HDAC6 as a novel therapeutic target for HSP-SPG4.

9	Salem Mosleh	Harvard University	***A universal developmental program across avian adaptive radiations***

Darwin's finches and Hawaiian honeycreepers are important examples adaptive radiations where the bird beak diversified into a multitude of forms performing various functions. By investigating how transverse sections to the beak centerline evolve with distance from the tip, we find that an experimentally motivated, geometry driven, growth law that captured the range of observed beak shapes in Darwin's finches and Hawaiian honeycreeper, and predicts developmental constraints — beak shapes that are not allowed based only on the developmental program of the beak. All together, our study illuminates how a minimal combination of geometry and dynamics allows for functional form to develop and evolve.

10	Alex Plum	University of California, San Diego	***Epiblast area homeostasis in gastrulation***

Gastrulation is a pivotal step in development, transforming the pluripotent epiblast into the three germ layers and forming the foundations of the body plan. In avian gastrulation, division expands the epiblast, ingression shrinks it, and cell number densities rise and fall. And yet, remarkably, the total area of the epiblast remains constant. Single-cell resolution data show that cells' average apical areas and their rates of division and ingression vary in space and time such that some regions expand while others shrink to compensate. We model these cell-level processes to understand how they might regulate one another locally to achieve area homeostasis globally. Building on an existing mechanochemical model that successfully predicts tissue flows of gastrulation, we model changes in epiblast and extraembryonic cell number densities to assess changes to the shape and area of their respective domains. This biphasic model allows us to probe various causal mechanisms that might explain in-vivo correlations between cell-level processes and account for the maintenance of the epiblast's total area.

11	Louis Prahl	University of Pennsylvania	***Independent control over cell patterning and adhesion on hydrogel substrates for tissue interface mechanobiology***

Replicating organizational principles that establish fine-scale tissue structure is critical to our capacity for building functional replacement tissues. Tissue boundaries such as epithelial-mesenchymal interfaces are engines for morphogenesis in vivo. However, despite a wealth of micropatterning approaches available to control tissue size, shape, and mechanical environment in vitro, fine-scale spatial control of cell composition within tissue constructs remains an engineering challenge. To address this, we augment DNA “velcro” technology for selective patterning of ssDNA-labeled cells with long-term culture on mechanically defined polyacrylamide hydrogels. We co-functionalize photoactive benzophenone-containing polyacrylamide gels (BP-PA gels) with spatially precise ssDNA features that confer temporary cell adhesion and with extracellular matrix (ECM) proteins that confer long-term adhesion. We find that co-functionalization does not compromise ssDNA patterning fidelity or cell capture, nor hydrogel mechanical properties or mechanosensitive fibroblast spreading, enabling mechanobiology studies of precise cell interfaces. We then co-pattern colonies of fibroblasts and epithelial cells to study interface formation and extracellular signal-

related kinase (ERK) activity at cellular contacts. Combining DNA velcro and ECM functionalization approaches provides independent control of initial cell placement, adhesion, and mechanical environment, constituting a new tool for studying biological interfaces and for programming multicellular interactions in engineered tissues.

12	Xiaochen Qin	Lehigh University	***Patient-Derived Non-small Cell Lung Cancer Organoid in Hydrogel Towards 3D Tissue-Engineering Vascular Drug Screening Platform***

