Program

Physics of Cancer Symposium

Leipzig, September 24-27, 2013

DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

Greeting to the program „Physics of Cancer 2013“

Does physics play a role in cancer research? Of course, would be the spontaneous response of most of my colleagues while thinking of linear accelerators and high-field MRI; perhaps also of mass spectroscopy, X-ray crystallography and STED microscopy.

Many cancer researchers consider physics responsible for the indispensable images of the internal organs in tumor diagnosis. Additionally, it provides a whole arsenal of radiation with which to fight cancer. It also delivers fantastic analytical tools. Ultimately something of an ancillary discipline, one that aids the “real” cancer scientists – molecular- and cell biologists, biochemists, clinicians – to achieve their results and successful cures.

However, a most basic observation demonstrates the significance of physical characteristics in the very definition of what we call a tumor: A woman feels a lump in her breast. This means nothing other than that she recognizes tumor tissue as distinct from surrounding healthy tissue due to mechanical characteristics.

Cancer cells are continually exposed to physical forces: Tractive and shearing forces, hydrodynamics and hydrostatic pressures. These have to be countered by their own forces. There is hardly any doubt that a connection exists between tumor mechanics and the biology of cancer cells. This could be the key to understanding why and when cancer cells divide, how they move or embed themselves in tissues.

The spectacular results in molecular biology – from decoding gene function to analyzing complex signaling cascades – may be responsible that for a long time it has almost been forgotten that there is another viewpoint in cells, tissues and organs, - that of the physicist.

Although molecular biology has in previous years led to many successful approaches to targeted cancer therapy, it remains incontrovertible that for many cancers there are still no effective treatments. The fresh approach of another discipline could provide new impetus and identify new ways of effectively combating cancer. We cannot afford to decline it.

Otmar D. Wiestler

(Chairman and Scientific Member of the Management Board, DKFZ)

1. Program overview

Tuesday, September 24, 2013

Time	No.	Speaker	Title/Field
14.00 - 14.15		Josef A. Käs University of Leipzig	Opening of the Symposium
	Session I: Mechanical properties of cancer cells and the tumor microenvironment		
14.15 – 14.45	1	Josef A. Käs University of Leipzig	The Physical Bounds of *In Vivo* Cell Motility
14.45 – 15.15	2	Dennis E. Discher University of Pennsylvania	Trafficking, sorting, and differentiation in relation to nuclear rheology
15.15 – 15.45	3	Fred MacKintosh UV University, Amsterdam	
15.45 – 16.15		**Coffee Break/Discussion**	
16.15 – 16.45	4	Sara Kaliman University of Erlangen	Novel growth regime of MDCK II model tissues on soft substrates
16.45 – 17.15	5	Kris Noel Dahl Carnegie Mellon University	Nuclear architecture, mechanics and movements
17.15 – 17.45	6	Muhammad Zaman Boston University	
17.45 – 18.00	7	Kristin Seltmann TRM and University of Leipzig	Keratins are major determinants of migration and invasion by regulating adhesion and cell stiffness
18.00 – 20.00	**Poster Session** with discussions, snacks and fingerfood - in front of lecture hall -		

Wednesday, September 25, 2013

Time	No.	Speaker	Title/Field
	Session II: Cell-cell and cell-matrix adhesions in cancer		
08.30 – 09.00	8	Jens Elgeti Forschungszentrum Jülich	
09.00 – 09.30	9	Ana J. García Sáez Max Planck, DKFZ and University of Heidelberg (Bioquant)	
09.30 – 10.00	10	Ulf Anderegg University of Leipzig	Cross talk of melanoma cells and peritumoral fibroblasts influences melanoma proliferation
10.00 – 10.30		**Coffee Break/Discussion**	
10.30 – 11.00	11	Arnoud Sonnenberg Netherlands Cancer Institute	
11.00 – 11.30	12	Luke Casserau University of California, San Francisco	
11.30 – 12.00	13	Rudolf Merkel Forschungszentrum Jülich	
12.00 – 12.15	14	Mithila Burute Cytoo Grenoble	Centrosome positioning during Epithelial to Mesenchymal Transition
12.15 – 12.30	15	Kristin Mills Max Planck Institute for Intelligent Systems, Stuttgart	Elasticity and the shape of prevascular solid tumors
12.30 – 14.30		Lunch	
	Session III: Cytoskeletal dynamics in cancer		
14.30 – 15.00	16	Bettina Weigelin Radboud University Nijmegen	Intravital tissue imaging of collective tumor cell invasion: interface guidance and resistance niches
15.00 – 15.30	17	Harald Herrmann DKFZ Heidelberg	Mechanistic principles of the nuclear lamina: How is lamin assembly generating tough geodesic structures and why do the four lamins segregate?

17.00 Get Together for the invited Speakers (Boat, Stelzenhaus)

Thursday, September 26, 2013

Time	No.	Speaker	Title/Field
	Session IV: Mechanics of single cell and collective migration during malignant cancer progression		
08.30 – 09.00	18	Ben Fabry University of Erlangen	Cell migration through narrow pores: matrix mechanics versus geometry
09.00 – 09.30	19	Ulrich Schwarz University of Heidelberg	
09.30 – 10.00	20	Buzz Baum University College London	The importance of being well rounded
10.00 – 10.30		**Coffee Break/Discussion**	
10.30 – 11.00	21	Jörg W. Bartsch University of Marburg	Signalling in the tumour microenvironment: Essential roles for extracellular ADAM proteases in malignant tumours
11.00 – 11.30	22	Robert H. Austin Princeton University	
11.30 – 12.00	23	Xavier Trepat Institute of Bioengineering of Catalonia, University of Barcelona	
12.00 – 12.15	24	Adrien Hallou University of Cambridge	Cancer metastasis: collective invasion and cell guidance in multicellular systems
12.15 – 12.30	25	Benjamin Bangasser University of Minnesota	Optimality of force transmission in a motor-clutch cellular adhesion model
12.30 – 14.30		Lunch	
	Session V: Role of the endothelium in cancer disease		
14.30 – 15.00	26	Rudolf Leube RWTH Aachen University	
15.00 – 15.30	27	Rodger D. Kamm MIT Boston	
15.30 – 16.00	28	Melody A. Swartz Ecole Polytechnique Federale de Lausanne	
16.00 – 16.30	29	Franziska Lautenschläger Universität d. Saarlandes	
16.30 – 17.00	30	Jürgen Behrens University of Erlangen	Macromolecular protein complexes in Wnt signalling
17.00 – 17.30	31	Matthias Rief TU Munich	

19.00 Get Together for the invited Speakers (Gewandhaus)

Friday, September 27, 2013

Time	No.	Speaker	Title/Field
	Session VI: Biomechanics and tumorigenesis		
08.30 – 09.00	32	Jörg Galle University of Leipzig	
09.00 – 09.30	33	Marija Plodinec University of Basel	
09.30 – 09.45	34	Gemma Lancaster Lancaster University	Identifying malignant melanoma through unique signatures in blood flow dynamics
09.45 – 10.00	35	Jing Guo Charite-University Medicine Berlin	High resolution magnetic resonance elastography of the in vivo human brain: Application to presurgical mechanical characterization of glioblastoma
10.00 – 10.45		**Coffee Break/Discussion**	
10.45– 11.15	36	Michael Murrell University of Chichago	
11.15 – 11.45	37	Christoph F. Schmidt University of Göttingen	Mechanosensing with primary cilia
11.45 – 12.15	38	Klemens Rottner University of Bonn	Molecular dissection of lamellipodium protrusion
12.15 – 12.45	39	Paul Janmey University of Pennsylvania	Pressure and stiffness sensing in glioma cells
12.45 – 13.00		The End	

2. Abstracts of invited and contributed talks

1.

The Physical Bounds of *In Vivo* Cell Motility

Josef A. Käs

Migration of cells through tissues is quintessential for wound healing, neuronal plasticity, and the functioning of the immune system. In disease it is also a key determinant of cancer metastasis and nerve regeneration. Mammalian tissues are a new state of active fluid matter. A broad range of different cell types demix like non miscible fluids building natural boundaries for migrating cells. At least to some extent the cells are hold back by an effective surface tension, which is determined by cell-cell adhesion and cell contractility. Individual cells in tissues behave very much like active soft colloids. Thus, cells have a high probability to get jammed when moving through tissues and collective cell assemblies are close to be frozen by the glass transition. Cells that effectively move through tissues and are able to transgress tissue boundaries are softer and more contractile than cells that stay local in tissues. Soft and contractile avoids jamming and is optimal to overcome boundaries. Naturally, softness has to have its limits. So neuronal growth cones are too soft to carry large loads and thus excessively weak to move efficiently e.g. through scar tissue, which is required for nerve regeneration. Whereas cancer cells optimize their biomechanical and contractile properties for metastasis during tumor progression. In synopsis, the physical bounds that the functional modules of a moving cell experience in tissues may provide an overarching motif for novel approaches in diagnosis and therapy.

4.

Novel growth regime of MDCK II model tissues on soft substrates

Sara Kaliman [1], Christina Jayachandran [2], Carina Wollnik [2], Damir Vurnek [1], Florian Rehfeldt [2], **Ana-Sunčana Smith** [1]
[1] Institute for Theoretical Physics Friedrich Alexander University Erlangen-Nürnberg
[2] 3rd Institute of Physic-Biophysics, University of Göttingen

Although single cell mechanosensing is a well studied phenomenon that has been abundantly reported in literature, it is believed that once cells grow in a monolayer their substrate sensitivity is lost. We oppose this by reporting a novel growth regime of MDCK II model tissues on soft substrates (0.6 kPa). On hard substrates the cluster growth is accompanied by cell densification process. We have analyzed this process and characterized the restructuring of the tissue organization that it induces. A monolayer on hard substrates reaches the steady state density in the bulk of the cluster while the edge of the cluster remains at a low density. We have found that this process is independent of the cell seeding method. In contrast, on soft substrates cells form 3D clusters that nucleate a very dense monolayer in the middle right upon reaching the critical cluster size. After that the clusters grow in elongated shapes keeping the same spatially homogeneous density independent of cluster size. The density of monolayers on soft substrates is twice the steady state density attainable on hard substrates. On all the substrates, the tissue undergoes an aging phenomenon whereby the morphology of the nuclei is changed, and the actin cortex restructured. The density in the bulk of the clusters becomes inhomogeneous which causes reduced static correlations in the tissue. We find that the problem of dome and tubule structure appearance in MDCK monolayers can be avoided by substituting glass substrates with hard (>3kPa) PA gels that induce the same growth regime. Furthermore, we report that tissue structure is well approximated by Voronoi tessellation constructed from the cell nuclei center of mass.

