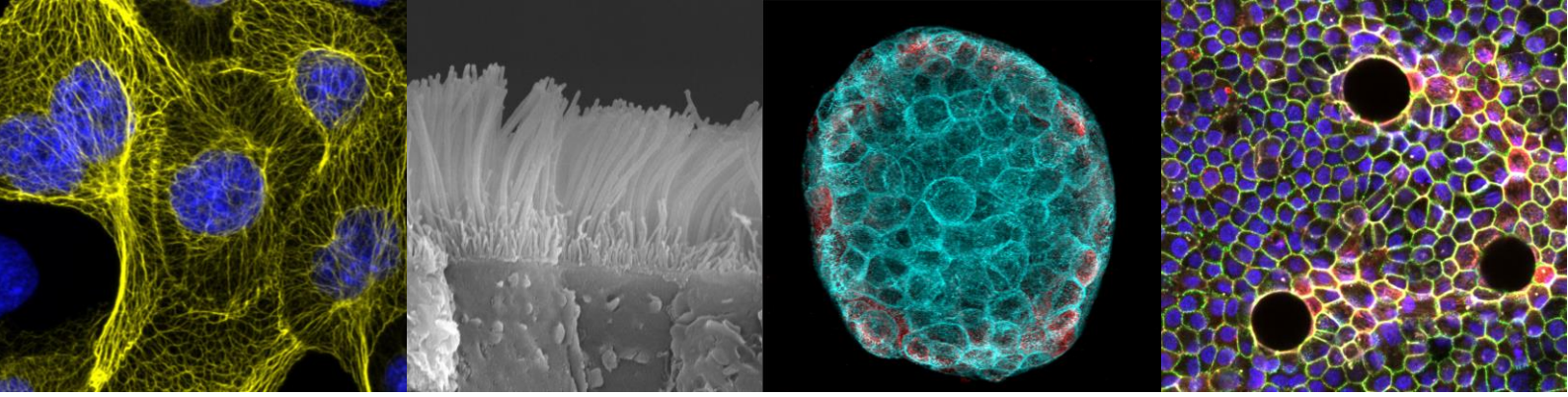

Bridging the Scales in Mechanobiology:

From Cytoskeleton to 3D Tissues

CYTAC meets ME3T

March 25-27, 2025

Göttingen

Cover image

Photo cc-by Ralf König

Bridging the Scales in Mechanobiology:
From Cytoskeleton to 3D Tissues
CYTAC meets ME3T Symposium
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16:00	**Stretch & Coffee Break** Foyer & Emmy Noether Room
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Keynote Talks

Mariaceleste Aragona

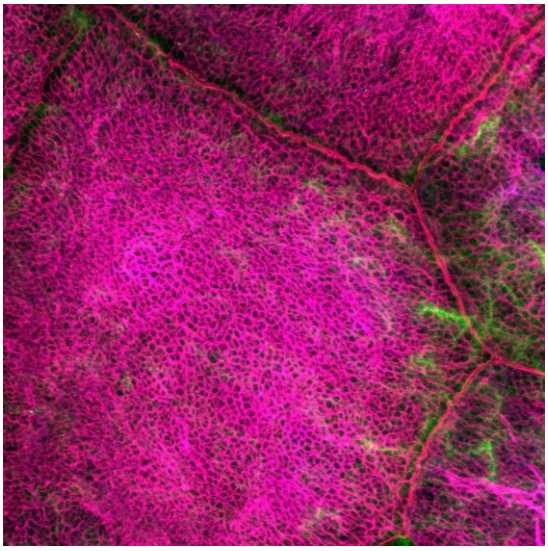
Novo Nordisk Foundation Center for Stem Cell Medicine

Faculty of Health Sciences

University of Copenhagen

Regulation of tissue architecture by mechanotransduction and stem cell dynamics to sustain homeostasis and repair

In the Tissue Architecture Laboratory, we study how biomechanics and signaling pathways shape tissues and guide cell fate decisions. We focus on the molecular mechanisms that determine stem cell identity and control stem cell fate in regenerating adult epithelia. We focus on tissues that are naturally experiencing various mechanical stresses, e.g. stretching and compression, such as the skin and the urinary tract. The lecture will present unpublished work on stretch-mediated skin expansion and the creation of in vitro bladder models to study urinary cycles and bladder urothelium regeneration. Our goal is to understand how different cell types work together to build tissues with specialized structures and functions, aiming to develop replacement organs for regenerative therapies.



Art and science by Oscar Axelsen "Cellular Shelter: Under protection of umbrella cells"

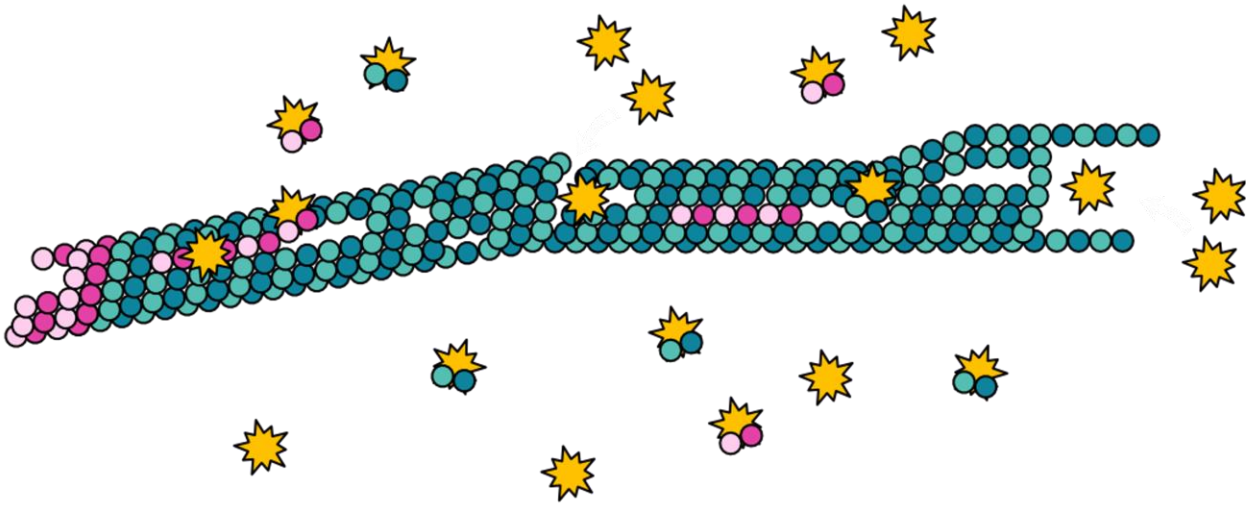
Laura Aradilla Zapata

Biophysics of the Cytoskeleton Lab

Saarland University

Are microtubules mechanical stress antennae?

Microtubules are rigid, hollow biopolymers and key components of the cytoskeleton, essential for cellular processes such as cell division, intracellular transport, and migration. Despite their high bending rigidity, microtubules frequently adopt highly curved conformations, suggesting that they experience significant mechanical forces from both intracellular and extracellular sources. Here, I will examine whether microtubules function as mechanosensors, thereby tuning cellular mechanosensitivity. I will explore the response of individual microtubules to mechanical forces, highlighting its relevance in a cellular context. Understanding how microtubules combine high rigidity with force sensitivity promises new insights into their role as mechanosensors.



Abstracts of Talks

Mapping nano scale muscle structure with X-ray diffraction

Hendrik Bruns

Institute for X-Ray Physics, University of Göttingen

Muscle contraction relies on the nanoscale structure of the sarcomere. It is driven by motor proteins arranged in filaments that are acting in unison to generate a macroscopic force. This molecular structure can be resolved by X-ray diffraction (XRD), that is sensitive to the regular arrangements of proteins inside the sarcomere. Cardiac muscle tissue is more disordered compared to skeletal muscle preventing effective XRD measurements. We have performed XRD experiments on individual cardiac muscle cells to overcome this challenge and were able to reveal their molecular structure. We complement these measurements by scanning XRD (sXRD) which reveals the two dimensional organisation of specific molecular features such as the actomyosin spacing inside the cell. In addition, we were able to perform sXRD experiments on human skeletal muscle tissue from patients with Duchenne-Muskeldystrophie (DMD), a severe genetic disease that eventually leads to a loss of muscle in young children. We used sXRD to identify the distribution of muscle and collagen inside the tissue, and mapped the quality of the molecular organisation of the muscle tissue inside the samples.

Keratin networks in epithelial cells under strain

Ruben Haag, Ruth Meyer and Sarah Köster

Institute for X-Ray Physics, University of Göttingen, Friedrich-Hund-Platz 1 37077 Göttingen, ruben.haag@uni-goettingen.de,

The cytoskeleton is mainly made up of microtubules, actin and intermediate filaments (IFs). The composition of the IF-network is cell-type specific and influences the viscoelastic properties of cells. In epithelial cells, the keratin IF network connects to desmomes in the cell membrane, while in the cell center keratin IFs can bind to the nuclear lamina via plectin proteins. The keratin IF network thus forms a mechanical link from the nucleus to the cell membrane. In in-vitro experiments, it was previously observed that IFs, unlike actin filaments, resist being stretched to high strains. We now ask whether this force-extension behavior of IFs is also relevant in whole cells and, more specifically, if mechanical signals from outside the cell are transmitted to the nucleus via the keratin IF network. To answer this question, we stretch cells both uniaxially to linear strains of 80 % and equibiaxially to area strains of 87 %. During stretching, we image the nuclei, deconvolve the images to recover their 3D shape, segment the nuclei and track each nucleus during stretching. This procedure allows us to investigate their deformation at increasing strain.

We compare wild type epithelial cells to keratin knockout cells to study the influence of the keratin IF network on the nuclei. Our results suggest, that the keratin network helps to adapt the nucleus shape to mechanical perturbation.