Introduction: Lung cancer is the leading cause of cancer death worldwide and is extremely heterogeneous across individuals, therefore, it drives a need for personalized medicine. The patient-derived organoids contain more heterogeneity and tissue architecture of the parental tumor than tumor cell lines, therefore providing the possibility of personalized medicine. Organ-on-a-chip platforms have been developed to be a compatible microenvironment of in vivo tumor. However, most 3D organoid models are inadequate to accurately recapitulate the roles of biological barriers and the extracellular matrix (ECM) involved in the organ-level drug response. Here, we present a vascularized bilayer microfluidic device enabling drug sensitivity test from patient-derived lung cancer organoid line (LCO) under a continuous physiologically relevant flow condition. As a proof of concept, the long-term culture of the microfluidic system is facilitated by our in-house developed bilayer device through active nutrition/drug supply import and toxin removal. Furthermore, we demonstrated the patient derived LCO lines for drug testing for efficient responses to the drug under a physiologically-relevant fluidic flow, thus providing an advanced personalized medicine platform under the vascularized-tumor-microenvironment to predict drug delivery in vivo.
Results and Discussion: We received fresh surgical discarded tissues with lung adenocarcinoma. We first manually minced the tissue into 1-2 mm pieces, followed by slicing the tissue further into 200*200*200 um3 using McIlwain Tissue Chopper. The organoid establishment and drug efficacy tests can be assessed using dead and live staining under confocal microscopy. Small organoids have started forming after 3 days and are continuously monitored over 2-3 weeks. The growth of the organoid can be measured by tracking individual organoid area changes. Organoids generated from mechanical dissociates were found to grow significantly faster than those from enzymatic dissociated. In our previous published work, Paclitaxel of a high concentration has negative effects on the healthy cocultured EC cells. To evaluate the efficacy of the patient-derived organoid to the anticancer drugs, we perfused the paclitaxel and doxorubicin as the model drugs into the system for 72 hours. The organoids were stained with live/dead and DAPI stains. Our result shows that the organoid was sensitively responded in the device to as little as 10nM of drug concentration.
Conclusions: Our tissue preparing method allows to efficiently establish organoid lines from as little as 0.01g of tissue. The 3D vascularized drug screening device provides an opportunity to produce hundreds of uniform organoids in a short time span. The in vitro antitumor drug screening platform presented consisted of two inlets with a single drug gradient or two-drug combination therapy.

13	Skandha Ramakrishnan	Drexel University College of Medicine	***The functional level of spastin is essential to axonal regeneration in vertebrate animals.***

SPAST, also called SPG4, encodes spastin, a multi-functional protein. Vertebrate SPAST has two start codons that produce a longer isoform called M1-spastin and a slightly shorter isoform called M87-spastin (M85 in rodents). Lines of evidence demonstrated that M87-spastin is a potent microtubule-severing protein that cuts long microtubules into short pieces, while M1-spastin is associated with the morphogenesis and homeostasis of ER. Previous studies on Drosophila showed a dose-dependent detriment in the ability of amputated axons to regenerate when spastin is reduced. Here, we sought to determine whether those observations extend to vertebrate axons and whether such functional impairment is isoform specific. Indeed, when spastin is either functionally inhibited or depleted in cultured rodent cortical neurons, the regenerative capacity of severed axons was significantly reduced and so too were neuronal activities, as indicated by impaired network firing and defective calcium signaling. Such defects were rescued in the spastin-depleted neurons in dose-dependent fashion by re-expression of M87-spastin, but not M1-spastin. Thus, we conclude that axonal regeneration, including the capacity of injured neurons to re-establish connections, depends on sufficient levels of M87-spastin. Furthermore, our findings suggest controlled overexpression of spastin as a novel therapeutic strategy for augmenting axonal regeneration after injury.

14	Sreejith Santhosh	University of California, San Diego	***Mechanics and a Turing mechanism pattern the early chick embryo***

Gastrulation is a morphogenetic process in early embryogenesis characterized by large-scale coordinated cell migration and differentiation. Before the onset of gastrulation, cell fate differentiation breaks circular molecular symmetry in the chick-embryo epiblast, a flat disk of ~60 thousand cells. At this stage, actomyosin cables are aligned azimuthally throughout. However, how these mechanical and molecular patterns arise and interact is not understood. We derive a theoretical framework that couples the cell division rate to the tissue mechanics, which predicts observed mechanical patterning in the epiblast. We then show that a Turing mechanism coupled with the mechanics can recapitulate the cell fate symmetry breaking in a pre- gastrulation chicken

epiblast. We verify our predictions with new chicken experiments in vivo and existing literature on pre-gastrulation patterning in cut embryo segments.