5.

Nuclear architecture, mechanics and movements

Kris Noel Dahl
Associate Professor
Department of Biomedical Engineering
Department of Chemical Engineering

The sequencing of the human genome has provided a wealth of scientific information, but this information is limited by the poor understanding of the mechanisms which control gene expression. The genome within the nucleus is a complex, self-assembled polymeric structure with unique rheological properties. The structure and mechanics of the nucleus is altered in cells undergoing phenotypic changes. For example, cells with a broader spectrum of gene expression patterns, including stem cells and cancer cells, have much softer nuclei whereas aged cells have stiffer nuclei. We measure the mechanics of these different cell types, and we also examine the role that force and cytokine treatment play in altering nuclear mechanics and gene expression. Motor activity from the cytoskeleton impact the driving force for nuclear and subnuclear movement, and altered chromatin condensation shifts the resistance and propagation of forces. The mechanics of the nuclear interior is capable of responding proportionally to mechanical and chemical stimuli. We find distinct temporal regimes of subnuclear movements as nuclei adapt to applied force by stiffening. Thus, nuclear structure and mechanics may be an important regulatory factor in directing or modulating global changes in gene expression.

7.

Keratins are major determinants of migration and invasion by regulating adhesion and cell stiffness

Kristin Seltmann[1], Anatol Fritsch[2], Josef A. Käs[2], John E. Eriksson[3] and Thomas M. Magin[1]
[1] TRM Leipzig & Institute of Biology, Division of Cell and Developmental Biology, University of Leipzig, Philipp-Rosenthal-Straße 55, 04103 Leipzig, Germany
[2] Division of Soft Matter Physics, Department of Physics, University of Leipzig, Leipzig, Linnéstr. 5, 04103 Leipzig, Germany
[3] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Biocity, POB 123, FI-20521 Turku, Finland.

Cell motility is crucial during skin morphogenesis, wound healing, inflammation and malignant progression. These processes require remodeling of the keratin cytoskeleton, involved in cell-cell and matrix adhesion in several epithelia including the epidermis. However, the role of keratins in migration and invasion is only partially understood.
Here, we address this issue in murine keratinocytes depleted of all keratins by genome engineering. The absence of the entire keratin cytoskeleton leads to loss of plectin from hemidesmosomes and higher β4 integrin dynamics in keratin-free cells. To investigate the functional consequences, migration and adhesion assays were performed. These revealed that in the absence of keratins, keratinocytes adhere much faster and migrate ~2 times faster compared to wildtype cells. In addition, invasion was also increased in a Boyden chamber assay. In contrast to prediction, keratin-free cells showed a much higher cell deformability using the optical stretcher with minor contribution of actin. Re-expression of the single keratin pair K5/K14 fully reversed the above phenotype. Our data uncover a novel role of keratins in the maintenance of hemidesmosomes upstream of plectin and thereby influencing adhesion, directed migration and invasion with implications for epidermal homeostasis and pathogenesis. This study supports the view that downregulation of keratins observed during epithelial-mesenchymal transition directly contributes to the migratory and invasive behavior of tumor cells.

10.

Cross talk of melanoma cells and peritumoral fibroblasts influences melanoma proliferation: Regulation of hyaluronan synthesis in fibroblasts

Anderegg U, Willenberg A, Saalbach A, Simon JC
University of Leipzig, Dept. of Dermatology

Hyaluronan (HA), an ECM component, plays a pivotal role in tumor progression. Stroma-derived HA may support tumor cell proliferation and migration. Previously we have shown that soluble mediators from tumor cells of the Malignant Melanoma elevate the synthesis of HA in stromal fibroblasts through the induction of HA-Synthetases (HAS) 1 and 2. Based on these findings we aimed to identify the functional impact for the tumor cells and the signaling pathways that are involved in this paracrine tumor-stroma interaction.
Using co-culture experiments we could demonstrate that melanoma cell lines *Bro* and *HT144* show increased cell proliferation when grown on a layer of HA-secreting fibroblasts. Blocking of HA-synthesis in fibroblasts with HAS2 specific siRNA abrogated this effect. In media transfer experiments we analysed melanoma-derived mediators that induce HAS1 and HAS2 mRNA in fibroblasts treated with melanoma cell conditioned medium (MMCM). Stimulation experiments with candidate factors, siRNA transfections and function blocking antibodies were used to identify the MM-derived mediators and their respective receptors on fibroblasts.
We show that soluble mediator proteins in MMCM elevate HAS1- and HAS2 mRNA expression in fibroblasts resulting in a significantly increased HA synthesis. This induction is highly sensitive to inhibitors of PI3- and MAP-kinase signaling pathways.
The growth factors PDGF-AA and PDGF-CC induce HAS2 expression in fibroblasts. Furthermore, silencing PDGF-A and PDGF-C mRNA in melanoma cells and/or blocking PDGFR-α on fibroblasts could reduce the observed stimulation of HA synthesis by MMCM. Additionally, we show that TGF-β_1 is the stimulating mediator for HAS1 and silencing of TGFβ_1 in Bro cells abrogated this induction.
Taken together, melanoma-derived mediators like TGFβ_1, PDGF-AA and PDGF-CC stimulate HA-synthesis in stromal fibroblasts thus enhancing tumor cell proliferation of malignant melanoma. An outlook on the impact of matrix components on tumor cell growth will be given.

14.

Centrosome positioning during Epithelial to Mesenchymal Transition

Mithila Burute1,2, Qingzong Tseng1, Magali Prioux1, Fabrice Senger1, Odile Filhol-Cochet1, Matthieu Piel3, Manuel Thery1
1 LPCV/iRTSV/ DSV/CEA, Grenoble, France
2 CYTOO, Grenoble, France
3 Institute of Curie, Paris, France

Contact: mithila.burute@cea.fr

Epithelial to mesenchymal transition (EMT) is a morphogenetic event that takes place during specific stages of embryo development and early stages of tumour formation. Study of EMT is of key importance for understanding phenotypic changes that occur in a group of cells during cancer progression. Proper tissue functions directly depend on the establishment of cell polarity. Epithelial, non-motile cells establish an apico-basal polarity whereas mesenchymal, migrating cells display a front-rear polarity. Polarized distributions of cell-cell and cell-matrix junctions direct the asymmetric spatial organization of cell internal compartments to establish cell polarity. Centrosome position is a good indicator of cell polarity due to its active role in organization of microtubules and orientation of internal traffic of endocytosed and secreted proteins. However, centrosome positioning during EMT has not been explored in detail so far. We used micropatterning method to study polarity changes during EMT using a minimal model of tissue composed of two cells, which can form cell-cell and cell-Extra cellular Matrix (ECM) junctions. We found that nucleus-centrosome axis is preferentially positioned towards the cell-cell junction in epithelial cells (MCF10A) while upon induction of EMT by TGFβ, the resulting mesenchymal cells showed loss of centrosome positioning towards the junction. This implicated an important role of cell-cell junction in centrosome positioning and in governing cell polarity during EMT. Tissue metastasis is an advanced stage of cancer progression and involves cell migration. Hence we are investigating phenomenological relevance of centrosome repositioning during EMT in promoting cell migration of mesenchymal cells with the help of new dynamic surface coatings offering the possibility to release the cells from confined environment of micropatterns and analyse their scattering properties away from the original cell cluster. Another important aspect of tissue architecture is matrix rigidity. The matrix rigidity and thus forces exerted by cells on ECM promote changes in the cancer tissue. Hence we are currently studying the effect of physiologically relevant stiffness of substrate on centrosome positioning. Interestingly, we observed that cells on softer substrate tend to retain their centrosome positioning towards junction even after undergoing EMT. We are further investigating possible role of intercellular and intracellular forces in establishing cell polarity using Traction force microscopy.

15.

Elasticity and the shape of prevascular solid tumors

Kristen Mills1, Shiva Rudraraju2, Ralf Kemkemer1,3, Krishna Garikipati2
1 Max Planck Institute for Intelligent Systems, Department of New Materials and Biosystems, Stuttgart, Germany
2 University of Michigan, Mechanical Engineering, Ann Arbor, Michigan
3 Reutlingen University, Reutlingen, Germany

Contact: mills@is.mpg.de

It is well known that cell behavior, both in health and disease, is influenced by the mechanical environment. In the environment of a solid tumor, increased ECM deposition and rearrangement stiffen the tissue. Additionally, growth of the tumor due to unchecked cell proliferation induces increased mechanical stress. This altered mechanical environment likely has an important interplay with tumor progression. We work with an in vitro model for tumor growth based on a bioinert hydrogel whose stiffness can be varied over the range of healthy and diseased tissues (100s of Pa to 10s of kPa). In this model, where the mechanical interactions between tumor cells and the hydrogel are isolated, we have found that tumors grow robustly to form ellipsoids. This is remarkable because tumors with defined boundaries in vivo are often described as being ellipsoidal in shape. Using the theory of elasticity we show how certain ellipsoidal shapes minimize the relevant free energy, hence providing a purely mechanical explanation for this growth behavior. This result may be of great importance to understand the shape progression of early solid tumors in vivo and is an important step in understanding the processes underlying solid tumor growth.

16.

Intravital tissue imaging of collective tumor cell invasion: interface guidance and resistance niches

Bettina Weigelin

17.