Cell-cell junctions during transepithelial trophoblast invasion

Liubov Izmaylova, Irmgard Classen-Linke and Rudolf E. Leube

Institute of Molecular and Cellular Anatomy (MOCA), Uniklinik RWTH Aachen, Wendlingweg 2, Aachen, 52074, Germany

Embryo implantation into the uterine endometrium is a distinct and highly species-specific process. Transepithelial invasion is essential for successful implantation, yet it remains one of the most enigmatic events in humans. To gain an understanding, it is important to define the exact cellular events and forces driving this process. It is initiated by adhesion of two epithelial cell layers, i.e., the embryonic trophectoderm and the endometrial surface epithelium, resulting in transepithelial invasion of the developing blastocyst and its subsequent invasion into the uterine stroma.

To investigate the role of cell-cell junctions and their associated cytoskeletal elements in this process, we developed an invasion model consisting of human trophoblast-derived spheroids (AC-1M-88 cells) attaching to and invading through a monolayer of human endometrial epithelial cells (adenocarcinoma-derived Ishikawa cells or primary human cells). Endometrial epithelial cells were grown on soft polyacrylamide hydrogels with a defined stiffness of 4 kPa to mimic the stiffness of healthy, non-pregnant endometrium.

This model enabled the 3D visualization of the invasion process. We monitored tight and adherens junctions as well as desmosomes by immunohistochemistry using confocal microscopy and transmission electron microscopy at invasion sites. We were able to detect the formation of heterologous junctions between endometrial and trophoblast cells.

In summary, our findings strongly indicate that the identified heterologous junctions between embryonal and maternal epithelia play a crucial role in the early stages of human embryo implantation.

Decoding Apical Cell Cortices: A Journey Through Image Analysis

Niklas Klatt and Andreas Janshoff

Institute of Physical Chemistry, University of Göttingen, Germany

Epithelial cells play a vital role in tissue formation and maintenance, responding to diverse forces that influence processes such as growth, migration and remodeling [1]. These cells are characterized by polarity, defined by distinct apical, lateral and basal membrane regions, and by differential expression of actin isoforms at the apical and basal surfaces [2]. Their mechanical response is largely determined by the structure and dynamics of their viscoelastic actin cortex [3].

In particular, γ-actin, which is mainly localized in the apical cortex, assembles into more rigid networks, whereas β-actin, which is located mainly in stress fibers, adopts a more flexible organization. The effects of the lack of the normally apically localized γ-actin isoform on the architecture of the apical actin cortex were analyzed by comparing wild-type and γ-actin-KO cell lines. For this purpose, apical cell cortices were isolated by means of the sandwich cleavage method [4].

Using a range of image analysis methods, we obtained insights into the mesh size, entanglement length as well as homo- / heterogeneity of the underlying actin network.

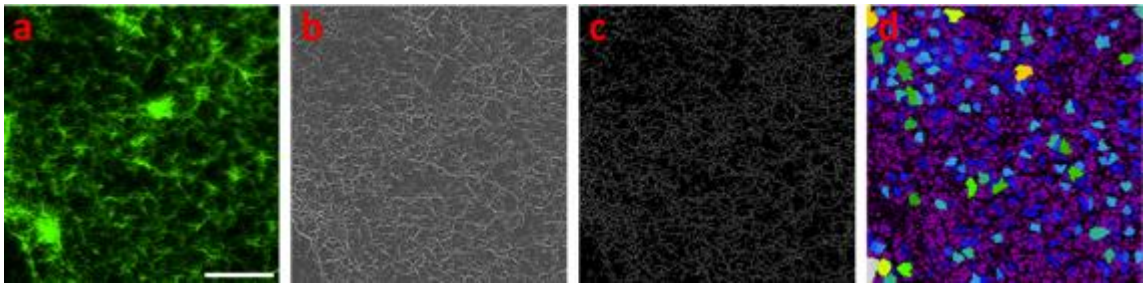


Figure 1: Typical pathway of analyzing the mesh size of apical cell cortices. An exemplary STED image of an apical cell sheet (a) was analyzed using a tube filter (b) and followed by skeletonization of the image (c). Obtained image after subsequent watershed segmentation (d). Scale bar 5 µm.

References

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Multicellular in vitro systems reveal Piezo1-mediated mechanotransduction in airway and lung

Alessa Pabst[a*], Andreas Ludwig [a] and Hannah Kubiza [b*], Lena Thiebes [b]

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*These authors contributed equally

Epithelial cells in both airway and lung parenchyma are constantly exposed to mechanical stresses such as stretch and wall shear stress. The mechanosensitive ion channel Piezo1 is one of the most prominent candidates to transduce these mechanical forces into intracellular signals by e.g. activating ADAM proteases. Beyond intracellular signaling, epithelial cells also engage in intercellular crosstalk with surrounding cell types at the alveoli-capillary interface as well as with the supporting stroma in the airways.

To mimic mechanical airway stimulation typical for tidal breathing, uniaxial stretch of bronchial epithelial and mesenchymal cell co-cultures in textile embedded fibrin gel scaffolds was performed at air-liquid-interface or with Piezo1-specific agonist Yoda1. An airway-specific stretch amplitude of 10 % with a frequency of 0.25 Hz was successfully applied to epithelial-mesenchymal co-cultures for 4 hours. Preliminary results show a visual alignment of cells in and against stretch direction, respectively, and Piezo1-mediated IL8 mRNA expression changes.

For investigations of the Piezo1-ADAM axis in the alveolar compartment, mechanical and chemical stimulation of the endothelial-epithelial interface was induced by either fluid shear stress to mimic the blood flow, stretch to mimic the breathing process or Yoda1. Applying physiologically high fluid shear stress of 15 dyne/cm2, as well as Yoda1, induces a strong increase in the activity of ADAM10/17 followed by the release of transmembrane proteins in endothelial cells. Moreover, a changed Piezo1 activity in epithelial cells mediates the ADAM-dependent release of signaling molecules involved in the induction of an inflammatory response in endothelial cells.

Utilization of physiologically relevant in vitro models and investigation of Piezo1-dependent cell interactions show the importance of Piezo1 mechanotransduction in the field of respiratory research. The mentioned results will pave the way to further investigate Piezo1-dependent molecular mechanisms, which could be relevant in onset and progression of common chronic lung diseases.

Age-Associated Cell Density Reduction Compromises Retinal Epithelium Biomechanics and Phagocytosis

Teodora Piskova[a,b], Aleksandra N. Kozyrina[a,b], Vasudha Turuvekere Krishnamurthy[a,b], Ragul Ravithas[a,b], Giedre Astrauskaite[e], Mohamed Elsafi Mabrouk[d], Sebastian Schepl[a,b], Alexandra Raab[a,b,c], Lok Sze Yam[a,b], Wolfgang Wagner[d], Massimo Vassalli[e] and Jacopo Di Russo[a,b,c]

[a] Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Germany, [b] Interdisciplinary Centre for Clinical Research, RWTH Aachen University, Germany; [c] DWI-Leibniz-Institute for Interactive Materials, Aachen, Germany; [d] Institute of Stem Cell Biology, University Hospital of RWTH Aachen, Aachen, Germany; [e] James Watt School of Engineering, University of Glasgow, Glasgow, UK

The mechanical properties of tissues are essential for their proper function and overall health. Tissue mechanical homeostasis maintains these properties within an optimal range, yet this balance is consistently challenged by natural remodeling processes associated with aging, including cellular turnover. This issue is particularly pronounced in postmitotic tissues, where the inability of cells to divide requires distinct temporal adaptations. The retinal pigment epithelium (RPE) is paradigmatic of this adaptation - it compensates natural apoptotic events by dynamic morphological reconfiguration and cytoskeleton remodeling. We used apoptosis-generated density reduction (AgeD) to establish an *in vitro* model that mimics the structural phenotype of human RPE with increasing age. The model recapitulates the reduced cell height, shortened apical microvilli and actin cytoskeleton remodeling as observed *in vivo*. This age-associated structural phenotype presents a significant biomechanical shift evidenced by tissue stiffening and enhanced elastic properties. AgeD-monolayers exhibit higher force at cell-cell junctions, demonstrated by vinculin and phospho-myosin light chain accumulation. Transcriptional profiling revealed global changes of the expression of actin-associated genes, including increased expression of actin nucleator subunit ARPC2 and actin bundlers (filamin-A, fascin-1), as well as decreased expression of formins (m-Dia1, m-Dia2, DAAM-1, DAAM-2) and membrane linker ezrin.

Functionally, AgeD-monolayers perform photoreceptor fragment phagocytosis differently. On average, cells engulf larger photoreceptor fragments and show less fragmentation, evidenced by reduced total number of internalized particles. Pharmacological inhibition of actin nucleator Arp2/3 during phagocytosis rescued the fragmentation in AgeD-monolayers, while formin inhibition in control monolayers led to larger average size of internalized fragments.

Altogether, we demonstrate that the age-associated structural phenotype of a postmitotic epithelium correlates with the establishment of a new mechanical equilibrium. In this new homeostatic state, the tissue possesses a different actin-remodeling capability, which leads to qualitatively different execution of phagocytosis. Besides valuable insight into postmitotic mechanobiology, our work delivers novel insights into the age-related changes of the mechanical homeostasis in the retina.

Quantification of stress generation and relaxation in model epithelium

Madhura Ramani[a], Kevin Höllring[a], Rudolf Merkel[b], and Ana Sunčana Smith[a,c]

[a] PULS, FAU Erlangen-Nürnberg, Erlangen, Germany; [b] IBI-2: Mechanobiology, Institute of Biological Information Processing, Forschungszentrum Jülich, Germany; [c]Group for Computational Life Sciences,Ruder Bošković Institute, Zagreb, Croatia

Mechanical forces influence cells through a process known as mechanotransduction, where cells within a tissue receive and respond to physical stimuli. Epithelial tissues are continuously subjected to external mechanical forces, and disruptions in tissue morphology and architecture due to mechanical stress are linked to various pathological conditions, including cancer. Understanding the effect of stretch deformation on tissue structure and function is therefore essential. In this study, we examine the response of epithelial tissue by culturing Madin-Darby canine kidney (MDCK) cells on a PDMS substrate, which is subsequently subjected to uniaxial stretch stress using a motorized stretching device. By applying uniaxial stretch stress to homeostatic tissue, we investigate the cellular and tissue deformation. Our focus is to quantitatively assess changes in cell morphology, particularly cell area, at both the tissue and single-cell level during stretch. Our approach offers valuable insights into the mechanical feedback mechanisms of epithelial tissue subjected to uniaxial stretch, contributing to the broader understanding of tissue biomechanics and the progression of mechanically influenced diseases.