15	Friedhelm Serwane	LMU	*Mechanical and electrophysiological characterization of neural organoids*

Stem-cell derived neural organoids mimic particular aspects of the human brain and have made the exploration of neuronal network formation and function accessible in vitro. The underlying behaviour of neurons to connect to networks has been shown to be modulated by mechanical signals, such as the elastic modulus of the neuronal tissue.
My group is developing tools for the mechanical and electrophysiological characterization of neuronal organoids. I will present a minimal-complexity setup for volumetric imaging of their network activity(1). To extract Ca-signals we combine a lightsheet microscope as an add-on to a standard inverted microscope with computational tools, hereby providing an accessible system to the community. As a proof of principle, we demonstrate imaging of spontaneous activity in 3D. As a next step, we apply statistical models to quantify the network behaviour.
Changes in the tissue mechanical properties are one biophysical hallmark associated with tumour growth in the brain. We map the mechanical properties of glioblastoma cerebral organoids using ferrofluid droplets as mechanical actuators(2). We find significant differences in tissue elastic modulus in tumourous and non tumourous regions.
Mechanical and electrophysiological measurements performed in neural organoids inform researchers about the interaction between mechanics and function in the central nervous system.
1 Wysmolek et al., Scientific Reports, 2021
2 Serwane et al., Nature Methods, 2016

16	Xiaohuan Sun	Drexel	*Probing mechanisms for axonal degeneration in primary tauopathy using patient derived cerebral organoids*

In tauopathies, such as Frontotemporal Dementia (FTD), tau loses association with microtubules (MTs) and forms neurofibrillary tangles. Tau is an abundant MT-associated protein in neurons, which essentially regulate MT properties. Because pathological tau binds less avidly to MTs, which was thought to reduce the levels and stability of axonal MTs. However, this idea is based on the dogma of functional tau as a MT stabilizer, which was challenged by some recent studies via tau-knockdown strategies. The situation is further confounded in tauopathies because the pathological tau may elicit certain gain-of-toxicities on axonal MTs. To investigate this matter in a more disease-relevant scenario, we used 1) cerebral organoids generated from an FTD-patient derived induced pluripotent stem cells bearing a tauP301S mutation and their isogenic control corrected by CRISPR-Cas9; and 2) postmortem brain tissues from a cohort of FTD patients, as our models. Elevated soluble tau was identified in tauP301S organoids during the early development with an increased ratio of mature/immature isoforms (4R-tau/3R-tau); whereas an overt reduction in soluble tau was identified in late-stage developed organoids and postmortem FTD brains accompanied by increased aberrant PTM-modifications of tau. Strikingly, MAP6, a bona fide MT stabilizer that competes with tau's effects on MTs, presented opposite changes in its levels in tauP301S organoids at the early- and late-developed stages. Such bi-phasic changes in tau and MAP6 were consistently reflected by the predicted changes in MT dynamics. Interestingly, certain neurodegenerative features recapitulated in tauP301S organoids were ameliorated by some novel corrective strategies. We conclude that the bi-phasic changes in tau pathology with the subsequent MT defects may underlie the progressive neurodegeneration in tauopathy. Thus, therapies to correct tau and MT abnormalities in tauopathy must be vetted for the specific stages of the disease.

17	Carolina Trenado-Yuste	Princeton University	*Tumor progression and cell-cell coordination in 3D breast cancer spheroids*

Breast cancer is the most frequent cancer in women and the second-leading cause of cancer -related deaths worldwide. Although our ability to diagnose cancer has improved, it remains challenging to predict whether a specific tumor will evolve to become metastatic, or whether it will remain a benign lesion. Thus, new studies of cancer progression are needed to predict patient-prognosis from the morphology of early-stage tumors and decrease cancer-related deaths.
Here, we take advantage of 3D engineered culture models and computational models of breast tumors, to identify the key parameters of the early tumor that can be used to predict its dynamics and progression at later stages. We generate 3D breast cancer spheroids consisting of either epithelial-like or mesenchymal-like cells to examine dynamic changes in tumor-like anatomy and behavior. We characterize the individual cell migration within the tumor spheroids and identify both individual cell progression, as well as the cell proliferation and death in the different regions of the tumor. We are developing a 3D agent-based computational model to enable predictions of tumor progression from the morphology at earlier stages. The model consists of an initial, disordered network with embedded cells and accounts for cell migration, proliferation, and death within the tumor. We aim to use this combination of experimental and computational approaches to define the minimal set of parameters that can be used to predict tumor progression.