Mechanistic principles of the nuclear lamina: How is lamin assembly generating tough geodesic structures and why do the four lamins segregate?

Harald Herrmann
B065 Functional Architecture of the Cell, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, Heidelberg, Germany

Four nuclear intermediate filament (IF) proteins - lamin B_1, lamin B_2, lamin A and lamin C - are essentially involved in the formation of the metazoan nuclear envelope in man. The knockout in mice for each of these proteins is lethal either around or shortly after birth. Nevertheless, these experiments revealed that the knockout of a single lamin is compatible with propagation of cells in a petri dish. Consequently, in order to explore if lamins are at all essential at the single cell level, a complete knockout of all four lamins has been performed in the lab of Yixian Zheng (Kim et al.: Proliferation and differentiation of mouse embryonic stem cells lacking all lamins. Cell Res. (2013) *in press;* doi: 10.1038/cr.2013.118). These experiments essentially demonstrated that lamins are not needed for cellular life outside of developmental programs, but that the formation of whole organs indeed requires the expression of lamins. Obviously, as soon as tissues and organs are formed in an organismal context*, C. elegans* to mice, lamins appear to have complex, multiple and essential roles. Corresponding to these central functions, a plethora of human mutations for lamin A and lamin C has been discovered that cause various severe diseases. Often, blebbing of the nuclear envelope is a characteristic event in cells taken from patients indicating that an unbalanced structural organization becomes a problem, in particular when the cells are cultured *ex vivo*.
As prerequisite to understand such effects, one has to know the regular assembly pathway of A-type lamins. As all intermediate filament proteins, they exhibit a high degree of sequence similarity in the three heptads at either end of the central α-helical rod. These parts of the molecule have been shown to longitudinally overlap by 3 nm, and therefore this domain is thought to be crucially involved in the elongation mechanism of dimers to filaments. At the next level of assembly, growing chains associate laterally in an anti-parallel fashion. Already at this stage, the four different kinds of lamins are sorted out within the nucleus as shown by high-resolution microscopy and immunoprecipitation studies with cellular fractions obtained by differential extraction procedures. Nevertheless, according to biochemical results with “mini-lamin” fragments the different types of lamins can longitudinally associate with each other, at least in vitro (1). Hence, the cellular segregation of A- and B-type lamins is at least partly mediated by the segregation of both protein species during and after mitosis, as B-type lamins are staying membrane-bound whereas A-type lamins are entirely soluble and are transported into the nucleus only after a functional nuclear envelope, including a lamina made out of B-type lamins, has formed. In the future, we will investigate the factors that keep these proteins separate during and after translation.

(1) Kapinos LE, Schumacher J, Mücke N, Machaidze G, Burkhard P, Aebi U, Strelkov SV and Herrmann H. (2010) Characterization of the head-to-tail overlap complexes formed by human lamin A, B1 and B2 "half-minilamin" dimers. *J. Mol. Biol.* **396**, 719-731.

18.

Cell migration through narrow pores: matrix mechanics versus geometry

Ben Fabry, Department of Physics, University of Erlangen-Nuremberg, Germany

Cell migration through a 3-dimensional (3D) matrix is sterically hindered by small pores, but it is currently unknown how pore size and matrix stiffness influence migration speed. Here we study the migration of MDA-MB-231 breast carcinoma and HT1080 fibrosarcoma cells in two different systems. First, we use 3D self-assembled collagen biopolymer networks. Pore sizes between 3 to 8 µm and effective matrix stiffnesses between 100 to 1000 Pa are obtained by varying the collagen concentration from 0.3 to 2.4 mg/ml. In addition, the stiffness of the networks can be increased by ~3-fold, without altering collagen concentration or pore size, by adding glutaraldehyde (GA) as a crosslinker. In a second set of experiments, we use a microfluidic device to investigate cell migration through narrow channels. Channels are fabricated in polydimethylsiloxane rubber using soft lithography. A series of 15 channel segments (20 µm length, 3.7 µm height, with a decreasing width from 10 to 1.7 µm) are separated by 20x20µm chambers so that cells can spread and relax between channel crossings. Cell nuclei are stained with Hoechst 33528, and images are taken every 5 min.
Cell migration in the 0.3 and 0.6 mg/ml collagen gels (8 µm and 5.6 µm pores) is strongly enhanced by GA stiffening of the collagen matrix, opposite to previous findings in 2D. By contrast, migration in the denser 1.2 and 2.4 mg/ml collagen gels (3.8 and 3 µm pores) is reduced by GA stiffening of the collagen matrix. These results demonstrate that 3D cell migration is enhanced by higher matrix stiffness as long as the pore size does not fall below a critical value where it causes excessive steric hindrance.
In the microfluidic device, where the channel walls have a virtually infinite stiffness compared to cell stiffness, cells move faster in channels coated with fibronectin compared to channels coated with collagen, in contrast to findings on flat 2D substrates where cell migration speed is lower on fibronectin. For channels narrower than 9 µm, the nucleus of both cell lines undergoes considerable elongation. Surprisingly, nuclear speed increases in narrower channels. Moreover, nuclear speed is higher when exiting the channel compared to entering the channel. However, if the overall height of both channel and chamber is increased from 3.7 to 7.4 µm, the migration speed also increases.
These data, taken together, are consistent with the notion that the deformability of the cell nucleus is the limiting factor during migration through narrow openings. As the nucleus becomes wedged against the channel or pore entrance, the cell builds up tractions to deform the nucleus. This process requires strong adhesions on a sufficiently stiff matrix. Once fully deformed, the nucleus glides through the pore or channel with less resistance and thus higher speed. By this mechanism, the cell can dramatically lower the resistance posed by narrow pores and thereby maintain a high speed even in the presence of high steric hindrance.

20.

The importance of being well rounded

Buzz Baum

As cells progress through mitosis they undergo profound changes in cytoskeletal organisation and cell shape. These include cell rounding upon entry into mitosis, alignment of the metaphase spindle with cortical cues in response to asymmetries in the environment, and polar relaxation and cell division following the onset of anaphase. These changes need to be coupled to the cell cycle machinery. Here, I will discuss our attempts to uncover the molecular, cellular and physical mechanisms driving the changes in the actin-based cortex that underlie these events. In addition, I will explore the importance of being well- rounded, for both normal and cancer cells.

21.

Signalling in the tumour microenvironment: Essential roles for extracellular ADAM proteases in malignant tumours

Uwe Schlomann1, Catharina Conrad1, Garrit Koller2, Christopher Nimsky1, Jörg Walter Bartsch1
1 *Marburg University, Department of Neurosurgery, Marburg*
2 IPS*, King's College London, London*
Contact: jbartsch@med.uni-marburg.de | Website

For tumour development and progression, tumour cells are in close communication with other cells types such as immune cells and fibroblasts. In particular, proteolytic release of membrane proteins from the tumour cell membrane creates signals that are essential in tumour formation and tumour progression. In the past years, ADAM (A Disintegrin And Metalloprotease) proteases have been identified in numerous malignancies. Their targeting appears attractive given that some ADAM proteases are dispensable for normal physiology.
We have identified a particular member of the ADAM protease family, ADAM8, that is highly induced in malignant tumour cells including those from pancreatic and breast cancer and from brain tumours. When tumour cell lines expressing ADAM8 were compared to those ones carrying ADAM8 knockdowns introduced by small hairpin RNA interference (shRNA), significant changes in cell membrane ß1 integrin localisation and downstream ß1 integrin signalling were observed, suggesting that ADAM8 contributes to the membrane distribution of ß1 integrin. In agreement with this hypothesis, significant differences between ADAM8 wild-type and ADAM8 knockdown cells were seen for cell migration and invasiveness, both, into extracellular matrix as well as in endothelial layers. With respect to cell-cell interactions, ADAM8-expressing cells caused angiogenesis of HUVEC cells </i in vitro>, whereas ADAM8 knockdown cells failed to do so. </i In vivo>, knockdown of ADAM8 caused reduction of tumour load, as shown in pancreatic and breast cancer. Molecular modelling of functional ADAM8 domains led to identification of a domain that is responsible for the observed ß1 integrin interaction. Mimicking this interaction using a peptidomimetic small cyclic peptide, we were able to demonstrate that the </i in vivo> function of ADAM8 can be inhibited, i.e. interaction with and signalling via ß1 integrin. By application of this peptide in mouse models for pancreatic cancer, reduced tumour load and increased survival of mice was observed. This example shows that it is feasible to target molecules successfully in the tumour microenvironment

24.

Cancer metastasis: collective invasion and cell guidance in multicellular systems

Adrien Hallou, Joel Jennings, Alexandre Kabla
University of Cambridge, Department of Engineering, Bioengineering Group, Trumpington Street, Cambridge, CB2 1PZ, United Kingdom
Contact: ah691@cam.ac.uk | Website

Metastasis, a complex and multistep process, accounts for more than 90% of cancer fatalities. The specific features of malignant cells such as uncontrolled growth, altered metabolism and invasive behaviours constitute the hallmarks of cancer [1]. Recent studies have highlighted the importance of the tumour microenvironment on these signatures of cancer and on the onset of metastasis [2]. All these factors have been associated with numerous biological determinants resulting both from an accumulation of genes mutations and a malfunction of numerous regulatory signaling pathways. Owing to the heterogeneity in these factors across different cancers and patients, no common mechanistic pathway leading to metastasis induction and progression has yet been characterized [1,2,3].

In contrast to these tremendous variations of genotype and phenotype, most solid tumours display similar collective invasion behaviours [3]. The cohesive multicellular structures formed during invasion such as cell sheets, strands or clusters display stunning morphological similarities and identical mechanical behaviours across most cancer pathologies and patients [3,4]. This homogeneity in terms of morphology and migration behaviours suggest that the invasion stage of metastasis could be mainly controlled by physical interactions between cells and simple mechanical phenomena [4].