Expanding the mechanobiological toolbox: light-tunable hydrogels for spatiotemporal manipulation of tissue mechanics

Felix Reul[a], Teodora Piskova, Vasudha Turuvekere Krishnamurthy, Aleksandra Kozyrina, Iulia Scarlat, Lok Sze Yam, Alexandra Raab, Yuxin Ji, Cédric Bergerbit, Laura De Laporte and Jacopo Di Russo

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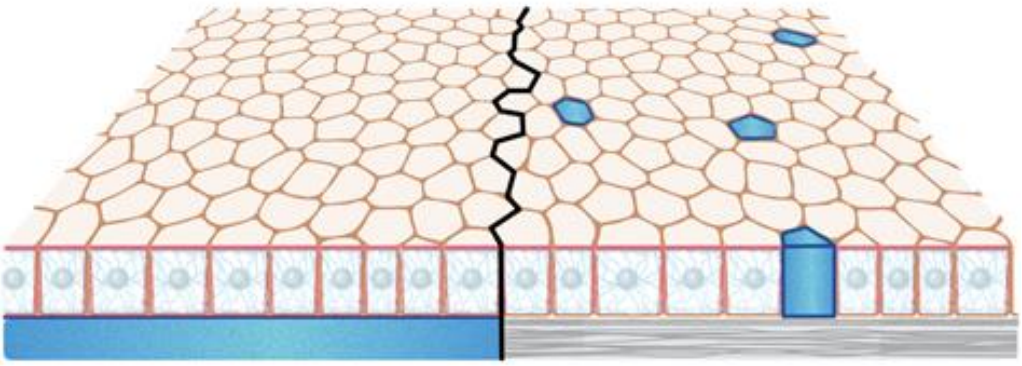
During aging, and disease, gradual extracellular matrix (ECM) remodeling as well as naturally occurring cell loss creates heterogeneity that influences cellular mechanotransduction and alters tissue mechanics. To better understand the mechanobiological aspects of such events, in vitro systems are needed to mimic such changes while minimizing complexity. My work aims to develop hydrogel-based tools to dissect the distinct role of mechanics in age-dependent outer retina remodelling both from the ECM and the intercellular perspective. To investigate the influence of ECM bulges (Drusen) formation, I established a light-tunable hydrogel, which can produce on-demand stiffness and topography changes in a live culture by triggering photodegradable moieties in the hydrogel's network. As proof of concept, I replicated the formation of ECM bulging and demonstrated that in-culture variations of bulges size and height affect morphometric parameters, actin cytoskeleton organization, and cell polarity. All of these are key hallmarks of functional impairment in the outer retina. To study the mechanical consequences on monolayer remodelling, as observed in ageing, I developed hydrogel-based light-tunable phantom cells. These phantom cells are functionalized with recombinant E-Cadherins and form hybrid junctions with adjacent cells. After disrupting the force balance within the monolayer by softening the phantom cell a biphasic tissue level response could be observed through actin cytoskeleton reorganization and nuclei deformation. In summary, I present a light-tunable hydrogel platform that allows precise spatial and temporal control over dynamic cell-matrix and cell-phantom cell interactions, offering novel insights into the mechanobiology of outer retinal remodelling during ageing and disease.



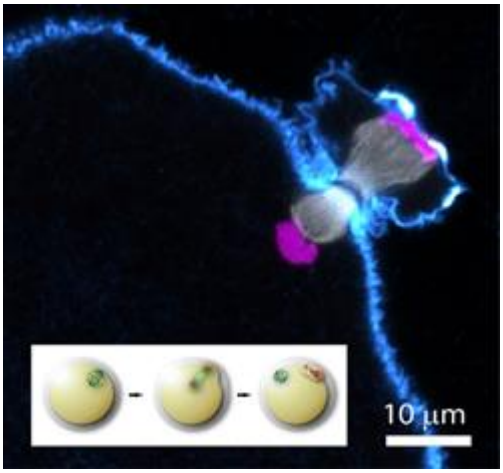
Light-tunable hydrogels with their respective application. Left: Light-tunable substrate mimic to recapitulate in-culture ECM remodelling. Right: Incorporated light-tunable phantom cells to challenge homeostatic stress balance on demand.

Functions of cytoskeletal filaments in mammalian oocyte division

Elena Todisco[a], Marios K. Martzoukos[a], Katarina Harasimov[a], Debojit Saha[a], Tommaso Cavazza[a], Eike U. Mönnich[a], Melina Schuh[b]

[a]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany, [b]Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany. melina.schuh@mpinat.mpg.de.

The oocyte is the largest cell in the mammalian body. It is formed through two rounds of meiotic division, producing a haploid cell and two small polar bodies. Mammalian female meiosis, in fact, is extremely asymmetrical, allowing the oocyte to eliminate the surplus of chromosomes while retaining essential cytoplasmic resources for early embryonic development. In mice, actin and myosin are known to drive spindle positioning to ensure asymmetry, however human oocytes display unique characteristics that suggest a potentially different mechanism [1]. At the same time, chromosomes must also be accurately segregated to ensure euploidy in the egg. While F-actin has been shown to assist microtubules in this process, a more direct role for its presence in the meiotic spindle has yet to be defined [2]. These critical processes have been associated to age-related infertility and pregnancy loss. In this study, we aim to underpin the specific cytoskeletal components, which ensure both spindle positioning and chromosome segregation are executed faithfully in mammals.



Example of mammalian oocyte during anaphase I. Actin in cyan, Microtubules in grey, DNA in magenta. Cartoon shows overview of meiosis I in oocytes.

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In vitro cytoskeletal networks through active microrheology at high strains

Shanay Zafari[a], Kristian Angeli Pajanonot[a], Pallavi Kumari[a], Raffaele Mendozza[b], Peter Sollich[b] and Sarah Köster[a]

[a]Institute for X-Ray Physics, University of Göttingen, Germany, [b]Institute for Theoretical Physics, University of Göttingen, Germany

The cytoskeleton is crucial in maintaining cell shape and overall structural integrity. It consists of three types of filaments, actin filaments (AFs), intermediate filaments (IFs), and microtubules (MTs). Force-strain curves of single IFs indicate nonlinear behavior with exceptional extensibility and remarkable resistance against rupture at high strains [1], while AFs break at low strains. Although rheology experiments have shown the extensibility of IFs networks [2], their nonlinear behavior remains unclear. A key gap in our understanding is whether these networks respond to external forces in a nonlinear manner, particularly under different strains and strain rates. Here, we show that the mechanical properties of vimentin and actin networks can be investigated through active microrheology with optical tweezers. We find that these two networks exhibit different responses to mechanical stress. Moreover, our results reveal that pure actin networks display fluid-like behavior, whereas pure vimentin networks show solid-like characteristics. These findings set the stage for a comprehensive study of combined networks, aiming to understand the role of IFs in composite cytoskeletal networks at high strain.

References

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Abstracts of Posters

P1 Structure and mechanics of strained membrane-bound F-actin

Laura Brinkmann, Fabien Büld, Nouhad El Jouni and Claudia Steinem

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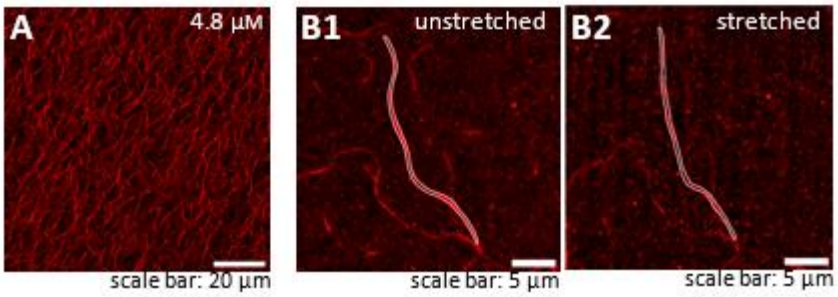
The mechanical response of cells, particularly under higher strains, is significantly influenced by intermediate filaments, which enhance cellular resilience and adaptability. While the actin cortex, tightly associated with the plasma membrane, governs cell shape, motility, and mechanical signalling, its interplay with vimentin intermediate filaments remains poorly understood. [1] Recent evidence suggests that actin and vimentin closely interact, yet their mechanical and structural interactions under strain are largely unexplored.[2]

To systematically investigate this interaction, we are developing a bottom-up in vitro model system that enables controlled studies of actin-vimentin crosstalk under mechanical stress. As a first step, actin filaments (A) were directly bound to a polydimethylsiloxane substrate to examine their strain response in the absence of vimentin. Optimized polymerization conditions yielded long, well-defined filaments, which were subjected to uniaxial stretching at different speeds. Laterally aligned filaments exhibited straightening and contour length extension (B1, B2).

Future experiments will incorporate vimentin intermediate filaments into the system to explore their influence on actin mechanics. This in vitro approach will provide fundamental insights into how cytoskeletal components coordinate under mechanical stress.