18	Evan Underhill	Princeton University	*Characterizing and Directing Signaling Gradients during the Growth of mESC-Derived Gastruloids*

When provided with a particular mechanochemical environment, collections of embryonic stem cells are capable of spontaneously generating complex 3-dimensional structures in vitro. One such structure, termed the “gastruloid,” acts as a cell culture-based model of vertebrate posterior elongation. Here, we employ this model to uncover connections between signaling pathway activity and the manifestation of final tissue shape. Using pharmacological inhibition and targeted pathway hyperactivation, we demonstrate that gradients in Ras/Erk and PI3K/Akt signaling are necessary to initiate and sustain unipolar tissue elongation. This work provides fundamental insight into the signaling drivers of vertebrate morphogenesis and opens the possibility of rationally directing the growth of novel tissue forms.

19	John Viola	University of Pennsylvania	*Probing the effects of mechanical transients in the nephron progenitor niche using organoid and 3D co-culture models*

Nephrogenesis is the process by which collections of mesenchymal progenitor cells within a stem cell niche form the highly organized filtration units of the kidney. The nephron progenitor stem cell niche must be able to balance differentiation and self-renewal of nephron progenitor cells as new nephrons are formed continuously throughout development from the progenitor pool. Understanding how this balance is maintained for continuous nephron formation is crucial for creating nephron-dense and structurally organized kidney organoids or replacement tissues. In vivo, the nephron progenitor niche exists in a highly dynamic environment at the actively branching tips of a rapidly growing epithelial network and is surrounded by stromal cells. Therefore, biochemical and physical properties within the niche that oscillate due to the migration and branching of the adjacent epithelial tissue are promising targets to regulate this balance in cell outcomes. We have shown through modeling and physical measurements that newly branching tips must overcome mechanical resistance, which generates increased mechanical stress within the niche that then dissipates over time. Analysis of scRNA-seq datasets of the developing mouse kidney shows that early differentiated nephron progenitors have a rapidly changing cytoskeletal state potentially reflecting a rapidly changing external mechanical environment. We introduced mechanical perturbations to human stem cell - derived nephron organoids to mimic the mechanical transients seen in vivo and observed increased nephron differentiation events. We next isolated nephron progenitor and stromal cells from the mouse embryonic kidney and found that the inclusion of kidney stromal cells changes the nephron progenitor differentiation response to mechanical perturbations. Crosstalk between these two cell types that impacts nephron formation has been identified in other work, however the nature of this crosstalk in the context of oscillating mechanical environments remains unexplored. In next steps we will use co-culture organoid models in controlled mechanical contexts to understand how these cell types interact within the niche over developmental time to balance nephron formation events. Understanding these properties would allow for engineering control of nephron formation in vitro leading to the development of potential replacement tissues for the 10% of adults that experience kidney disease.