Using a newly introduced computational framework [5], we study the collective dynamics of a cancer cell population enclosed in a mechanically resistive tissue. We observe that simple physical rules are sufficient to account for some of the common morphologies of invading tumours, including regimes of individual and collective invasions [6]. Moreover, the introduction of a few fibroblast-like cells, with an enhanced ability to remodel the tumour microenvironment, leads to a strong increase in the invasiveness of the cancer cell population. Their invasion behaviour is here associated with the frequent occurrence of “cell fingers”, guided by fibroblast-like cells at their leading edge [6].

Overall our in silico experiments reproduce the phenomenology of invasion across the different cancer pathologies and provide new insights on the mechanisms controlling this complex phenomenon [6]. Based on simple biophysical hypotheses and generic cellular interactions, we believe that our approach will help to unfold the different biological contributions to metastasis and to disentangle the links between genes, environment and malignancy.

[1] D. Hanahan, R. Weinberg, Cell (100, 1, 57-70) (2000)
[2] J.A. Joyce, J.W. Pollard, Nature Cancer Reviews (9, 4, 239-52) (2009)
[3] P. Friedl, K. Wolf, Nature Cancer Reviews (3, 5, 362-74) (2003)
[4] P. Friedl et al., Nature Cell Biology (14, 8, 777-83) (2012)
[5] A.J. Kabla, Journal of the Royal Society Interface (9, 77, 3268-78) (2012)
[6] A. Hallou, J. Jennings, A.J. Kabla, To be submitted (2013)

25.

Optimality of force transmission in a motor-clutch cellular adhesion model

Benjamin Bangasser1, Steven Rosenfeld2, David Odde1
1 Department of Biomedical Engineering, University of Minnesota, 312 Church St, Minneapolis, MN, USA
2 Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH, USA
Contact: bang0078@umn.edu | Website

Microenvironmental mechanics play an important, but variable, role in determining cell morphology, traction, migration, proliferation, and differentiation with potential impacts on tumor development, growth, and invasion. Interestingly, some cell types have shown increasing migration and traction force as a function of substrate stiffness, while others have shown decreasing migration and traction force. These seemingly contradictory results may be explained by a motor-clutch model of cellular adhesion and force transmission [1] which exhibits a maximum in traction force with respect to stiffness and may be tuned to different stiffness optima. Both stochastic [2] and deterministic [3] castings of the motor-clutch model provide a basis to explain the tuning of cells to different microenvironmental mechanics. A sensitivity analysis of the stochastic model suggests that molecular motors and adhesion clutches must approximately balance each other to achieve stiffness sensitivity. Consequently, individual parameters changes, which favor only the motors or the clutches, have little effect in shifting the stiffness optimum because the system loses stiffness sensitivity altogether. However, dual parameters changes, such that motors and clutches remain balanced, can shift the stiffness optimum over several orders of magnitude. This optimum occurs on the stiffness at which the time for all clutches to bind equals the cycle time of adhesion load and fail. At stiffnesses above this optimum, fewer than the maximum clutches bind, so the clutches are not utilized to their fullest extent. At stiffnesses below the optimum, clutches spontaneously fail at low loads because of the long cycle time, again resulting in an inefficient use of clutches. This determinant of the optimum stiffness was applied in conjunction with the deterministic motor-clutch model to derive a dimensionless quantity defining model behavior at any particular stiffness. A second dimensionless quantity derived from the deterministic model describes the overall stiffness sensitivity of the model system. Used together, these two dimensionless quantities describe both the existence and position of an optimum stiffness for cellular force transmission. Since both molecular motors and adhesion molecules are upregulated in some cancers, these findings suggest that tumor cells may tune their force transmission mission ability to different microenvironmental stiffnesses which may facilitate tumor cell migration and invasion. The dimensionless quantities identified may also help explain how a particular drug treatment will affect the force transmission ability of tumor cells.

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30.

Macromolecular protein complexes in Wnt signalling

Jürgen Behnrens

Nikolaus-Fiebiger-Center, Chair of Experimental Medicine II, University Erlangen-Nürnberg, Glückstr. 6, D-91054 Erlangen

The canonical Wnt/□eta-catenin pathway is a central regulator of embryonic development, stem cell behaviour, and cell proliferation and mutational activation of the pathway is a major cause of cancer. Wnts are secreted glycoprotein's that bind to pairs of frizzled/LRP%76 receptors which leads to inhibition of the degradation of the cytoplasmic protein □eta-catenin. Association of □eta-catenin with TCF transcription factors results in activation of specific target genes that mediate the biological consequences of the pathway. □eta-Catenin degradation in the absence of Wnts is mediated by a specific destruction complex containing scaffold components APC and Axin/Conducin and kinases GSK3b and CK1 which phosphorylate □eta-catenin thereby earmarking it for ubiquitination and proteasomal degradation. How □eta-catenin degradation proceeds and how it is inhibited is a major topic in current Wnt signalling research. We will characterize the □eta-catenin destruction complex by deciphering its interaction with a class of recently discovered membrane associated proteins of the Amer1/WTX family, and with upstream components at the plasma membrane that interact in a differential way with the scaffold proteins axin and conductin thereby allowing specific feedback regulation of the pathway.

34.

Identifying malignant melanoma through unique signatures in blood flow dynamics

Gemma Lancaster1, Marco Rossi2, Margherita Pesce2, Aneta Stefanovska1
1 Department of Physics, Lancaster University, Lancaster, United Kingdom
2 Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
Contact: g.lancaster1@lancaster.ac.uk

Malignant melanoma is an aggressive form of skin cancer, known for its capacity to proliferate and metastasize rapidly, and for its resistance to conventional cancer treatments. Early diagnosis remains essential for a positive prognosis. Atypical melanocytic naevi continue to present a significant diagnostic challenge to dermatologists, due to their similar appearance to melanoma lesions. In these cases, excision and histological examination are currently the only methods capable of unequivocally ruling out melanoma. Therefore, the development of noninvasive alternatives is an active area of research.
86 patients were recruited in Pisa, 8 with psoriasis, 11 with malignant melanoma, 33 with atypical naevi and 34 with benign naevi. Laser Doppler flowmetry (LDF) tracings were recorded for 30 minutes, at lesion centres, lesion margins and on healthy skin contralateral to the lesion centre. Data were analysed by applying sophisticated analysis techniques, based on the previously established coupled oscillator approach to the cardiovascular system [1], to detect oscillations within the blood flow, attributed to various processes including the heartbeat, breathing rate, activity of smooth muscle cells, vessel innervation and endothelial activity [2].
Higher blood perfusion is expected in malignant melanoma when compared to non-cancerous naevi, due to increased vasculature following angiogenesis [3], and inflammatory effects [4]; this increase has been confirmed in the current study. However, the increase was found not to be uniform across the observed frequency spectrum, 0.005 Hz (endothelial) – 2 Hz (heart rate), revealing important information on the physics of cancer blood flow dynamics. Both melanoma and psoriasis are characterized by excessive cell proliferation and angiogenesis, with both groups showing significantly higher blood flows than both benign and atypical naevi. Differences in their blood flow dynamics were observed in the frequency intervals associated with neurogenic (0.021 – 0.052 Hz) and myogenic activity (0.052 – 0.145 Hz), with melanoma being lower than psoriasis in both intervals. This could indicate differences in cellular metabolism, in particular the suppression or damage of the mitochondria in cancer. Cancer cells are known to predominantly use glycolysis for energy production in hypoxic conditions [5]. It is possible that the excess lactate produced may alter the pH of the tumour microenvironment [6], in turn affecting vessel reactivity [7].
In addition to providing new insights into cancer dynamics, the results are also of great practical importance. Investigations with the Wilcoxon rank sum test showed that the melanoma centre to contralateral power ratio was significantly higher ($P<0.05$) than both benign and atypical naevi at all frequencies except in the myogenic interval and also shows no significant differences between atypical and benign naevi at any frequency. Significantly discriminatory ratios in the cardiac (0.6 – 2 Hz) and neurogenic intervals were used to develop a diagnostic test. This noninvasive method, based on the observation of tumour vasculature in vivo, can

distinguish malignant melanoma from non-melanoma, including atypical naevi, with 100% sensitivity and 90% specificity.

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35.

High resolution magnetic resonance elastography of the in vivo human brain: Application to presurgical mechanical characterization of glioblastoma.

Authors: Jing Guo[1], Kaspar-Josche Streitberger[1], Martin Reiss-Zimmermann[2], Florian Freimann[3], Jürgen Braun[4], Karl-Titus Hoffmann[2], Ingolf Sack[1]
[1]Department of Radiology, Charite- University Medicine Berlin, Berlin, Germany.
[2]Department of Neuroradiology, University Hospital Leipzig**,** Leipzig, Germany
[3]Department of Neurosurgery, Charité - Universitätsmedizin Berlin, Berlin, Germany
[4]Department of Medical Informatics, Charite- University Medicine Berlin, Berlin, Germany