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P2 PEDOT:PSS/Gelatine conductive hydrogel blends for two-photon lithography patterning

Marco Buzio [a,b]*, Nevena Stajkovic[b], Valeria Criscuolo [a,b], Francesca Santoro [a,b,c]

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In the expanding field of organic bioelectronics, conductive hydrogels are emerging as key materials for the development of tissue-like and seamlessly integrated electronic devices. Hydrogels, which are hydrophilic cross-linked polymeric networks, serve as an effective bridge between traditional rigid electronics and soft-nature biological tissues and organs, thus addressing the inherent mechanical mismatch. These polymers are typically insulators but can be engineered to obtain electroconductive blends. Polymeric electroconductive materials, such as poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), are introduced as electroactive component to craft pristine or doped polymeric conductive hydrogels overcoming conductive inorganic materials limitations. Despite the enormous progress in the organic bioelectronics field, engineering of 3D patterning of conductive polymer materials at micro and nanoscale and the combination of biological and physical stimuli to better mimic the biological environment remains a challenge. This project aims to develop an electroconductive, photopatternable hydrogel composite by combining a methacrylate-modified gelatin matrix with the conductive polymer PEDOT:PSS to enhance the integration between bioelectronic devices and biological tissues. The use of advanced photopatterning techniques, particularly two-photon polymerization lithography (TPL), is of high relevance for engineering 3D surface topographies at the micrometer scale that can promote cellular adhesion while reducing interface impedance during signal transduction. Although PEDOT:PSS is inherently non–photo-crosslinkable, its incorporation into a photo-curable hydrogel matrix has opened new avenues for fabricating conductive materials with high spatial resolution. To evaluate the performance of this composite, its material properties, such as mechanical characteristics and electrical conductivity, are evaluated using nanoindentation and electrochemical impedance spectroscopy. Before seeding, cell culture substrates were patterned with the hydrogel formulation, effectively bridging the mechanical and functional disparity between rigid electronic devices and the flexible, dynamic nature of soft tissues. This approach facilitates the development of bioelectronic systems that integrate more seamlessly with biological tissues.

P3 Quantification of Actomyosin Network Dynamics – Activity Generation in Minimal Model Systems

Nilay Cicek, Kim Stöbener and Andreas Janshoff

Institute of Physical Chemistry, Tammannstraße 6, 37077 Göttingen, Germany, nilay.cicek@uni-goettingen.de, kim.stoebener@uni-goettingen.de

Cellular motility is defined as the movement of a cell accompanied by energy consumption. This is mainly accomplished by myosin motor proteins that interact with actin filaments and convert chemical energy into kinetic energy via ATP hydrolysis. Thereby, the motors slide filaments past each other, leading to the emergence of internal flow, contraction and directed motion events that can reshape the actin cytoskeleton and even lead to motility [1]. In these processes, other actin-binding proteins (ABPs) that change the network architecture by branching, bundling or crosslinking of actin filaments and thereby alter the network dynamics of this active matter are also involved. We are investigating actomyosin architecture, viscoelasticity, and activity outside of the complicated environment of cells by using passive and active microrheology as well as imaging techniques in vitro. To develop a deeper understanding of cellular motility, a higher level of complexity is added by the encapsulation of actomyosin networks into giant unilamellar vesicles (GUVs) and water-in-oil (W/O) droplets to create a minimal model of living cells. The interaction between the activity of gel and confinement will enable us to investigate the emergence of internal flow generated by the active matter and its consequences for self-propulsion.

References

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P4 Microtubule dynamics in the presence of actin networks

Saheli Dey, Claudia Marcelli, Komal Bhattacharyya, Tiago Mimoso, Stefan Klumpp and Sarah Köster

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The eukaryotic cytoskeleton is a composite network of biopolymers of variable bending rigidities and turnover times. It drives many important biological processes such as cell division and motility. In response to external and internal stimuli, the cytoskeleton rapidly rearranges itself. This property is achieved with the aid of microtubules and actin filaments, which are dynamic components of the cytoskeleton. Since these biopolymers co-exist in a cell, our focus lies on understanding microtubule dynamics in a composite network. Using a bottom-up approach, we investigate whether actin networks influence microtubule dynamics. Furthermore, we regulate the actin network architecture by assembling actin monomers either into a filamentous or a bundled network. Total internal reflection fluorescence (TIRF) microscopy serves as an essential tool to capture the dynamics of the microtubules in presence and absence of different actin networks [1]. Based on our analysis of kymographs, we quantify the polymerization and depolymerization rates of microtubules. Moreover, rescue and catastrophe frequencies indicate the influence of actin filaments on the stability of microtubules. Till now, studies have shown interaction between these two cytoskeletal filaments in the presence of motor proteins or cross-linkers [2]. Complementary to those results, our study provides insight into direct filament-filament interactions and will answer the question of what effect actin network architecture have on microtubule dynamics.

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P5 Light-responsive, actuating p(NIPAm-*co*-NEAm) microgels and microgel rods to mimic 3D epithelial deformation and 'train' skeletal muscle *in vitro*

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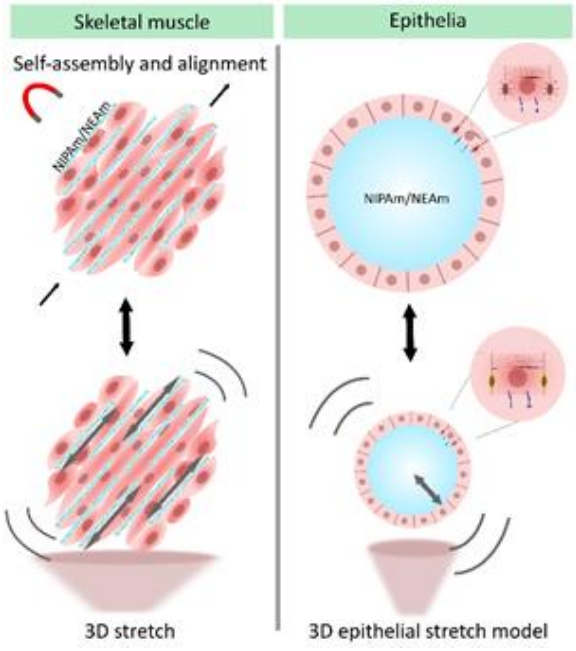
Many tissues in the body experience a cyclic mechanical deformation, such as the muscle, lung and intestine. To study the effects of these deformations, several in vitro systems have been developed, most of them applying stretch by relying on a motor or the application of a vacuum to a stiff, flat PDMS sheet. However, this does not resemble the soft, 3D environment that the cells experience in vivo. Therefore, we developed a light-responsive, actuating (NIPAm-co-NEAm) system [1,2,3]. The microgels are produced through microfluidics and are either spheres (Ø 140 µm) or rods (Ø 80 µm, length: 160 µm). Due to their thermoresponsive nature [4] and the incorporation of gold nanorods (AuNRs), the microgels can be collapsed upon irradiation with near-infrared light, as the AuNRs heat up due to plasmon resonance. When the light is off, the heat dissipates and the microgels quickly reswell again, allowing reversible deformation at frequencies up to 5 Hz.



Light-responsive, actuating p(NIPAm-co-NEAm) microgel rods (left) and spheres (right) are used to 'train' a skeletal muscle construct (left) or mimic 3D epithelial deformation (right).

We show the production and cell-adhesive protein functionalization of the microgels. We characterize the low stiffness (~1-100 kPa) of the microgels through nanoindentation, and observe their thermoresponsive behavior by laser actuation. Next, MDCK epithelial cells form a mature monolayer on the spherical gels, whereas C2C12 skeletal muscle cells are grown on the rods. We aim to investigate how cyclic deformation alters the mechanical state of the epithelial monolayer through cytoskeletal remodeling and activation of mechanosensitive proteins. Additionally, we intend to form a 3D construct of skeletal muscle cells and rods, and 'train' the construct to improve myogenesis.

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P6 The mechanical heterogeneity of the intracellular active viscoelasticity

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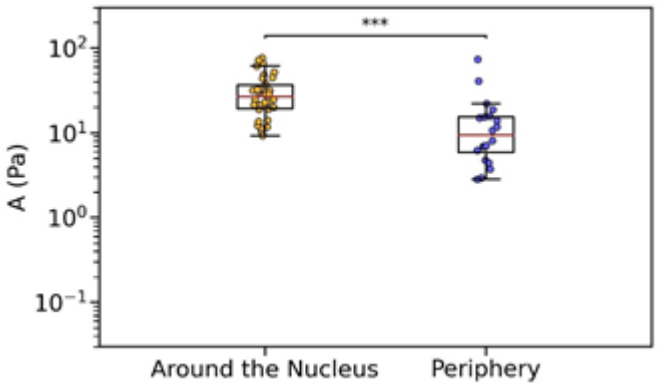
Mechanical properties of cells are crucial for biological functions like migration and division. While the mechanical properties at cell cortex level has been extensively studied, intracellular mechanical properties have only recently become experimentally accessible. Using optical tweezers for active-passive microrheology, we directly measure viscoelastic properties of cytoplasm. Despite the importance of intracellular mechanics in biological functions, we observe significant variation of these properties within a single cell type. To explain this, we propose that heterogeneity arises from local and temporal variations in cell compositions. To test this hypothesis, we create polarized cells using micropatterns [1] for spatially registered microrheology experiments [2]. Given the random distribution of probe particles in the cytoplasm, we aim to generate a 2D map of viscoelastic properties and examine its potential correlation with local composition of cytoskeletal elements. The preliminary results show that the cytoplasm is stiffer around the nucleus as compared to the periphery of the cell in low frequency regime (Fig.).



Stiffness of the cytoplasm in low frequency regime (A) is higher around the nucleus

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P7 Intracellular mechanics in migrating cells

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To fulfill their incredibly large number of different tasks, biological cells have developed mechanisms to adapt their physical properties and appearance [1, 2]. The proper control of these changes is crucial, as they are not only essential for healthy cells, but can also distinguish healthy from diseased cells [3]. Important examples related to such changes in mechanical properties are cell shape variation or cell migration. It is still not clear whether the changes in these mechanical properties are due to passive or active processes. Investigating and understanding these processes is the core of this work. For this, I will analyze the behavior of migrating cells, which are induced to move alternately on patterns and within channels. To connect the observed dynamics with the underlying mechanical properties and activities I will use the new quantity of mean back relaxation (MBR). Findings in this area could provide information for the big question of whether the mechanical properties of cells can be predicted by their activity.