20	Sifan Yin	Harvard University	*Three-dimensional chiral morphodynamis of chemomechanical active shells*

Morphogenesis of active shells such as cells is a fundamental chemomechanical process that often exhibits three-dimensional (3D) large deformations and chemical pattern dynamics simultaneously. Here, we establish a chemomechanical active shell theory accounting for mechanical feedback and biochemical regulation to investigate the symmetry-breaking and 3D chiral morphodynamics emerging in the cell cortex. The active bending and stretching of the elastic shells are regulated by biochemical signals like actomyosin and RhoA, which, in turn, exert mechanical feedback on the biochemical events via deformation-dependent diffusion and inhibition. We show that active deformations can trigger chemomechanical bifurcations, yielding pulse spiral waves and global oscillations, which, with increasing mechanical feedback, give way to traveling or standing waves subsequently. Mechanical feedback is also found to contribute to stabilizing the polarity of emerging patterns, thus ensuring robust morphogenesis. Our results reproduce and unravel the experimentally observed solitary and multiple spiral patterns, which initiate asymmetric cleavage in, *Xenopus* and starfish embryogenesis. This study underscores the crucial roles of mechanical feedback in cell development, and also suggests a chemomechanical framework allowing for 3D large deformation and chemical signaling to explore complex morphogenesis in living shell-like structures.

21	Muhmmad Sulaiman Yousafzai	NIH	*Defining the functions of myosin 2 isoforms in epithelia using intestinal organoids*

Mouse intestinal organoids provide a physiological context for the investigation of fundamental aspects of epithelial cell biology (e.g. cell:cell adhesion, maintenance of barrier function, cell division, and extrusion of apoptotic/aneuploid cells) because they retain a folded, crypt-villus topology in the form of a three-dimensional, regenerative, stem cell-driven tissue model. Here we imaged the localization and dynamics of endogenous myosin 2A (M2A) and myosin 2C (M2C) using organoids made from GFP-M2A and GFP-M2C KI mice. M2A localizes along the lateral surface of organoids, consistent with its known roles in supporting cadherin-based cell: cell adhesion at adherent junctions (AJs) and ZO-1-based cell: cell adhesion at tight junctions (TJs). M2A also localizes in stress fibers at the basal surface, consistent with a role in supporting integrin-based cell: ECM adhesion as well. Interestingly, M2A is seen not only in the cleavage furrow of dividing enterocytes, but also in evenly-spaced strings of cortical

mini-filaments that span the long axis of the dividing cell. shRNA-mediated knockdown of M2A in GFP-M2A KI organoids leads to the appearance of a multilobed phenotype that scales with the degree of KD (the dimmer the GFP-M2A signal, the more multilobed the organoid appears). Current efforts are directed at understanding the physical basis of this robust phenomenon. Finally, endogenous M2C localizes exclusively at the apical plane of an organoid, where it appears as a belt of mini-filaments that resides directly across from ZO-1 in TJs and is brightest at tricellular junctions. Current efforts are directed at creating confluent 2D organoids from WT and M2C KO mice to assess the role of M2C in maintaining barrier function using a variety of permeability assays.

22	**Chunzi Liu**	**Harvard University**	***The role of mechanical forces on diseased cell models and morphogenesis process***

The mechanical forces that cells experience play a crucial role in the development of multicellular organisms and the progression of diseases. This study aimed to investigate the impact of mechanical forces on diseased cell models and morphogenesis processes. We established a mucin-deficient ocular epithelial cell model for the diseased cell model to mimic the ocular surface conditions for a subset of dry-eye patients. We utilized rheology and interfacial science to characterize how frictional forces and interfacial tension impact the epithelial cell adhesions against external fluid phases. Our results demonstrate that the cortical tension of the cytoskeletal components, instead of the adhesive forces at the cell adherens junctions, was the predominant contributor to the mechanical integrity of the epithelial sheet, consistent with previous reports. In addition, the mucin-like glycoproteins on the ocular epithelial cells drastically reduce the frictional force imposed by the eyelid during spontaneous blinking motions and, therefore, function as a molecular barrier for external mechanical perturbation. To explore how mechanical forces can guide the morphogenesis process, we focused on the formation of cortex convolution patterns. We used a simple two-layer PMDS gel system to demonstrate that complex folding patterns can arise from a mismatch in substrate growth rates. Reconstruction of the gel system and the mature cortex convolution patterns showed qualitative agreements. In future work, we would like to perform a detailed morphometric comparison between the gel system and the adult cortex folding patterns and study the evolution of cortex convolution patterns among primate species.