Contact: jing.guo@charite.de

Magnetic Resonance Elastography (MRE) [1] is a unique modality for non-invasively measuring mechanical properties of the in vivo brain. The method relies on the external excitation of shear vibrations in the brain, their acquisition by MRI and the reconstruction of images of viscoelastic constants - so called elastograms. This principle was recently proven sensitive to the consistency of meningeoma [2], the maturation of the brain [3] as well as to the disseminated disruption of brain parenchymal tissue integrity related to Alzheimer's disease [4], multiple sclerosis [5], and hydrocephalus [6]. Biophysical experiments on single cells further motivate the use of mechanical constants as radiological markers of tumor malignancy [7].
MRE could sever as a probe for the presurgical non-invasive assessment of the mechanical property of tumors. However, classic MRE suffers from limited spatial resolution due to artefacts related to ill-posed parameter reconstruction. In this work, we introduce 3D multifrequency MRE (3DMMRE) combined with a novel wave field reconstruction method to generate highly resolved maps of the viscoelastic properties of the brain and demonstrate the method in 15 patients with glioblastoma.
In 3DMMRE wave data at 7 vibration frequencies are acquired (30 Hz to 60 Hz with 5 Hz increment) with motion encoding gradient applied along all three axes of the scanner coordinate system for full wave field acquisition. For reconstruction, multifrequency dual elastic-visco inversion (MDEVI) algorithm was applied to the full set of wave images. The algorithm as outlined in [8] is capable of reconstructing the phase ϕ and magnitude $|G^*|$ of the complex shear modulus $G^*=|G^*|(\cos\phi + i\cdot\sin\phi)$ by solving $\Delta\mathbf{x}_{mn}\cdot\mathbf{x}_{mn}=-|\Delta\mathbf{x}_{mn}||\mathbf{x}_{mn}|\cos\phi$ for ϕ ($\mathbf{x}_{mn}$ is a two-element vector of the real and imaginary part of the m^{th}-component of the wave field at n^{th}-frequency) and by solving the magnitude-Helmholtz equation $|G^*||\Delta u^*_m(\omega_n)|=\rho\omega_n^2|u^*_m(\omega_n)|$ for $|G^*|$. ϕ is sensitive to the underlying tissue network structure as can be quantified by the fractal network dimension. $|G^*|$ reflects the ratio between peak stress and peak strain in the tissue and is thus determined by both storage and loss properties.
Incorporating the full 3D-multifrequency wave filed information into MDEVI, we found that resolution and consistency of viscoelastic parameters maps of the glioblastoma are greatly improved. In particular, ϕ-maps revealed the mechanical heterogeneity of the tumor in great detail, which is beneficial for characterizing glioblastoma considering the common presence of cysts, necrosis and hemorrhages.
To summarize, 3DMMRE combined with MDEVI reconstruction enables us to high resolution MRE, which could contribute to an early noninvasive clinical assessment of tumor entity and tumor growth in terms of infiltration and mechanical heterogeneity.

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36.

Michael Murrell

Myosin II motors drive contractility of the cortical actin network, enabling shape change and cytoplasmic flows underlying diverse physiological processes ranging from cell division and migration to tissue morphogenesis. Yet, despite its importance, the mechanisms that describe contractility and the generation of mechanical forces within the cortex are not well understood. We recapitulate contractility in vitro, through the development of a minimal model of the cell actomyosin cortex by coupling a two-dimensional, cross-linked F-actin network decorated by myosin thick filaments to a model cell membrane. Myosin motors generate both compressive and tensile stresses on F-actin and consequently, induce large bending fluctuations. Over a large range of crosslinking, we show the extent of network contraction corresponds exactly to the extent of individual F-actin shortening via buckling. This demonstrates an essential role of buckling in facilitating local compression to enable mesoscale network contraction of up to 80% strain. Buckled F-actin at high curvatures are prone to severing and thus, compressive stresses mechanically coordinate contractility with F-actin severing, the initial step of F-actin turnover. Finally, the F-actin curvature acquired by myosin-induced stresses can be further constrained by adhesion of the network to a membrane, accelerating filament severing but inhibiting the long-range transmission of the stresses necessary for network contractility. Thus, the extent of membrane adhesion can regulate the coupling between network contraction and F-actin severing. These data demonstrate the essential role of the non-linear response of F-actin to compressive stresses in potentiating both myosin-mediated contractility and filament dynamics.

37.

Mechanosensing with primary cilia

Christoph F. Schmidt
Co-authors: Chris Battle, Carolyn Ott, Nikta Fakhri, Jennnifer Lippincott-Schwartz

Third Institute of Physics - Biophysics, Georg August University, Göttingen, Germany

Primary cilia are ubiquitous, microtubule-based organelles that play a role in sensory transduction in a variety of eukaryotic systems. They interrogate the extracellular environment and signal to the cell interior through chemosensation, osmosensation and mechanosensation. Form and function of primary cilia are intricately linked, yet little is known about the mechanical and dynamical properties of cilia that support cilia sensory function. We have examined structural and dynamical features of primary cilia of kidney epithelial cells (MDCK), which are mechanosensors: rigidity, interdoublet coupling, basal attachment, and positional fluctuations. We probed the mechanical response of the cilia using optical trapping and found weak interdoublet coupling. Relaxation dynamics point towards a limited hinge in the cell cortex. Video tracking of spontaneous fluctuations of cilia points towards active driving, in contrast to the current dogma that the primary cilium is immotile.

38.

Molecular dissection of lamellipodium protrusion

Klemens Rottner[1,2], Anika Steffen[1], Stefan Koestler[1], Jennifer Block[1], Cord Brakebusch[3], Jan Faix[4], J. Victor Small[5], and Theresia E.B. Stradal[2,5]

[1]Institute of Genetics, University of Bonn, Karlrobert-Kreiten Str. 13, D-53115 Bonn, Germany
[2]Helmholtz Centre for Infection Research (HZI), Inhoffenstrasse 7, D-38124 Braunschweig, Germany
[3]Biomedical Institute, BRIC, University of Copenhagen, DK-2200 Copenhagen, Denmark
[4]Institute for Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany
[5]Institute of Molecular Biotechnology, Austrian Academy of Sciences, Dr. Bohr-Gasse 3, A-1030 Vienna, Austria
[6]Institute for Molecular Cell Biology, University of Münster, Schlossplatz 5, D-48149 Münster, Germany

Cell migration as observed for instance in metastatic cancer cells is frequently initiated by protrusion of thin leaflets of cytoplasm or of rods called lamellipodia and filopodia, respectively. Lamellipodia are built of dense networks of actin filaments and are also called 'ruffles' when lifting up- and backwards during protrusion and retraction.
It is commonly agreed that lamellipodia formation and turnover are controlled by continuous polymerization and depolymerisation of actin filaments, but the precise temporal and spatial features of regulation of these processes are still controversial.
In recent years, we have experienced major progress in our understanding of the function and biochemical activities of key regulators of actin assembly and disassembly, including Arp2/3 complex, its activators or members of the ADF/cofilin family.
Here, our recent efforts to separate essential from modulatory factors in lamellipodium protrusion will be discussed. Based on our experiments, we conclude that protrusion of lamellipodial actin networks in mammalian cells requires activation of Rac subfamily GTPases, either Rac1, 2 or 3, accompanied by recruitment and coincident activation of both WAVE- and Arp2/3-complexes. Furthermore, continuous Arp2/3 complex activity is essential for initiation, maintenance and turnover of branched, lamellipodial actin networks, but in addition, for recruitment of other lamellipodial regulators including capping protein and cofilin. Finally, the precise functions of small GTPases other than Rac in lamellipodium protrusion or of formins are less firmly established. However, we provide evidence that the Rho-GTPase Cdc42 promotes this process through activation of FMNL subfamily formins, which are capable of capturing and elongating actin filaments generated *in vitro* through branching by Arp2/3 complex.
Current and future efforts are aimed at defining the precise biochemical activities that operate *in situ*, both at the network and at the individual filament level, to shed more light on the molecular regulation of lamellipodia-based cell migration.

3. Abstracts of Posters

Talks 7,14,15,24,25,34 and 35 are additionally presented as posters:

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P8

Challenges in probing cellular adhesion forces using atomic force microscopy

Steve Pawlizak, Tobias Thalheim, Anatol Fritsch, Josef A. Käs

University of Leipzig, Faculty of Physics and Earth Sciences, Institute of Experimental Physics I, Soft Matter Physics Division, Linnéstr. 5, 04103 Leipzig, Germany
Contact: pawlizak@uni-leipzig.de | Website

How do cells find each other to form specific complex structures, such as compartments and organs, while segregating themselves from other cell populations which, in turn, form their own structures? Why do cancer cells initially stay in their original compartment, but later become metastatic invading other, distant tissues?

An understanding of the cellular interactions and physical principles underlying cellular organization processes is of great importance. A common model system demonstrating the formation of simple cellular aggregates involves mixing two different populations of suspended cells. The mixture will eventually segregate into two phases, whereas mixtures of the same cell type will not. Different physical properties of the interacting cells explaining this behavior have been suggested, such as cortical tension or cellular adhesiveness. The latter mechanism, also know as the *differential adhesion hypothesis (DAH)*, predicts that in equilibrium those cells with the lower adhesiveness must surround the cells having the higher adhesiveness in order to minimize the free energy in the system and to maximize the total cell-cell binding strength.

In addition to aggregation and segregation experiments with different cell populations, *atomic force microscopy (AFM)* can be used to directly measure the adhesion forces between two cells. The combination of both techniques could help to support the validity of the DAH. However, a contemporary issue concerning such AFM data is normalization which is very challenging since the contact area is not accessible using conventional transmitted light techniques. A commonly accepted approach is limited to pre-selection of cells that have roughly the same size. Here, we will discuss several other approaches which might help to estimate the diameter of the contact area.