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P8 Tracking Formins as They Add Single Monomers to Actin Filaments

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The actin cytoskeleton is a versatile and dynamic network that is comprised of many substructures with differing architectures, and consequently differing mechanical properties. Regulatory proteins that interact with actin control the shape and dynamics of these structures by defining the rate and localization of filament nucleation, the rate of assembly and disassembly, and interactions with other cellular structures. Formins, one class of such regulatory proteins, are required for the formation of several actin structures with distinct architectures and cellular functions. For example, the three fission yeast formins fus1, for3 and cdc12 are responsible for constructing the fusion focus [1], interphase actin cables [2], and the cytokinetic contractile ring [3], respectively. As a protein family, formins are defined by two conserved catalytic domains formin-homolgy 1 and 2, that enable them to nucleate actin filaments, catalyze their assembly and processively move alongside the filament tip as the filament grows and shrinks [4]. While structural biology methods have provided us with static molecular structures of formin-decorated filament tips [5, 6], the molecular dynamics of their growth have so far been difficult to access experimentally. Here, I show stochastic simulations of formin-catalyzed filament elongation, following two competing mechanistic models. I show that the spatiotemporal resolution of Minflux microscopy is sufficient to track formin movement at the speeds I observe in living cells. This finding serves as the basis for a detailed investigation of formin dynamics in living cells using Minflux microscopy. The aim of this study would be to connect differences in the molecular mechanism between different formins to the differences in the actin structures they build.

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P9 ECM Shear Strain Leads to Mechanosensitive Ion Channel Activation and Apoptotic Cell Extrusion in Early Breast Gland Development

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Human epithelial breast gland cells experience various mechanical extracellular matrix (ECM) stresses that regulate tissue development and homeostasis. To analyze cellular mechanoadaptation to ECM-transmitted shear strain, we developed a magnetic shear strain device. This system applies defined cyclic shear strain (≤15%, 0.2 Hz) over long time periods on developing, basoapically polarized human breast gland spheroids (MCF10A cell line) embedded in a laminin-rich EHS matrix (<60 Pa) with physiological rigidity. Our assay enables, for the first time, the investigation of instant cell response mechanisms via 4D live-cell imaging.

We demonstrated that spheroids increase their mechanical strain resistance during development, i.e., through basement membrane (BM) scaffold maturation and basoapical polarization. In poorly developed spheroids, long-term cyclic strain caused frequent apoptotic cell extrusion (71%), hindering proper differentiation. In contrast, matured spheroids remained widely insensitive to this mechanoresponse (12%), indicating altered mechanoadaptation mechanisms [1]. Notably, our data suggest distinct yet complimentary function modes of Piezo1 and TRPV4 channels in mediating increased Ca^{2+} influx-driven apoptosis upon cyclic straining. Preliminary data indicate reduced Piezo1 expression in highly matured spheroids, while TRPV4 remains unchanged.

Our novel device revealed that BM maturation and cell polarization protect breast gland tissue from aberrant ECM strain-induced and Piezo1/TRPV4-mediated cell death and loss of homeostasis. Furthermore, Piezo1 downregulation in matured spheroids may contribute to strain resistance. This in vitro approach enables the investigation of mechanobiological regulatory circuits in 3D multicellular clusters within mechanically active, tissue-like microenvironments.

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P10 Keratin Network Defects: A Gateway to Lysosomal Dysfunction?

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Intermediate filaments participate in various cellular processes, including differentiation, proliferation, and cell signaling, though the precise mechanisms remain unclear. Growing evidence suggests that intermediate filaments interact with organelles and contribute to subcellular organization by positioning and retaining them appropriately. In epithelial cells, keratins are the major intermediate filaments. Here, we show that mutations in the keratin network influence the localization and functionality of lysosomes.

To investigate subcellular localization, micropatterning was used to standardize cell shape. Cathepsin B and mitochondrial turnover assays were performed to assess degradation activity, while lysosomal pH was measured using a ratiometric biosensor. Compared to healthy cells, human keratinocytes carrying a Krt6aN171K or a Krt16R127C mutation showed accumulation of lysosomes at one side of the nucleus. Furthermore, lysosomes of the mutant cell lines displayed an increase in lysosomal pH, decreased lysosomal activity and reduced mitochondrial turnover. Overall, these findings support a significant limitation of lysosomal function.

This study offers new perspectives on the function of intermediate filaments that go beyond their role in protecting against mechanical stress. However, the exact relationship as well as the potential binding protein has yet to be determined.

P11 Impact of hydrogel-based 3D environment and externally applied mechanical stress on iPSCs

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The interplay of cellular and mechanical cues shapes cell fate decisions. Cells embedded in hydrogels exhibit an abundance of these cues that play a role in driving gene expression. Hydrogels, which are hydrophilic 3D substrates of polymer fibres, are utilized to embed cells with the objective of mimicking the *in vivo* microenvironment. In this study, we want to better understand the influence of the mechanical cues of hydrogels on iPSCs and whether the cells remain pluripotent or differentiate towards one of the three lineages namely endoderm, mesoderm, and ectoderm. For this purpose, we selected collagen and polyethylene glycol (PEG) hydrogels of varying stiffnesses to decipher whether properties of the gel and presence/absence of cell binding motifs would impact the survival of the iPSCs. To this end, we determined the actin-cytoskeleton interactions via immunofluorescence and analysed the cell differentiation patterns within the hydrogels through RT-qPCR and DNA methylation of pluripotency and germ-layer specific CG dinucleotides. On maintenance of iPSCs in collagen, there was formation of cellular aggregates from single iPSCs. We observed the expression of endodermal marker GATA6 in collagen gels along with the pluripotency marker OCT4, indicating the onset of differentiation. A preliminary analysis of the apical marker ZO-1 also indicated the formation of luminal structures when cells were embedded in collagen. When iPSCs were embedded in PEG hydrogels, a rapid decline in viability was observed unless they were embedded as 3D aggregates known as embryoid bodies. Furthermore, undirected differentiation of the embryoid bodies indicated endodermal differentiation in hydrogels of intermediate stiffness, suggesting that hydrogel stiffness guides cellular differentiation. Additionally, we investigated the application of an external shear stress to embryoid bodies for a defined period of time post-aggregation. The resulting embryoid bodies depicted increased expression of mesodermal marker (Brachyury) in comparison to the control embryoid bodies. All in all, we were able to show that mechanical cues, both emerging from the microenvironment and applied externally, can be crucial for cellular differentiation.

P12 Time-efficiency improvement of microgel production via particle replication in non-wetting scaffolds for interlinking under physiological conditions

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Previous work from the De Laporte group demonstrated that mechanical guidance cues offered by aligned rod-shaped microgels in a soft hydrogel matrix (Anisogel) promote directed and improved neurite growth. To address challenges with cell infiltration in early in vivo tests, Anisogel 2.0 features a scaffold of directly interlinked aligned microgel rods. The system is designed to interlink under physiological conditions in presence of cell culture media to ensure minimally-invasive injectability.

The initial phase of this project involved optimizing the lab scale microgel production process using the particle replication in non-wetting templates (PRINT) technique. By refining the harvesting, as well as the sterilization method, the production time per batch was reduced from 5-7 days down to 2-3 days while significantly increasing yield and maintaining crucial characteristics, such as stiffness, cell compatibility and sterility.

The current phase of the project focuses on interlinking these microgels into aligned scaffolds. This will be achieved by using a system of recombinant proteins that form a covalent isopeptide bond upon contact (SpyTag/SpyCatcher). The system reacts with high specificity and speed upon contact in presence of cell culture media. Microgels of varying compositions, stiffnesses, and dimensions are functionalized with epoxy groups to introduce reactive sites on the surface that can sequentially bind SpyTag/SpyCatcher proteins. Various spacer molecules and interlinking procedures are being explored to optimize protein accessibility on the surface, which is required for an efficient interlinking of the microgels.

P13 Microtubule dynamics are defined by conformations and stability of clustered protofilaments

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Microtubules are dynamic cytoskeletal polymers that add and lose tubulin dimers at their ends. Microtubule growth, shortening and transitions between them are linked to GTP hydrolysis. Recent evidence suggests that flexible tubulin protofilaments at microtubule ends adopt a variety of shapes, complicating structural analysis using conventional techniques. Therefore, the link between GTP hydrolysis, protofilament structure and microtubule polymerization state is poorly understood. In this work, we investigate the conformational dynamics of microtubule ends using coarse grained modeling supported by atomistic simulations and cryo-electron tomography. We show that individual bent protofilaments organize in clusters, transient precursors to a straight microtubule lattice, with GTP-bound ends showing elevated and more persistent cluster formation. Differences in the mechanical properties of GTP- and GDP-protofilaments result in differences in intra-cluster tension, determining both clustering propensity and protofilament length. We propose that conformational selection at microtubule ends favors long-lived clusters of short GTP-protofilaments that are more prone to form a straight microtubule lattice and accommodate new tubulin dimers. Conversely, microtubule ends trapped in states with unevenly long and stiff GDP-protofilaments are more prone to shortening. We conclude that protofilament clustering is the key phenomenon that links the hydrolysis state of single tubulins to the polymerization state of the entire microtubule.

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P14 Mechanobiological challenges related to hydrogel-based bioprinting technology for manufacturing novel 3D cell culture models

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Hydrogel-based bioprinting technology is one of the most widely used techniques in research to realize three-dimensional tissue models using various dispensing methods such as microextrusion and drop-on-demand. Most commonly, each technique utilizes an external mechanical force to dispense cell-laden hydrogel-based bioink through a nozzle and build the desired structure in a layer-by-layer approach. The resulting forces during the bioprinting process can alter the structural, physical and mechanical properties of the construct as well as the resulting post-printing response of the embedded cells within the hydrogel. This project aims to quantify these forces using computational fluid dynamics as well as perform experiments that investigate how different printing approaches or parameters can affect the cellular behavior and inner structure of bioprinted constructs. To achieve this in microextrusion methods, fibrous hydrogels such as collagen were printed non-planarly in a submerged bath to induce fiber orientation in three dimensions. The resulting anisotropy of the hydrogel structure and the cell response was quantified to prove that the internal structure of the bioprinted construct can be modified for the same geometry. For drop-on-demand systems, a novel approach that utilizes acoustic droplet ejection (ADE) to generate hydrogel-based tissue models was developed in our laboratories. Using this nozzle-free method, physiologically relevant cell densities can be achieved in printing with higher-viscosity bioinks, which remain a challenge for most drop-on-demand methods. A parameter study was performed on the acoustic bioprinter to determine optimal parameters for highest accuracy to be able to realize complex patterns and three-dimensional models with the system while preserving high-cell viability due to the lack of a nozzle.

P15 Anisotropic PEG-hydrogel system for aligned three-dimensional neuronal growth and motor neuron differentiation

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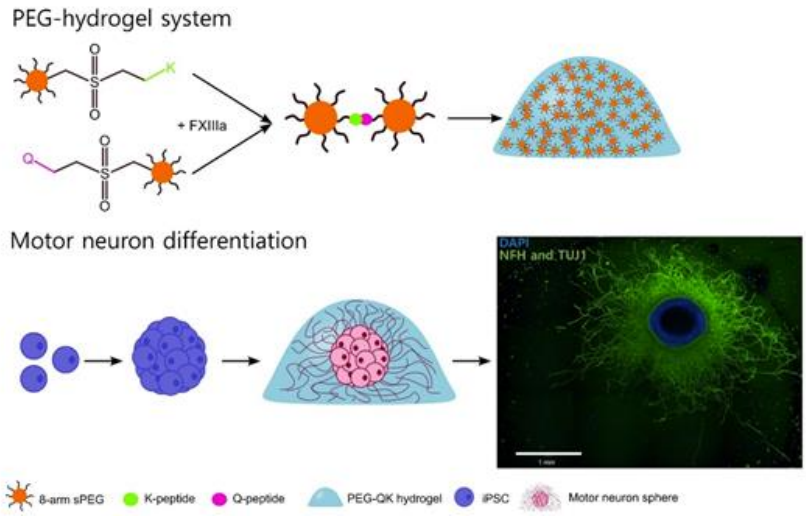
Spinal cord injury (SCI) is a severe neurological trauma characterized by irreversible damage to the spinal cord's neural tissue, which disrupts signal transmission between the brain and the body. Soft biomaterials can be utilized both for developing SCI disease models and for promoting the regeneration of damaged spinal cord tissue. In this study, an enzymatically crosslinked polyethylene glycol (PEG) hydrogel was employed as a soft matrix to support aligned and three-dimensional neurite outgrowth. To enable the analysis of human neuronal growth within this hydrogel system and to distinguish between motor neuron development, induced pluripotent stem cells (iPSCs) were differentiated into motor neurons within the hydrogel matrix. During differentiation, the hydrogel biomodified with IKVAV and fibronectin provided a chemically defined matrix, facilitating reproducible neuronal differentiation. In addition to facilitating three-dimensional nerve outgrowth, the alignment of neuronal growth was achieved in this study through the application of the Anisogel system. The PEG-Anisogel comprises magnetically alignable microgels functionalized with superparamagnetic iron oxide nanoparticles (SPIONs), enabling the induction of anisotropy within an otherwise isotropic hydrogel system [1, 2]. By utilizing this system in combination with the differentiation of iPSCs into motor neurons, this study presents a platform for the three-dimensional, aligned growth of human neurons.



PEG-hydrogel system with motor neuron differentiation. Confocal Z-stack image of 20.000 cell motor neuron sphere in PEG-hydrogel functionalized with IKVAV and Fibronectin; Blue: Nuclei, Green: Neurofilaments. Scale bar 1mm.

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P16 Determination of active force densities in filament networks as an inverse problem for the Stokes equation

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Biological cells rely on the interaction of proteins to perform various forms of movement such as cell contraction, division, and migration. In particular, the protein actin can create long branching filament structures which the protein myosin can bind to and slide along on. These acto-myosin networks produce mechanical stress resulting in movement in the inside of the cell that can lead to self-propulsion [1].

We aim to reconstruct the active forces inside of the droplet from noisy measurements of the velocity field. This results in an inverse problem for the Stokes equation.

We depict the simplified physical process of the flow inside such cells generated by acto-myosin networks using a 2-dimensional droplet model with the Stokes equation for incompressible Newtonian fluids. We provide a rigorous mathematical framework of the problem as well as numerical simulations of the reconstruction of the forces and apply our methods to experimental. In particular, we aim to investigate what information can be extracted from incomplete data using the Helmholtz decomposition for the purpose of partial reconstruction.

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P17 So far, no effect of substrate stiffness on the differentiation and functionality of an airway in vitro model?

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In the lung parenchyma, altered tissue stiffness caused by fibrotic remodeling of the extracellular matrix (ECM) has been described in depth. Similar observations have been reported in the small airways of patients with chronic obstructive pulmonary disease (COPD). However, studying and manipulating tissue mechanics both *in vivo* and *in vitro* is challenging. As a result, our knowledge about the influence of substrate stiffness on the airways is still limited.

The airways are lined with a specialized mucosa, which comprises a pseudostratified, ciliated epithelium and a vascularized layer of supporting stromal tissue. To characterize the effect of substrate stiffness on the differentiation and functionality of the airway mucosa, we applied our previously described *in vitro* equivalent.

This model consists of primary respiratory epithelial cells cultivated on top of a fibrin hydrogel supplemented with bronchial mesenchymal cells. Full differentiation into a mucociliary phenotype was achieved by cultivation at air-liquid-interface for four weeks. The mechanical properties of the hydrogel matrix were tuned by employing different concentrations of fibrinogen (0.8-3.3 kPa). Cellular composition and ECM production was assed using gene expression analysis and (immuno)-histochemistry. Ciliation and ciliary activity were characterized using electron microscopy and measurements of ciliary beating frequency.

Surprisingly, the ciliary beating frequency, as well as the expression of marker genes for basal, secretory and ciliated cells were not significantly affected in a stiffness-dependent manner. Gene expression of the ECM proteins collagen type I and fibronectin demonstrated a positive trend with increasing stiffness. However, principal component analysis revealed a donor-dependent segregation of all samples. Interestingly, an increase in stiffness was accompanied by a reduction of variability between donors.

Overall, these results show promising directions, but remain inconclusive. Further efforts will examine the combination of altered substrate stiffness with pro-inflammatory mediators to induce early fibrosis-like changes within the model.

P18 Early response analyses of desmosomes and keratin intermediate filaments under strain in an epithelial monolayer

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Desmosomes and keratin intermediate filaments are integral components of cell junctions, providing mechanical stability and integrity and are therefore found in tissues experiencing high mechanical forces. But how exactly keratins stabilize intercellular adhesion of desmosomes against strain is poorly understood.

To analyze the early response of desmosomes and keratin intermediate filaments to mechanical strain, we developed a stretching device, which can be incorporated into the stage of a confocal microscope. This enables mechanical stress applied to cells, which are seeded on a PDMS substrate and at the same time live cell imaging in a high resolution with a reduced focal drift.

By using the Fluorescence Recovery After Photobleaching (FRAP) method we were able to analyze the exchange kinetics of the transmembrane desmosmal protein desmocolin2-GFP in a Madin-Darby Canine Kidney (MDCK) monolayer, before and after short time stretch application. Here, amplitudes of 20 % and 50 % were used. My data suggest a decrease of exchange kinetics compared to the unstretched monolayer, indicating a force activated adhesion. Upon desmosomes maturation from a calcium-dependent to a calcium-independent hyper adhesive state (72 h), the FRAP measurements show no difference in exchange kinetics before and after stretch, revealing an increased core stability of the desmosomes.

When the keratin network is subjected to a mechanical stimulus, a more intense filament cage is build around the nucleus to protect the nuclear core. Furthermore, the filament density changes and with an extended mechanical stimulus (60 %, 4 h cyclic stretch, 300 mHz), the orientation of the filaments changes perpendicular to the stretching direction with an additional rearrangement of the desmosomal plaqueprotein desmoplakin.

P19 Effects of Chemotherapy on iPSC-derived Sensory Neurons

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Chemotherapy-induced peripheral neuropathy (CIPN) is a major adverse drug event during cancer treatment affecting about 68 % of patients within the first month [1]. Drug classes associated with CIPN are, among others, platinum-based agents and vinca alkaloids [2]. Severity of symptoms such as pain differ between drugs and individuals and are to date not effectively treatable or preventable [2].

Here, we aim to establish an in-vitro model of CIPN which allows to analyze changes of sensory neurons causing those symptoms in a drug and patient specific manner. We differentiate nociceptors from induced pluripotent stem cells of three cancer patients via an NGN1-driven forced expression protocol (modified from [3]). After maturation for 10 weeks, those sensory neurons are treated with the chemotherapeutic drugs oxaliplatin, cisplatin and vincristine. Drugs were chosen from the patients' treatment schemes and concentrations are based on their expected plasma levels during chemotherapy. With live-cell imaging and multi-electrode array (MEA)-recordings, the neurite outgrowth and electric activity during differentiation and treatment are investigated. Preliminary results showed that vincristine strongly impairs the structural integrity of neurons, whereas oxaliplatin treatment increases the firing rate.