P9

Diffusive transport and spontaneous vortex formation in cell sheets invading µ-structured channels

Anna-Kristina Marel1, Matthias Zorn1, Christoph Klingner2, Roland Wedlich-Söldner2, Joachim Rädler1
1 *Fakultät für Physik & Center for NanoScience, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 München, Germany*
2 Cel*lular Dynamics and Cell Patterning Group, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany*

Contact: Matthias.Zorn@physik.uni-muenchen.de | Website

Collective cell migration in sheets cannot be sufficiently explained by simply adding up the random motion performed by individual cells outside of a social context. Instead, collectively migrating cells exhibit characteristic phenomena unknown from isolated cells. In order to provide a well defined geometry for examining such behavior, epithelial Madin-Darby canine kidney (MDCK) cells are confined to channels by micro-patterning methods. Using lift-off experiments, controlled starting conditions can be provided before allowing the cell sheets to migrate into the channels in a fluid like way. As the cells migrate onto the newly provided uncovered area, a density gradient develops, with lower density towards the front and high densities in the bulk closer to the channel entrance. For the sake of studying both large scale directional flow as well as noise phenomena on small scales, such as swirl formation, particle image velocimetry (PIV) analysis was performed on the migrating sheet, yielding a velocity field describing the cells' motion. In doing so, a trend in the velocities is found, opposing the density gradient, with the velocity increasing linearly as one gets closer to the cell front. Studying of the flow at large length and time scales via time averaging and coarse graining of the velocity fields reveals a net flux along the gradient in the cell density. This flux causes the density profiles of the cells in the channels to evolve over time, with the leading front moving at a constant velocity. This behavior has previously been described using a reaction-diffusion equation (Fisher-Kolmogorov). Indeed, fitting the analytical solution of the Fisher-Kolmogorov equation to the density profiles shows good agreement in the shape, while the resulting theoretical velocity derived for the cell front matches the leading edge's observed speed. Additionally, the resulting theoretical maximum carrying capacity for the system is in good agreement with the highest cell densities of MDCK cells found in literature, further confirming the applicability of this approach. These three controls lend credibility to the value of about 1000 µm²/h for the collective diffusion coefficient resulting from the fit. While at large time and length scales a smooth flowing behavior is found for the cells, the analysis of the system at small length and short time scales reveals a much noisier behavior. While this generally expresses itself in a less smooth PIV field and fluctuations in almost all measured quantities observed over time, one concrete manifestation is the formation of short lived vortexes spanning a few cell diameters, with life times of about 15 minutes. Mapping of vorticity fields allows localization of such swirls and shows a density dependence of the strength and frequency they form with. In addition to depending on the density of the cell layer, both these quantities are shown to decrease under presence of a directional flow when compared to a confluent layer without flow. Since description of the system via the Fisher-Kolmogorov equation

provided a value for the collective diffusion constant, comparing it to the diffusion of single cells is of interest. For this purpose, individual cell nuclei were tracked over time as representatives of their whole cell's movement. Performing a mean square displacement analysis on cells tracked in the bulk shows large discrepancies between the single cell diffusion coefficients (in the order of 20 $\mu m^2/h$) and the previously found collective diffusion coefficient that is about 50 times as high.

P10

DNA nanostructures as carriers of therapeutic biomolecules

Jessica Lorenz1, Christin Möser1, Julia Sandmaier1, Ines Neundorf2, David Smith1

1 *Fraunhofer Institute for Cell Therapy and Immunology (IZI), DNA Nanodevices unit, Perlickstraße 1, 04103 Leipzig, Germany*
2 Uni*versity of Cologne, Department of Chemistry, Insitute of Biochemistry, Zülpicher Straße 47, 50674 Cologne, Germany*

*Co*n*tact: jessica.lorenz@izi.fraunhofer.de*

*DNA i*s a powerful building block which allows the programmed self-assembly of molecular scaffolds, cages and multifunctional carriers with nanoscale dimensions by the nature of predictable base pairing. The so-called “DNA-origami” technique [1] is based on a process where a long “scaffold” strand (~7kb) hybridizes with hundreds of short synthetic “staple” oligonucleotides to form programmed 2D or 3D nanostructures. These structures can function as carriers to deliver functional biomolecules including proteins, antibodies or other therapeutic molecules to malignant or otherwise diseased cells. Our early work uses these DNA-based structures as scaffolds for the loading and transport of small, therapeutic anti-cancer drugs such as doxorubicin (Doxil®) or the microtubule targeting molecule paclitaxel (Taxol®) to cells. While displaying different mechanisms of action in target cells, both of these molecules associate non-covalently with double-stranded DNA and therefore can be attached to DNA origami-based carriers and delivered to cancer cells.
A smaller but very stable DNA nanostructure is the DNA-tetrahedron [2]. This molecule consists of four oligonucleotides which hybridize to form a wireframe tetrahedron with double-stranded edges of less than 10 nanometers. It self-assembles very rapidly, has an optimal size for the encapsulation of individual protein elements up to approximately 60 kDa and can also be used to deliver therapeutics to different cells.
Previous studies have shown that internalized DNA nanostructures were observed to co-localize with endosomal markers [3]. In order to access many cytosolic, nuclear or mitochondrial pathways which are potential targets for therapies, the ability to achieve a finer control compartmentalization is required. To study the dynamics of the active cellular uptake and internal trafficking of DNA-nanostructures, we attach short, mostly positively charged peptides derived from protein-transduction domains to the previous mentioned DNA tetrahedron. These so-called cell-penetrating peptides (CPPs) can be internalized in most cell types and, more importantly, allow the cellular delivery of conjugated or fused biomolecules [4]. By tracking the uptake dynamics and compartmentalization of fluorescently labeled tetrahedron-CPP-molecules, we hope to gain a greater understanding and control over how DNA-nanostructures enter cells, whether through passive or active means, which is a fundamental question for their eventual application to therapeutic strategies.

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P11

High contrast visualization of cell-hydrogel contact by advanced interferometric optical microscopy

takahisa matsuzaki1, gen sazaki2, masami suganuma3, tasuro watanabe3, motomu tanaka4,5, seiichiro nakabayashi1

1 *University of Saitama, department of chemistry, Sakura-ku, Saitama, 338-8570, Japan*
2 Uni*versity of Hokkaido, institute of low temperature science, N19-W8, Sapporo 060-0819, Japan*
3 Saitam*a cancer center, Research institute for clinical oncology, Kitaadachi-gun, Saitama 362-0806, Japan*
*4 Un*iversit*y of Heidelberg, institute of physical chemistry, physical chemistry of biosystems, D69120 Heidelberg, Germany*
*5 Univ*ersity o*f Kyoto, institute for integrated cell-material sciences (WPI iCeMS), 606-8501, Kyoto, Japan*

Contact: s13ds007@mail.saitama-u.ac.jp

Recently, a number of studies revealed that cells sensitively response to mechanical properties of their environments via adhesion sites (so called mechano-response)[1]. To study such cell mechano-response, hydrogels with tunable elasticity has been widely used as micromechanical environment models for cells. So far, total internal reflection fluorescence (TIRF) microscopy has been widely used to visualize cell adhesion molecules near substrate[2]. However, height resolution of TIRF (100-200 nm) is not sufficient to identify the physical contact between cells and substrate. Alternatively, the leading technique for visualizing cell-substrate contacts is reflection interference contrast microscopy (RICM), which detects interference of linearly polarized light reflected at cell-liquid (i.e., cell membrane-liquid) and substrate-liquid interfaces. If solid substrate (e.g., glass) is used, RICM is a powerful label-free technique to measure the thickness of water reservoir between cells and substrates with a resolution of ~ 2 nm[3], which is much finer than surface-sensitive fluorescence techniques such as TIRF microscopy. However, in case of hydrogel, contrast of RICM images becomes very poor because of the following two reasons. Firstly, the refractive index of hydrogels is very close to that of water, which results in low reflectivity at hydrogel-liquid interfaces. Secondly, the thickness of gels used for such studies is typically several μm or beyond, and thus the contrast of interferometric patterns is poor. Here, reflection interference contrast microscopy (RICM) was modified with a confocal unit, a low-coherent monochromatic light source, and high throughput optics to enhance interferometric signals from cell-hydrogel contact zones. The advanced microscopy clearly visualized physical contacts between cells and polyacrylamide hydrogels, and thus enabled the quantitative evaluation of the area of cell-hydrogel adhesion. By using the microscopy, we succeeded in evaluating the dependence of mouse melanoma (B16-F10) cells spreading on the hydrogel stiffness in a quantitative manner.

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P12

Surface tension governs the shape of mechanically confined cells in mitosis

Elisabeth Fischer-Friedrich1,2, Anthony Hyman2, Frank Jülicher1, Daniel Müller3, Jonne Helenius3

1 *Max Planck Institute for the Physics of Complex Systems, Dresden, Germa*ny
2 M*ax Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
3 D-BS*SE, ETH Zurich, Basel, Switzerland*

*Con*tact: efischer@pks.mpg.de

When animal cells enter mitosis, they alter their adhesion properties and round up, attaining largely spherical shapes. Previous findings indicate that this shape change is driven by an increase of the cell's internal hydrostatic pressure and cortical tension [1].
Here, we present significant improvements on this AFM-based approach by incorporating wedged cantilevers to facilitate proper axisymmetric cell confinement [2], confocal microscopy to determine cell shape, and a simple physical model in which the cell is considered as a liquid core surrounded by a cortical shell. The method allows a more precise determination of the intracellular hydrostatic pressure and enables calculation of an effective cell surface tension by application of the Laplace law.
We find that HeLa cells increase their hydrostatic pressure difference and surface tension from ≈50 Pa and 0.2 mN/m during interphase to ≈400 Pa and 1.6 mN/m in metaphase. We confirm that the cell increases its surface tension at the entry of mitosis driving a reduction of cell surface area at roughly constant cell volume and thereby promoting cell rounding.

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P13

Tracking of single cell migration in engineered 3D microenvironments

Jiranuwat Sapudom, Katja Franke, Michael Ansorge, Tilo Pompe

Universität Leipzig, Faculty of Biosciences, Pharmacy and Psychology, Institute of Biochemistry, Biophysical Chemistry Group, Johannisallee 21-23, 04103 Leipzig, Germany

Contact: jiranuwat.sapudom@uni-leipzig.de | Website

Different cell types dynamically interact in 3D tissues and matrices in order to regulate many physiological processes, like metastasis and wound healing. To better study the underlying mechanisms we developed a new method for automated *in vitro* tracking of living label-free cells in three-dimensional (3D) biomimetic matrices at single cell level. We aim to reveal characteristic cellular characteristics including migration patterns, proliferation and differentiation features under various exogenous microenvironments.

Using our tracking algorithm we could quantify single cell cellular behavior over a long period of time in 3D collagen matrices without a fluorescent labeling of cells. Various primary cells were analyzed such as fibroblasts, macrophages, melanoma cells and hematopoietic stem cells. By analyzing individual cell trajectories of hematopoietic stem and progenitor cells, it was possible to identify subpopulations of cells presenting different migratory characteristics. Matrix specific migration patterns of macrophages showed distinct features allowing predictions for the prospective cell fate.