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P20 UV-micropatterning for high-throughput generation of embryoid bodies

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Embryoid bodies (EBs) are three-dimensional aggregates of pluripotent stem cells. Due to their self-organized differentiation, they are of growing interest for drug screening and regenerative medicine. However, current methods for EB formation are difficult to implement for standardized, large-scale production in automated cell culture. We recently demonstrated that geometrically confined iPSC colonies spontaneously detach after 5-7 days of culture. The confinement within circular coated areas of vitronectin generated by micro-contact printing resulted in 3D aggregates of controlled size [1]. In this study, we have further improved the efficiency of the approach by using ultraviolet (UV)-micropatterning for the generation of homogeneous EBs. The micropatterning process involves generating a passivated surface, which is then selectively exposed to UV light to locally degrade cell-repellent molecules. The irradiated regions of the surface are then coated with vitronectin to enable cell adhesion and growth. Similarly to micro-contact printing, the iPSCs detached from these UV-microstructured substrates as EBs. We are currently investigating the effects of storage, cell seeding density, and size of the material structures on self-detachment of the cellular aggregate. Furthermore, we analyze the capability of self-organization into the three lineages of endoderm, mesoderm, and ectoderm in comparison to EBs generated with gold standard methods like AggreWell™ or Spin-EBs. Our UV-micropatterning method provides a homogeneous and automatable generation of large numbers of EBs. It can provide a standardized tool to investigate self-organization and differentiation potential for quality control of iPSC lines, such as PluripotencyScreen.

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P21 Bayesian Inference of Direct Interaction Strength Between Actin and Vimentin Intermediate Filaments

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The cytoskeleton is a composite network of actin filaments, microtubules, and intermediate filaments (IFs) that supports cell structure and enables key functions such as migration and intracellular transport. The interactions among these filaments are crucial regulators of such cellular functions. While direct interactions between two single vimentin IFs [1] and between a microtubule and a vimentin IF [2] have been demonstrated, the strength and nature of direct interactions between actin filaments and vimentin IFs remain unclear.

In this study, we numerically simulated the direct interaction between a single actin filament and a single vimentin IF using Cytosim, an open-source software addressing the cytoskeleton [3]. By applying Bayesian inference to the simulation data, we successfully estimated key interaction parameters. To validate this approach, we applied Bayesian inference to published experimental data on vimentin IF–vimentin IF interactions [1]. Our results align with previous findings [1], while offering a more precise parameter estimation. With this validated framework, we now aim to characterize the direct interaction properties between actin and vimentin IF.

Our findings highlight how Bayesian inference, in combination with numerical simulations, serves as a powerful tool for complementing experiments by uncovering interaction parameters that may not be directly measurable through experimental methods. This study provides new insights into actin–vimentin interactions, contributing to a more comprehensive understanding of cytoskeletal mechanics.

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P22 Mechanical Properties of Intermediate Filament Networks

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The mechanical strength and dynamics of cells are essential for sustaining life. For instance, during simple activities such as breathing or walking, cells are subjected to significant tensile stresses as they are stretched, sheared, or compressed. The cytoskeleton - a cross-linked composite network of actin, microtubules, and intermediate filaments - plays a central role in determining the cells' mechanical properties. While actin and microtubule networks have been studied extensively, this work focuses on intermediate filaments, such as vimentin and keratin. Compared to actin, intermediate filaments exhibit much smaller persistence lengths, but are much more stretchable with highly nonlinear elasticity. Extending the existing work [1] about freely-jointed chain (FJC) models by nonlinear stretching elasticity, a simplified model has been developed to investigate the mechanical and physical properties of cross-linked intermediate filament networks. Analogous to experimental approaches, the mechanical properties of the model are tested by applying normal and shear strains or stresses and analyzing the resulting responses.

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P23 Influence of ezrin mutations on the formation and mechanics of minimal actin cortices

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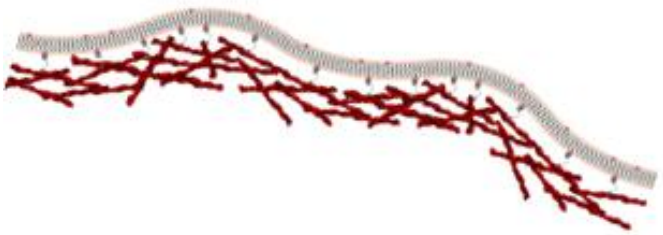

Actin is a highly conserved and versatile protein, making up 15 % of the total cellular protein and the actin cell cortex is a thin layer of a filamentous-actin network located just beneath the plasma membrane, encasing the entire cell with a dense, cross-linked network forming a mesh-like structure. This dynamic system undergoes constant polymerization and depolymerization, interacts with other cellular components like actin binding proteins, motor proteins, and signaling proteins, and contributes to cellular stability and motility.

Ezrin is a member of the ERM protein family, and it links the plasma membrane to the actin cortex of the cytoskeleton within the cell. Ezrin has been shown to be overexpressed in several diseases, including cancer, where it promotes dynamic remodeling of the actin cortex, facilitating increased cell motility, promoting cancer metastasis.

The precise amino acids involved in the interaction between ezrin and actin remain elusive, so this project aims to introduce point mutations into the actin-binding domain of ezrin and quantify their effects on binding affinity. By identifying essential residues, this study may provide insights into potential therapeutic targets for modulating ezrin-actin interactions.

This is realized by reconstituting minimal actin cortices on solid-supported lipid bilayers (SLB), using the physiological ezrin-PIP2 linkage. Confocal laser scanning microscopy (CLSM) in combination with a fluorescence micrograph analysis algorithm were used to assess the impact of ezrin mutations and anchor lipid concentration on actin cortex formation and drastic changes in actin-binding ability were found, when introducing just a single point mutation into the actin-binding domain of ezrin. Additionally, colloidal probe atomic force microscopy (CP-AFM) is employed to investigate the binding affinity of the different ezrin mutants by determining the dissociation rate constant of the ezrin-actin interaction.

P24 The oncogenic mechanobiological coupling of HRas-driven breast cancer invasion.

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Overactivation of the oncoprotein HRas is associated with increased tumor aggressiveness and invasiveness of breast cancer. A hallmark of cancer invasion is cell penetration of the basement membrane (BM) barrier and transmigration into the microenvironment. Previously, we demonstrated that tumor-like ECM stiffening and aberrant EGF signaling trigger actomyosin-driven cell contractility, eventually leading to increased mechanical BM stress and disruption [1]. This invasive transition of basoapically polarized, non-transformed breast gland spheroids (MCF10A-derived) is mediated by EMT-like cytoskeleton reorganization with formation of highly contractile actin stress fibers [2].

Inspired by these findings, we now investigated the impact of tumor-like HRas overactivation on BM disruption and cell invasion. Therefore, we generated BM-covered MCF10A_ER:HRasG12V spheroids, in which HRas hyperactivation can be pharmacologically induced at any developmental stage. We developed a traction force microscopy-based invasion assay to quantify the invasive outcome and the mechanical BM stress caused by actomyosin cell contractility. Activated HRas led to a significant invasiveness with a gradual increase on soft and rigid ECM stiffnesses. Unexpectedly, HRas-driven invasion was independent of elevated myosin II cell contractility, EMT-like actin stress fiber formation and MMP-driven BM-proteolysis.

In contrast, highly resolved live cell imaging analyses revealed opposite cytoskeletal changes that preceded activation, leading to mechanical BM deformation and local disruption events. Firstly, HRas-activated cells that contacted the BM scaffold increased compactness by reinforcing the cortical actin cytoskeleton. Secondly, cortical pulling densified the entire BM scaffold barrier. The local outward pushing of distinct cells, initiating BM breaching and transmigration followed this eventually.

Our study demonstrated that on tumor-resembling ECM stiffness, this newly discovered HRas mechanism resulted in a 100%-invasive outcome for originally non-invasive breast gland spheroids. At present, we are exploring the molecular bases of these processes.

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P25 Response of confined vimentin filament networks to applied strain

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Eukaryotic cells undergo high strains during cellular processes such as division, motility, and wound healing. The mechanical integrity of cells under these conditions is maintained by the cytoskeleton, mainly comprising actin filaments, intermediate filaments (IFs), and microtubules. Among these three filament types, IFs are the most extensible ones [1]. However, the role of IFs in modulating the mechanical response of cells under strain still remains unclear. In vitro studies on single vimentin IFs show that they exhibit tensile memory and can dissipate more than 70% of the input energy [2]. Since these filaments form networks in cells, it is crucial to extend this analysis to study network behaviour. Here, we present a microfluidic device that is compatible with fluorescence microscopy. We image reconstituted networks of vimentin encapsulated in microfluidic droplets. The device design, consisting of alternatively relaxed and constricted channels, allows the application of cyclic strain to these networks. Thus, this approach enables us to probe the mechanical properties of vimentin IF networks in a confined environment with the ability to manipulate the buffer conditions as well as the degree and nature of strain.

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P26 Myosin Processivity: Dependence on ATP and Effect on Cytoskeletal Network

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Motors proteins like myosin, kinesin are a major source of activity in cellular mechanisms like cell division and perform tasks such as maintaining cellular structure and transporting cargo within the cell. These motors form complexes of multiple motors and cooperate and give rise to complex behaviors not seen in single-motor dynamics. Myosin motors form medium sized (~100 motors) assemblies called myosin minifilaments that bind to and move along actin filaments. The mechano-chemical cycle of individual motors in the motor assembly is dependent on ATP. We are exploring the concentration of ATP as a control parameter for the processivity (walking distance) of myosin minifilaments through stochastic modelling. Here we propose processivity as a parameter to tune the activity in system. On the cellular scale, we explore the effect of processivity on cytoskeletal network structures at large. Preliminary results show that decrease in ATP concentration increases the processivity of myosin assemblies. However, the velocity of motor assembly decreases with decreasing ATP. Thus, an optimal trade-off between processivity and velocity must be maintained for efficient assembly performance. To study the effect of processivity on network level structures, we use the simulation package Cytosim. The simulation shows that higher processivity leads to a more pronounced contraction of the actin network.

P27 3D hiPSC-derived in vitro model to decipher the role of mechanics in retinal function

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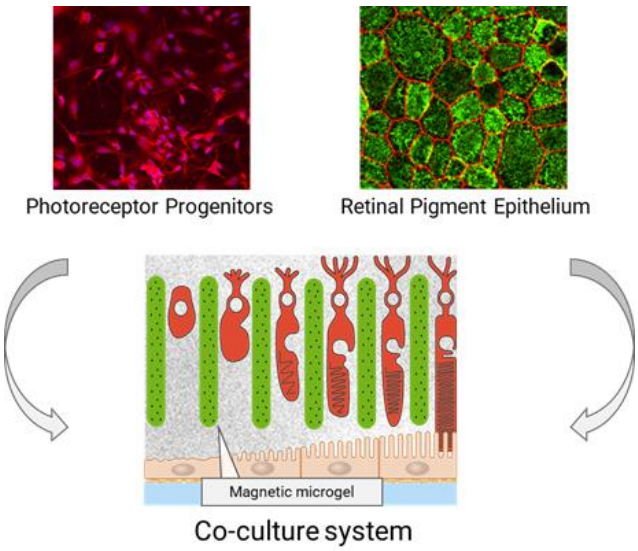
The retinal pigment epithelium (RPE) is a single layer of pigmented cells situated between the neurosensory retina and the choroid. On its basal side, the RPE adheres to an extracellular matrix (ECM) layer, while its apical side interacts with photoreceptor outer segments (POS), playing a vital role in maintaining their homeostasis and visual function. In recent research conducted by our group, we demonstrated that the contractility of ECM-defined RPE influences its functionality [1]. The current project seeks to further explore the role of ECM in retinal mechanobiology using a human-relevant model. We culture hiPSC-derived RPE on a synthetic and tunable polyacrylamide gel. On top of the RPE, we co-culture hiPSC-derived Recoverin+ photoreceptor progenitors along with magnetoreceptive microgels embedded in a hyaluronic acid hydrogel [2]. By varying the volume percentage of microgels within the hydrogel, we mimic either the anterior (ora serrata) or central/peripheral region of the retina. With this co-culture model established, we will systematically alter the synthetic RPE substrate to examine its effects on RPE-photoreceptor interactions, which are essential for vision.



Schematic of the outer retina co-culture model

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P28 Mechanics of chromosome capture by actin and microtubules in oocyte meiosis

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The accurate capture and alignment of chromosomes by spindle microtubules are crucial processes in cell division to prevent chromosome loss and aneuploidy. Somatic cells employ dynamic astral microtubules utilizing a "search and capture" mechanism, characterized by the dynamic growth and shrinkage of microtubules, along with lateral kinetochore attachments for efficient chromosome capture. In the distinctive context of oocytes, which exhibit large voluminous nuclei (70µm) with dispersed chromosomes and a meiotic spindle primarily localized near the cortex, the conventional mechanism is insufficient.

Previous research in starfish oocytes has shown a mechanism involving a contractile F-actin network. This network facilitates the transport of chromosomes within the capture range of aster microtubules (30µm) and simultaneously delays kinetochore-microtubule attachments, preventing chromosome loss. Interestingly, the mechanism governing chromosome aggregation in jellyfish oocytes (which are devoid of clear centrosomes at cortex) seems like that of starfish. They organize dynamic aster microtubules far cortex accompanied by F-actin shell contraction, crucial for efficient chromosome transport and capture. Though the role of actin seems conserved in transporting chromosomes to microtubules in both species, there are other mechanisms which govern the aster movement in jellyfish oocytes. Such as, aster microtubules are not anchored and formed far from cortex on nuclear envelope; after capturing chromosomes, the whole asters are moved to cortex followed by spindle assembly and polar body elimination.

Our findings indicate that aster movement in jellyfish relies on cytoplasmic flows and cortical actin changes. Detailed studies in starfish and jellyfish oocytes reveal conserved and divergent mechanisms for chromosome capture. This comparative analysis provides valuable insights into the dynamics insights of oocyte meiotic division.

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P29 Impact of adhesion on a Minimal Actin Cortex

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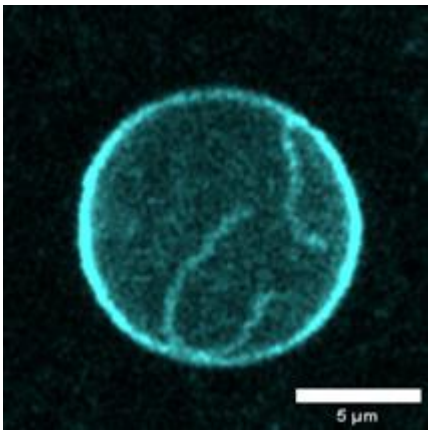
The cytoskeleton, a dynamic network vital for cellular functions like cell division, motility and morphogenesis, includes actin in its versatile architectures. In this work, the structure and mechanical properties of the actin cortex, a thin crosslinked network, that is bound to the cell's membrane, are investigated under adhesion.

To address this research question, my aim is to introduce an actin cortex into rapid-emulsion derived giant unilamellar vesicles (GUVs). Linkage of the actin network to the inner leaflet of the lipid membrane will be assured by the crosslinking protein ezrin and the receptor lipid phosphatidylinositol-4,5-bisphosphate (PIP2). Adhesion of GUVs will be applied using the improved light induced dimer (iLID) protein pair, by binding the Stringent starvation protein B (SspB) to the outer leaflet of the GUV and the corresponding part of the protein pair (iLID) to a planar substrate. Upon illumination, binding affinity of both proteins is increased drastically.

This approach allows for precise spatio-temporal control of adhesion and can therefore provide an unprecedented insight into the response of the actin cortex to adhesion and vice versa. Earlier work by BARTELT ET AL.[1] shows, that using the iLID protein pair and partial illumination of the GUVs can even induce a crawling behavior. Later, the systems complexity can be increased by addition of more cytoskeletal components like crosslinkers and motor proteins.



Actin bundle in a GUV

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P30 Development of a 3D Oriented Material Construct to Establish a Human Innervated Skin Model

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Mechanical pain on skin is transduced in epidermis by crosstalk between keratinocytes and nerve fiber endings, however it is unknown how keratinocytes and neurons communicate in this process. In this study, we aim to develop an *in vitro* innervated skin model to study the crosstalk between sensory neurons and keratinocytes. The model is shown in Fig.1. A multi electrode array (MEA) is used as the substrate and sensory neurons are cultured directly on top so that the neurons' activity can be monitored. An Anisogel [1], which contains magnetically oriented microgels, is cast on top of neurons with the aim to vertically guide growing neurites towards a keratinocyte monolayer on top of the Anisogel. When this is achieved, light-actuating microgels [2] will be incorporated into the keratinocyte layer to exert native-like mechanical force on the keratinocytes. This will enable us to study neuron activity changes caused by mechanical stimulation of the keratinocytes through MEA recording and better understand how neurons communicate with keratinocytes.

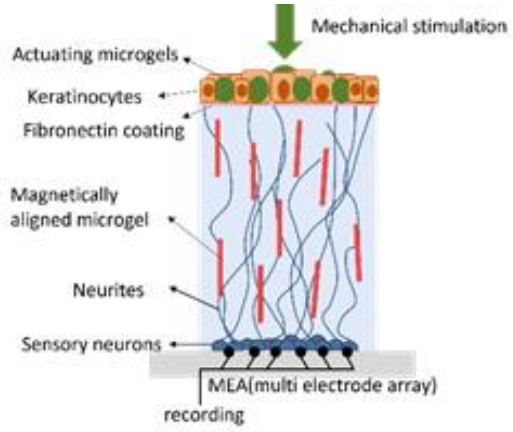

Figure 1. Illustration of 3D skin model

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P31 In vitro cytoskeletal networks through active microrheology at high strains

Shanay Zafari[a], Kristian Angeli Pajanonot[a], Pallavi Kumari[a], Claudia Marcelli[a], Raffaele Mendozza[b], Peter Sollich[b] and Sarah Köster[a]

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The cytoskeleton is crucial in maintaining cell shape and overall structural integrity. It consists of three types of filaments, actin filaments (AFs), intermediate filaments (IFs), and microtubules. Force-strain curves of single IFs indicate nonlinear behavior with exceptional extensibility and remarkable resistance against rupture at high strains [1], while AFs break at low strains. Although rheology experiments have shown the extensibility of IFs networks [2], their nonlinear behavior remains unclear. A key gap in our understanding is whether these networks respond to external forces in a nonlinear manner, particularly under different strains and strain rates. Here, we show that the mechanical properties of vimentin, actin and microtubules networks can be investigated through active microrheology with optical tweezers. We find that these three networks exhibit different responses to mechanical stress. Moreover, our results reveal that pure actin networks display fluid-like behavior, whereas pure vimentin networks show solid-like characteristics. These findings set the stage for a comprehensive study of combined networks, aiming to understand the role of IFs in composite cytoskeletal networks at high strain.

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Method Talks

Ruben Haag	Automated analysis of cell stretching and compression experiments
Elena Honscheid	Protons Gone Wild: Measuring Lysosomal pH Like a Pro
Alejandro Gómez Montoya	Acoustic Droplet Ejection for gentle cell transfer
Sophia Götz & Hajaani Manoharan	Application of Mechanical Forces in Vitro: In-house developed Shear and Stretch Devices
Mert Karpat	Monolayer compression of Keratinocytes
Jonas Penning	Lees-Edwards boundary conditions
Jannis Fischer & Mohammad Eskandari	Shaping the cells with micropatterning
Marios Martzoukos	Degradation of endogenous proteins by Trim-Away
Pratima Sawant	Droplet microfluidics
Raffaele Mendozza & Sanjay Bhandarkar	Active Soft Glassy Rheology as a model for cytoskeletal mechanics
Carolina Itzin & Iris Doolaar	Colloidal microgel building blocks for tissue engineering in 3D


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