The efficacy and accuracy of our newly developed single cell tracking technology will facilitate the *in vitro* long-term study of label-free cells in 3D biomimetic matrices to reveal cell fates in different physiological settings. The method offers a less invasive and economical approach for biomedical as well as pharmaceutical studies and can be applied to a variety of questions.

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Tension homeostasis of epithelial cells by surface area regulation: Model and application

Bastian Rouven Brückner, Anna Pietuch, Stefan Nehls, Andreas Janshoff
Georg August University of Göttingen, Institute of Physical Chemistry, Tammannstr. 6, 37077 Göttingen, Germany

Contact: b.r.brueckner@chemie.uni-goettingen.de | Website

Mechanical properties of cells are important during the whole cell cycle as well as in many biological processes such as locomotion, cell division, and oncogenesis. Against this background it has recently been shown that malign cells appear softer than benign ones[1]. The complex interplay between plasma membrane and underlying cytoskeleton contributes to the mechanical behavior as well as the membrane properties itself. To describe the mechanical response of cells upon deformation with an external probe in an adequate way, a tailor-made model which takes the shell-like architecture of cells into account is mandantory. The commonly used Hertz model describes the elastic deformation of a homogenous continuum[2]. It is useful to obtain a bulk Young's modulus but excludes the properties of the cellular plasma membrane and the intricate structure of the whole cell. In contrast to Hertzian mechanics, here we describe an approach relying on a modified tension model from Discher and coworkers[3]. Our model additionally takes the complex architecture of confluent epithelial cell monolayers into account. Force spectroscopy comprising indentation and tether pulling experiments was applied to map the membranes overall tension as a set-point for regulation and the available surface area at the same location. Evidence for the validity of our model is given by obtaining the same mechanical properties, mainly independent from the geometry of the indenter[4]. Thereby, we can show, how cells quickly adapt to a mechanically challenging situation as experienced during osmotic stress or interfering with membrane-cytoskeleton attachment sites[5]. Various stimuli target membrane reservoir availibility, actomyosin-integrity, membrane-cytoskeleton attachment sites, and hydrostatic pressure to provoke a tension-driven response of the apparent area compressibility modulus. This can readily be transformed into available membrane surface area. We found substantial changes of the mechanical properties and available membrane surface area of epithelial cells due to interference with membrane-cytoskeleton attachment sides.

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P15

The 3D vertex model for epithelial mechanics

Silvanus Alt, Guillaume Salbreux, Frank Jülicher

Max Planck Institute for the Physics of Complex Systems Dresden, Nöthnitzer Strasse 38, 01187 Dresden, Germany

Contact: silvanus@pks.mpg.de

Understanding how mechanical forces drive epithelia deformations is crucial to shed light on processes involved in morphogenesis. Here we introduce a 3D vertex model, which represents cells in epithelia in three dimensions by a network of vertices joined by triangulated surfaces. Surface and line tensions, arising from forces generated in the actomyosin cytoskeleton, and the cells' intracellular elasticity act on the vertices to generate cellular deformations. Using this framework, we are interested in understanding the 3D cell shape in epithelia as well as the 3D deformations of epithelia, such as folding, formation of furrows, and invagination of structures.
On this poster, I focus on the physical mechanism for the formation of cysts, out-of-plane bulges which form in the Drosophila wing discs as a result of ectopic expression of a wide range of transcription factors. The 3D vertex model quantitatively captures the observed tissue deformations by considering an increase in line tension at the boundary of the cyst. The increase in tension at the boundary results in a planar pressure driving out of plane deformations. We propose that this constitutes a general mechanism for both invagination and evagination of epithelia.

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Quantitative evaluation of cancer cell adhesion to organosilane monolayer by reflection interference contrast microscopy

kosaku ito1, eisuke kakinuma2, kentaro masuda2, takahisa matsuzaki1, masami suganuma3, motomu tanaka4, seiichiro nakabayashi1, takashi tanii2, hiroshi yoshikawa1
1 *Saitama Univ., Saitama, Japan*
2 Was*eda Univ., Tokyo, Japan*
3 Saitam*a Cancer Center, Saitama, Japan*
*4 Un*iv. Hei*delberg, Heidelberg, Germany*
Contact: s12mc105@mail.saitama-u.ac.jp

Cell adhesion is a crucial step for cancer metastasis and its quantitative insight is important to understand malignancy of cancer. Recently, Tanii et al. have developed a unique method for the evaluation of cancer adhesive ability by using an organosilane monolayer template (OMT) [1], where an array of cell-adhesive spots (aminosilane) were micropatterned. Since cancer cells can be orderly arranged in the array, counting number of cells that adhere to the OMT can be used as a measure for cell adhesive ability. However, the physical contact between cancer cells and organosilane monolayer remains very unknown, though it is more quantitative and direct information for cell adhesive ability. In this work, we evaluated cancer cell adhesion to OMT by reflection interference contrast microscopy (RICM).
RICM was used to evaluate area and shape of contact between cells and OMT. A monochromatic incident ray I0 (λ = 5*4*6 nm) from the substrate side is first reflected at the substrate/medium interface to give ray I1; the t*r*ansmitted ray is reflected further at the cell surface and give rise to the ray I2. Rays *I*1 and I2 interf*e*re and it is possible to estimate cell-substrate distance from its fringe using a calculation model [2]. An array of circular spots of 3-aminopropyltriethoxysilane (APTES) (diameter = 19 ± 2 μm) was fabricated on a glass substrate functionalized with n-octadecyltrimethoxysilane (ODS) by photolithography. B16 melanoma cells with high (F10) and low (F1) metastatic ability were seeded onto OMT in culture media with various concentrations of anticancer agent (-)-epigalocatechin-3-gallate (EGCG). After 24h incubation, the cells were visualized by RICM.
Both F1 and F10 cells adhered only to APTES regions, because the adsorption of bovine serum albumin in culture media on the ODS monolayer prevents cells from adhering and spreading outside the circular APTES region [1]. RICM clearly visualized the contacts between cells and APTES monolayer. The contact zone was defined by the intensity contrast: if the intensity difference from minimum (I–Imin) w*a*s lower than 50% of the maximum intensity difference (Imax–Imin*)*, th*i*s was defined as the adhesion zone. This threshold level corresponded to the cell-APTES separation distance of ~ 50 nm. The RICM analysis revealed that adhesion area of F10 (172 ± 54 μm2) was about 1.5 times larger than that of F1 (119 ± 62 μm2), while there was no significant difference in projection area of cells determined by bright field microscopy (F1: 358 ± 94 μm2, F10: 384 ± 110 μm2). In addition, we found that adhesion of F1 and F10 cells was lost at EGCG concentration of 40 μM and 60 μM respectively. We expect the combination of OMT and RICM is a powerful method for the quantitative evaluation of adhesive ability of cancer cells.
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P17

Damir Vurnek

Using as the model of choice the MDCK II immortalized cell line we identify the time scales on which the toxicity of an increased osmotic environment influences the survival and the morphology of the epithelial tissue. The full phase space of tissue viability is explored from the isotonic to elevated toxic conditions.
Morphology changes are first addressed only by choosing different seeding procedures. Here the cell density reaches the mean steady state of the same value, in dense and disperse seeding alike, indicating that the internal organization of the model tissue is independent of its growth history even on macroscopic scales. On the other hand, the results obtained on varying the osmotic stress show that changes in toxic molecular surroundings change the nuclei and actin structures not only on single cell length scales, but also at the tissue level. For example, the presence of urea increases the motility of the cluster cells, and at high urea concentrations the overall cluster area is doubled (with respect to the clusters grown in the same concentrations of NaCl).
In the first week elevated osmotic conditions suppress the growth, and the average cell density in the affected tissue clusters is smaller than in the controls. However, as time scales increase, adaptations to the osmotic stress occur, and the cell density reaches its mean steady state. Nevertheless, different microscopic and macroscopic traits in the affected tissues persist (such as nuclei elongations and shapes, and varying local densities).

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How are intermediate filaments integrated into cellular structures by synemin and nestin?

Petra Zugschwerdt[1], Norbert Mücke[2], Harald Herrmann[1]
[1]B065 Functional Cell Architecture; [2]B040 Biophysics of Macromolecules; German Cancer Research Center (DKFZ), Heidelberg, Germany.

Background: In addition to microtubules and actin filaments, intermediate filaments (IF) constitute one of the three principle filament types that represent the basic element of metazoan cytoskeletons. The integration into complex interconnected structures occurs, in the case of microtubules and actin filaments, largely by associated proteins. In the case of IFs, which employ also a set of associated proteins such as desmoplakin, plectin and BPAG 1, another principle to connect with other cellular filaments, complexes and organelles has evolved: In specialized tissues such as neurons and muscle, proteins with the principle structure of IF proteins but without the ability to form filaments on their own have evolved. These proteins need a "carrier" IF-protein to integrate into the cytoskeleton, i.e. neurofilament proteins NF-M and NF-H require NF-L and likewise it is assumed that the muscle IF-proteins synemin and nestin depend on desmin and/or vimentin to be presented in a filamentous form. However, it is not entirely clear if these proteins integrate into the filament structure or if they associate in an as yet not specified fashion.
Objective: We study how the highly specialized intermediate filament proteins synemin and nestin interact with desmin and vimentin intermediate filaments.
Methods: We have synthesized recombinant desmin, vimentin, as well as domain-deleted variant of desmin, vimentin, synemin and nestin. After purification und renaturation of the individual proteins, we have analyzed the structures formed by desmin and vimentin in presence of synemin or nestin under assembly conditions. The negatively stained samples were visualized by electron microscopy. Moreover, we analyzed the interaction of desmin and vimentin with synemin by analytical ultracentrifugation and sucrose gradient centrifugation.
Results: Our main finding is that synemin has a high binding affinity to desmin and vimentin under assembly conditions and thereby exhibits a negative impact on the filament forming process of desmin and vimentin under these in vitro conditions. Synemin as well as nestin do not form filaments by themselves. For synemin, we have determined the formation of globular structures with a distinct s-value under assembly conditions.
Conclusions: *In vitro*, synemin and nestin do not from *bona fide* intermediate filaments on their own but behave actually like IF-associated proteins. A more refined analysis on the co-assembly of these two proteins with desmin and vimentin is under way. In addition, the organization and generation of authentic synemin- and nestin-bearing desmin filaments will be analyzed with high-resolution microscopic methods to elucidate the patterns of IF-association exhibited by synemin and nestin in the living cell.

Keywords: intermediate filaments, cell structure, synemin, nestin

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Nanomechanics of basement membrane invasion under hypoxic conditions - reenacting metastasis in vitro

P. Oertle[1], V. Kubak[1], S. Benayat[1], W. Halfter[2], I. Näthke[3], M. Bentires-Alj[4], R.Y.H. Lim[1] and M. Plodinec[1]

[1]Biozentrum and the Swiss Nanoscience Center, Switzerland, [2]Department of Neurobiology, University of Pittsburgh, US, [3]Cell and Developmental Biology, University of Dundee, United Kingdom, [4]Friedrich Miescher Institute, Switzerland

Basement membranes (BM) are highly conserved structures in mammals that define the epithelial or endothelial lumen (e.g. blood vessels and mammary glands) thereby separating it from the surrounding connective tissue. Moreover, BM make up the foremost mechanical barrier against the propagation of cancer cells (Kalluri et al. 2003). In breast cancer, cells form a primary lesion constrained within the mammary ducts or lobules. Once the cells breach the BM, they are able to spread throughout the body and form distant metastases (Wirtz 2011). The understanding of this very first step of the metastatic process, the breaching of the BM, is as crucial as it is limited. Therefore, to contribute to the understanding of this process, we try to answer two questions: 1) what triggers the cells to breach the membrane, and 2) how do the 'triggered' - or malignant - cells breach the native human BM. Our recent findings show that malignant lesions contain populations of very soft cancer cells, which typically make up 10 - 15 % of the cells of the lesion. The same cells also show a very high metastatic potential (Plodinec et al. 2012).

We hence aim to understand how these soft malignant cells infiltrate the surrounding stroma by breaching the BM barrier. It is known that malignant cancer cells in the primary tumor thrive in a hypoxic environment (Höckel et al. 1996). Therefore we first asked, does the native BM behave as a physiological substrate for healthy epithelial cells? Next, we asked if the hypoxia drives the malignant cells into a soft phenotype that facilitates the penetration of the BM.

To answer these questions, we use the Inner Limiting Membrane (ILM), a bi-layered (collagen IV and laminin 521) BM from the human eye (Henrich et al. 2012) as a physiologically relevant substrate to grow epithelial cells (MDCK and MCF10A) and their transformants of different metastatic potential. To assess the physiological functioning of the healthy epithelial layer we measured trans-epithelial resistance to corroborate if the polarity of the layer is established. Using confocal microscopy and structured illumination microscopy (SIM) imaging we examined epithelial morphology and structural properties of BMs and cell cytoarchitecture. Subsequently, to test if hypoxia (low oxygen level) can drive epithelial cells into a soft phenotype, we exposed them to hypoxia for a week (1 % oxygen) and tracked the changes in stiffness using atomic force microscopy (AFM). We then performed a rescue experiment by reversing oxygen levels to normoxic conditions (20 %), again for a week, to examine if the cell stiffness changes due to hypoxia can be reversed.

Our experiments show that the ILM is an adequate substrate for healthy epithelial cells (MDCK and MCF10A) to establish a normal polarity. In addition, cells grown on ILM do not show F-actin stress fibers that are typically present as an artifact of culturing on infinitely stiff and non-physiological plastic substrates. The formation of focal contacts and tight junctions is visualized by laser scanning confocal microscopy of ZO-1 and beta1-integrin proteins. Finally we also show that cells under hypoxic conditions exhibit a decreased stiffness, as well as changes in morphology. In particular a clear distinction in stiffness between cell-cell junctions (4 kPa) and nuclear regions (2 kPa) is lost. In addition a specific amount of cells, about 15 %, turn more than 2-fold softer and show typical stiffness values of 0.8 kPa. Surprisingly, we were able to “rescue” this population of very soft cells to a normal stiffness phenotype of 2 kPa when cells were cultured in normoxic conditions again. However, the clear distinction between the nuclear and the junction region was not fully re-established.

These clear changes in the phenotype of hypoxic cells, together with the ILM as a native substrate for epithelial cells will enable us to study e.g. invasive MCF10A Her2/Her3 mutants attacking the BM, to understand what mechanical and biochemical tools these cells are using to finally breach the BM.

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Entropic contraction of actin networks

C.Schuldt, T. Golde, J. Schnauß, M. Glaser and J. Käs

Universität Leipzig, Faculty of Physics and Earth Sciences, Institute for

Experimental Physics I, Linnéstr. 5, 04103 Leipzig, Germany

Retraction at the rear of a cell is a fundamental part of its migration process. This contraction can be accomplished by actin-myosin interaction. However, myosin knock-out cells have been shown to be still capable of migrating. Alternatively, the depolymerization of the cytoskeleton was proposed to cause contractile forces only by a gain in entropy in the absence of molecular motors. This concept has been demonstrated on polymer meshworks of nematode's major sperm protein [1].

We study the depolymerization of actin networks. In particular, the mesoscopic details and the forces associated with this process are of interest. We employ a microrheology approach in conjunction with light induced softening of actin networks [2] to measure both softening and contraction of the depolymerizing meshwork.

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P21

Fluorescent Beads Disintegrate Actin Networks

Tom Golde, Carsten Schuldt, Jörg Schnauß, Dan Strehle, Martin Glaser and Josef Käs

We studied the influence of fluorescent polystyrene beads on both entangled and cross-linked F-actin networks - a widely used model system for semi-flexible polymer networks. Viscoelastic properties of semi-flexible polymer networks can be explored by embedding micrometer sized tracer particles probing the network on length scales as small as the mesh size - a technique known as *microrheology* [1].
The mean-squared displacement MSD = <)x^2(J)> of the tracer particles is used to obtain the complex shear modulus G*(T) [2]. Several particle trajectories can be measured simultaneously via *video particle tracking.* Image acquisition is performed with either fluorescent beads and an epifluorescent microscope or polystyrene beads imaged by bright field microscopy. We found that illumination of fluorescent beads with their approriate excitation wavelength leads to a drastic softening of actin gels.

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The generation of topological order in the nuclear lamina studied with GFP-lamin A

Kendra Maaß, Thorsten Kolb, Ina Peristere, Harald Herrmann
B065 Functional Architecture of the Cell, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg, Germany

Background: The nuclear lamina of metazoan organisms is made from two types of intermediate filament proteins, termed A-type and B-type lamins. In man, two B-type lamins, lamin B1 and lamin B2, are constitutively found within the nuclear lamina, whereas the major A-type lamins, lamin A and its smaller splice form lamin C, are differentially expressed parallel to routes of tissue differentiation during embryonic development. In contrast to B-type lamins, A-type lamins are also encountered in significant amounts within the nucleoplasm in the form of small soluble complexes, in addition to their lamina location. Surprisingly, lamin A and lamin C do completely segregate, both in the soluble and the lamina-bound form despite the fact that their amino acid sequence is identical with the exception of a tiny sequence stretch in the carboxy-terminal domain (1). High-resolution immunofluorescence microscopy studies revealed that A- and B-type lamins do by and large occupy independent territories, and the same holds true for lamin A and lamin C. Hence the question arises how this order is established and maintained through the expansion of the nucleus during interphase and what the functional consequences of the coexistence of the fours lamin systems are.
Objective: We aimed at studying the organization of the nuclear lamina and in particular how GFP-chimeras are topologically organized after forced expression.
Methods: We have studied the composition of the nuclear lamina by ectopic expression of fluorescently tagged A-type lamins in cancer cell lines. We performed an integrated approach using a combination of fluorescence microscopy, differential cell extraction with the subsequent GFP-specific “pull-down” (GFP-binder) and separation of individual cell fractions by sucrose gradient centrifugation followed by immunoblotting of protein samples separated by gel electrophoresis.
Results: Our main finding is that the GFP-lamin A chimera are soluble to large extent in buffers of both low and high detergent, in stark contrast to untagged lamin A. Upon overexpression, GFP-lamin A chimera formed huge aggregates within the nucleus. In contrast to endogenous lamin A, the majority of GFP-lamin A was extracted with buffers of both low and high detergent concentrations. Furthermore, when the GFP-lamin A chimera precipitated with “GFP-binder” only minor amounts of the endogenous lamin A and lamin C were co-isolated indicating they did not associate in the cell with one another, in particular did not form coiled coils. A separation by sucrose gradient centrifugation revealed that the complexes formed by GFP-lamin A differed in size from those recovered with the endogenous lamin A complexes.
Conclusions: The nucleus provides a complex set of molecular mechanisms that enables the functional organization of the four major lamin polypeptides as distinct structural entities in human cells.

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P23

Temperature Induced Sudden Loss of Cell Nuclei Integrity

Authors:
Enrico Warmt, Tobias R. Kieß_ling, Roland Stange, Anatol W. Fritsch, Josef A. Käs
Institut für Experimentelle Physik I, Universität Leipzig, Germany

Email:
enrico.warmt@uni-leipzig.de

Abstract:
Despite the high temperature stability of DNA itself, we have found a sudden loss of cell nuclei integrity at relative moderate temperatures ranging from 45 to 55 degree Celcius. Suspended cells hold in an optical double beam trap were heated under controlled conditions and nuclear shape was monitored. At specifi c critical temperatures an irreversible sudden shape transition of the nuclei was observed. These temperature induced transitions diff_er in character of shape change for diff_erent cell lines. The high connectivity of the nuclei to the cytosol becomes visible when the initial shape transition of the nucleus propagates toward the plasma membrane.

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Jörg Schnauß

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Steffen Grosser